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HIV-1 and Alcohol: Interactions in the Central Nervous System

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Abstract

The use of alcohol has been associated with both an increased risk of acquisition of HIV-1 infection and an increased rate of disease progression among those already infected by the virus. The potential for alcohol to exacerbate the effects of HIV infection is especially important in the CNS because this area is vulnerable to the combined effects of alcohol and HIV infection. The effects of alcohol on glial cells are mediated through receptors such as TLR4 and NMDAR. This causes the activation of signaling molecules such as IRAK and various members of the p38MAPK family and subsequent activation of transcription factors such as NF- κ B and AP-1. The eventual outcome is an increase in pro-inflammatory cytokine production by glial cells. Alcohol also induces higher levels of NADPH oxidase in glial cells which leads to an increased production of ROS. Viral invasion of the CNS occurs early after infection, and HIV proteins have also been demonstrated to increase levels of pro-inflammatory cytokines and ROS in glial cells through activation of some of the same pathways activated by alcohol. Both cell culture systems and animal models have demonstrated that concomitant exposure to alcohol and HIV/HIV proteins results in increased levels of expression of pro-inflammatory cytokines such as IL-1 β , and TNF- α , along with increased levels of oxidative stress. Clinical studies also suggest that alcohol exacerbates the CNS effects of HIV-1 infection. This review focuses on the mechanisms by which alcohol causes increased CNS damage in HIV-1-infection.

Keywords

Alcohol; HIV-1; Central Nervous System

INTRODUCTION

HIV-1/AIDS remains a major problem despite recent advances in antiretroviral therapy (ART). One of the factors that has been associated with accelerated disease progression and increased risk of the acquisition of infection is the use of alcohol or drugs of abuse. It has been estimated that over 50% of the general population are at least occasional users of alcohol and estimates of the proportion of the general population who are heavy drinkers are as high as 8% (Krupitsky et al., 2005). Increased alcohol use has been associated with an increase in risky sexual behaviors that potentiate transmission of STDs (Krupitsky et al., 2005). Along with the increased risk of acquisition of infection, alcohol use has also been

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associated with accelerated disease progression as measured primarily by decreased CD4+ cell counts in patients receiving ART (Samet et al., 2007; Wu et al., 2011). There have also been reports of increased viral load in some cases (Wu et al., 2011). For more insight into potential mechanisms behind this effect the reader is referred to a recent review on the topic (Kumar et al., 2012). Another possible explanation for these observations lies in reduced adherence to ART in those who use alcohol (Kim et al., 2007).

The combined effects of alcohol and HIV infection on the CNS have been a subject of interest for well over a decade (reviewed in (Nath et al., 2002; Persidsky et al., 2011). In this review we will first discuss the effects of alcohol on cells in the CNS, followed by a brief discussion of the impact of HIV-1 and HIV proteins on the CNS. The final section will focus on the combined effects of HIV and alcohol on the CNS as determined by *in vitro*, *in vivo* and clinical studies. The ultimate objective of this review will be a discussion of the combined effects of HIV infection and alcohol exposure on neuroinflammation.

Effects of alcohol on the CNS

Activation of the NF- κ B Pathway in the CNS by Ethanol – inflammatory pathways

In a mouse model of chronic alcohol exposure it was demonstrated that 10 daily doses of ethanol potentiate significant increases in TNF- α , MCP-1, and IL-1 β in response to LPS (Qin et al., 2008). These increases occurred in brain, serum and liver samples. However, all three proinflammatory cytokines/chemokines returned to basal levels after 24 h in the serum and the liver, while these levels remained elevated in brain for 7 days after the administration of LPS. In addition, the levels of the anti-inflammatory cytokine IL-10 in the brain were reduced at 7 days after LPS administration, possibly accounting for the long lasting increase in proinflammatory mediators. In subsequent studies using rat brain slice cultures, these investigators confirmed the induction of cytokines along with iNOS in response to ethanol and also documented ethanol-induced increases in the proteases TACE and α PA, which are involved in the processing of TNF- α and MCP-1 (Zou and Crews, 2010). Furthermore, ethanol was shown to increase DNA binding of NF- κ B in a dose and time dependent manner.

Blanco and colleagues (Blanco et al., 2005) examined ethanol-induced activation of signaling pathways mediated through TLR4 and IL-1R. Exposure of fetal astrocyte cultures to ethanol caused rapid (10 min-30 min) activation of IRAK, ERK, JNK, and p38 as well as induction of COX-2 and iNOS expression. The increase in phosphorylation of these signaling molecules, as well as the induction of iNOS and COX-2 at the level of mRNA, could be abrogated by the addition of antibodies to either TLR4 or IL-1R. Acute treatment with ethanol increases the phosphorylation of IRAK, p38, JNK, and ERK, all of which are downstream of TLR4; this resulted in increased production of TNF- α , NO, and IL-1 β in rodent microglial cells in wild type but not in TLR4-deficient mice (Fernandez-Lizarbe et al., 2009).

Although the role of TLR4 in ethanol-mediated neuroinflammation has been the subject of investigations for several years, the role of some of the other TLRs has not been extensively investigated. The role of TLR3 in neuroinflammation was investigated by treating mice with

ethanol for 10 days followed by poly I:C, a classic TLR3 agonist, administered intraperitoneally (Qin and Crews, 2012a). Treatment with only ethanol resulted in increased levels of TNF- α in the brain, while increased levels of IL-6 and MCP-1, were observed in both the blood and brain. Treatment with poly I:C caused increased levels of TNF- α , IL-1 β , IL-6 and MCP-1 in both the blood and brain. However, treatment with both ethanol and poly I:C caused increased levels TNF- α , IL-1 β , IL-6 and MCP-1, in both the blood and brain, that were significantly greater than when treated with either agent alone. This demonstrates that TLR3 ligands may exacerbate alcohol-induced neuroinflammation and suggests that alcohol may exacerbate neuroinflammation caused by viral infection of the CNS.

Oxidative Stress

Along with increased production of inflammatory cytokines, one of the other areas of concern regarding the effects of alcohol on the CNS focuses on oxidative stress. In one early investigation of the effects of ethanol on oxidative stress in the CNS, hamster microglia were treated *in vitro* with ethanol concentrations up to 200 mM for periods up to 48 h. Ethanol concentrations of 20 and 200 mM were effective at increasing superoxide anion production, with 20 mM ethanol producing the greatest effect after 24 h (Colton et al., 1998). This was the first demonstration that ethanol could mediate oxidative stress through microglia. Another early investigation of the effects of alcohol on glial cells demonstrated that intermittent ethanol exposure increases the number of microglia in the cerebellum of rats (Riikonen et al., 2002), which may exacerbate the increase in superoxide production.

Astrocytes have also been demonstrated to yield indicators of oxidative stress in response to acute ethanol exposure. The human astrocyte cell line A172 was shown to induce higher levels of iNOS activity when stimulated with cytokines in the presence of 50 mM ethanol than when stimulated with cytokines alone. Interestingly, 24 hr exposure to 200 mM ethanol resulted in inhibition of iNOS activity (Davis and Syapin, 2004a). A subsequent study by these investigators demonstrated that 50 mM and 200 mM ethanol produced differential effects on NF- κ B nuclear translocation and DNA binding activities in astrocytes stimulated with cytokines (Davis and Syapin, 2004b). While 200 mM ethanol caused higher levels of p65 nuclear translocation, 50 mM ethanol resulted in higher levels of cytokine-induced NF- κ B binding activities.

Acute ethanol exposure has also been shown to activate NADPH oxidase and induce ROS in the SH-SY5Y neuroblastoma cell line (Wang et al., 2012). Expression levels of the p47^{phox} and p67^{phox} subunits were significantly increased by ethanol exposure, and inhibition of the expression of the p47^{phox} subunit by siRNA reduced both the level of ROS and the level of ethanol-induced lipid peroxidation.

Animal models of chronic ethanol exposure have also demonstrated that oxidative stress is responsible for neuronal injury. Chronic feeding of ethanol to mice was demonstrated to cause neuronal loss and to increase various markers of oxidative stress such as decreased neurofilament staining in the cortex, increased levels of ROS in glial cells and increased levels of 3-nitrotyrosine protein adducts and iNOS expression in neurons (Rump et al., 2010). The levels of both markers of oxidative stress and markers of neuronal injury were reduced by co-administration of the antioxidant acetyl-L-carnitine with the ethanol.

Astrocytes have long been known to express CYP2E1 which metabolizes ethanol to acetaldehyde with the consequent production of ROS (Kumar et al., 2012). Treatment of astrocytes with ethanol or acetaldehyde increased levels of ROS and the inflammatory mediator, prostaglandin E2. Expression of cytosolic phospholipase A2, which is responsible for the production of arachidonic acid, could also be induced by exposure to ethanol or acetaldehyde (Floreani et al., 2010).

Chronic ethanol treatment of mice with ethanol for 10 days has been shown to increase the levels of markers of cell death such as caspase-3 and Fluoro-Jade B staining in neurons (Qin and Crews, 2012b). Along with the increase in neuronal death, the ethanol treatment resulted in the activation of astrocytes and microglia, an increase in the expression of NADPH oxidase (NOX) gp91^{phox} and increased detection of ROS. The increase in NOX expression was also observed in human brains of heavy drinkers that were obtained postmortem. This provided a direct link between increased ROS production from microglia and increased neurotoxicity.

Another mechanism responsible for alcohol-induced neuronal damage was recently shown to be mediated by IL-1 β (Zou and Crews, 2012). Ethanol treatment of HEC slices was demonstrated to substantially reduce DCX, a marker of neurons undergoing maturation. IL-1 β was also increased by ethanol treatment of HEC slices, while CREB and BDNF levels were inhibited. The effects of ethanol could be abrogated by the addition of antibodies to IL-1 β or an IL-1 receptor antagonist (IL-1R1a). Along with IL-1 β , the inflammasome proteins NALP1 and NALP3 were also increased in response to ethanol. Similar results with regard to IL-1 β and NALP1 and NALP3 were observed in post-mortem analyses of human brains.

NMDA receptors and ethanol

Another potential mediator of alcohol-induced neuronal damage is the NMDA receptor. The receptor is expressed as a heterotetramer comprised of GluNR1 and GluNR2 subunits. The heterotetramer comprised of 2 subunits of GluNR1 and 2 subunits of GluNR2B is commonly expressed at high levels in the striatum (Alfonso-Loeches and Guerri, 2011). Early work indicated that ethanol administration caused increased glutamate concentration and subsequent neurotoxicity that was presumed to be the result of excitotoxicity of the NMDA receptor (Smothers et al., 1997). While subsequent work indicates that neurotoxicity associated with binge drinking appears to be relatively independent of NMDA receptor activity and is more closely associated with oxidative stress (Collins and Neafsey, 2012), NMDA-mediated excitotoxicity is a key therapeutic target to reduce the effects of withdrawal from chronic exposure to ethanol.

The NR2B subunit has been the focus of several recent investigations aimed at determining the various behavioral and neurotoxic effects of ethanol on NMDA receptors. Using rodent models along with acute exposure of dorsal striatal slices, it was determined that NR2B is phosphorylated by Fyn kinase (Wang et al., 2007). Furthermore, the inhibition of the phosphorylation of NR2B was associated with decreased self-administration of ethanol. An NMDAR antagonist with selectivity against heterotetramers containing NR2B/NR2B dimmers was used to demonstrate that this conformation of NMDA plays a role in ethanol-

induced behavioral alterations as well as ethanol induced cell toxicity (Lewis et al., 2012). As these experiments were conducted using neonatal ethanol exposure the results are most applicable to the developing brain.

Ethanol and the blood-brain barrier

In a series of studies Persidsky and colleagues have examined the various mechanisms through which ethanol affect the blood-brain barrier (Persidsky et al., 2011). Using BMVEC in conjunction with human monocytes, they demonstrated that ethanol had numerous effects on the BBB, including causing increased transmigration of monocytes across the barrier, and an increase in phosphorylation of several proteins including myosin light chain kinase (MLCK), myosin light chain, claudin-5 and occludin. There was also an overall decrease in the expression levels of both claudin-5 and occludin. Thus, the overall result of the ethanol exposure was increased permeability of the BBB. Interestingly, treatment with an inhibitor of MLCK or pre-treatment with an inhibitor of ethanol metabolism reversed the effects of ethanol. These results suggested that the effects of ethanol were mediated by ethanol metabolism through MLCK.

HIV/HIV proteins and Ethanol: Effects on the CNS

Initial work suggesting the neurotoxicity of HIV

Some of the first indications that HIV-1 infection might be problematic with regard to the CNS were the presence of neurological complications in patients infected with the virus (Price et al., 1988). The cells infected in the CNS were shown to be microglia and perivascular macrophages (Pumarola-Sune et al., 1987). Although neurons are not infected by HIV, it is clear that they are subject to damage by HIV infection of the CNS. In a pioneering study that investigated the causes of HIV-1-induced neurotoxicity, Gendelman and colleagues (Genis et al., 1992) performed co-culture assays using monocytes and astroglia in which the monocytes were either uninfected, or infected with HIV-1. Although the infected monocytes when cultured alone failed to secrete neurotoxins, co-culture of infected monocytes with astroglia did produce neurotoxins including cytokines and leukotrienes. This work laid the foundation for the currently accepted paradigm that neuronal toxicity is the result of toxic cellular factors secreted by infected microglia, perivascular macrophages or astrocytes, as well as direct toxicity resulting from exposure to HIV-1 proteins secreted by infected microglia (Kaul and Lipton, 2006). Infection with HIV, or exposure of non-neuronal cells to HIV-1 proteins has been associated with increased levels of inflammatory cytokines such as IL-1 β , and IL-6, as well as the induction of chemokines like MIP-1 β , RANTES, and MCP-1. Exposure to HIV-1 proteins, or infection with HIV-1, has also been shown to induce increased production of markers of oxidative stress (reviewed in Kaul and Lipton, 2006).

A more comprehensive account of the direct effects of HIV-1 proteins on neurons is beyond the scope of this review. However, recent reviews have been published on the overall neurotoxicity of HIV-1 proteins (Mocchetti et al., 2012; Silverstein et al., 2012).

Effects of Ethanol and HIV infection or HIV proteins on the CNS

We will focus on the effects of HIV and alcohol on the CNS that have been associated with increased neuronal toxicity. These effects include, but are not limited to, increased production of inflammatory cytokines, increased production of ROS and other markers of oxidative stress and release of viral proteins (e.g. Tat, gp120) which have been demonstrated to be neurotoxic or to have deleterious effects on other cells in the CNS such as astrocytes or the microvascular endothelial cells that comprise the blood-brain barrier. A large portion of the work on the effects of HIV and HIV proteins has utilized mixed culture models such as hippocampal-entorhinal cortex (HEC) slices and other *in vitro* models, as well as animal models as described below.

One of the methods used to circumvent the inability of HIV-1 to infect rodents has been through the use of stereotactic injections of HIV proteins. Synergy between Tat and ethanol was demonstrated by using this approach (Flora et al., 2005). Injections of Tat into mouse hippocampus produced significant increases in oxidative stress in the hippocampus and corpus striatum of mice that were also injected i.p. with ethanol. The combined treatment also increased the levels of phosphorylated ERK, and DNA-binding activity of NF- κ B. The expression levels of IL-1 β , TNF- α , MCP-1, and ICAM-1 in the hippocampus and corpus striatum were also increased. Overall, Tat and ethanol synergized to increase oxidative stress and levels of proinflammatory cytokines in mouse brain exposed to both agents. Furthermore, the increase in MCP-1 and ICAM-1 may be involved in increased trafficking of monocytes across the blood-brain barrier, whose permeability is also increased. Canulation of an abdominal vein has also been utilized to administer gp120 to rats (Singh et al., 2008). The combination of ethanol and gp120 produced the greatest increase in free radical production; this was followed by rats treated with only gp120 and then by rats treated with only ethanol. The same pattern was followed with regard to levels of protein carbonylation. These results strongly suggest that ethanol exposure significantly exacerbates the oxidative stress induced by gp120 or Tat.

A mouse model of HIV-1 encephalitis was utilized to demonstrate the increased effect of ethanol on neuroinflammation caused by HIV (Potula et al., 2006). SCID mice were fed a Lieber-DeCarli diet containing 4% ethanol for two weeks before engraftment with human lymphocytes. Mice were then inoculated stereotactically with HIV-1_{ADA}-infected MDMs 8 days after engraftment and sacrificed at 7 and 14 days after inoculation. In the CNS, 1 week after inoculation ethanol-fed mice had more HIV-1 p24+ MDMs in the brain than did control mice. Ethanol-treated mice also exhibited microglial increases in terms of both number of cells and activated phenotype. By the 2nd week after inoculation, ethanol-fed mice had more CD68+ cells and more HIV-1 p24+ cells in the CNS. Ethanol-fed mice also exhibited increased levels of nitrotyrosine, a marker of oxidative stress, in the brain (Potula et al., 2006).

In terms of direct toxicity of HIV-1 proteins to neurons, the effects of Tat and gp120 have been extensively studied (Haughey et al., 2001; Kaul and Lipton, 1999). The effect of ethanol withdrawal upon Tat-mediated neurotoxicity was shown to be mediated through NMDAR in an *in vitro* system using rat organotypic slices. Treating the sections with either

Tat or NMDA during ethanol withdrawal resulted in significant increases in PI uptake. However, PI uptake could be reduced to levels equivalent to the control by using the NMDAR antagonist MK-801 (Self et al., 2004). A similar approach was utilized to evaluate the effects of ethanol on gp120-induced toxicity to human neurons, however the period of ethanol exposure was limited to 24 h (Chen et al., 2005) as opposed to the 10 day ethanol treatment utilized with the rat experiment (Self et al., 2004). In this case, ethanol also potentiated the toxic effects of gp120 and the effects of ethanol and gp120 were mediated through an NMDAR-dependent pathway. Injection of Tat into the hippocampus of rats during ethanol withdrawal has also been shown to increase behaviors seen in ethanol withdrawal. The use of the NMDAR antagonist MK-801 demonstrated that these effects were dependent upon NMDAR (Self et al., 2009).

Non-Human Primate Studies

There are currently two widely used non-human primate models of HIV infection that involve either simian immunodeficiency virus (SIV), or simian-human immunodeficiency virus (SHIV). The majority of studies involving alcohol and either model have focused on the effects of alcohol in the periphery. Molina and colleagues have published several studies demonstrating that alcohol contributes to muscle wasting in the latter stages of SIV infection (Molina et al., 2008). Chronic alcohol consumption has been shown to increase viral replication and result in decreased levels of CD8+ lymphocytes in the duodenum (Poonia et al., 2006). Using a model of binge alcohol consumption, the level of viral RNA during the initial phase of infection in alcohol-consuming animals was higher than in sucrose-treated animals (Bagby et al., 2003). Although viral RNA levels in alcohol-treated and sucrose-treated animals gradually equalized after a period of about 6 months, alcohol-treated animals exhibited a more rapid progression to end-stage disease (Bagby et al., 2006). A macaque-SIV/SHIV model was used with alcohol-treated animals addicted to alcohol along with control animals (Kumar et al., 2005). The CD4+ cell counts in the infected and alcohol-addicted animals was lower than in the infected, but non-alcohol treated controls. Although the initial viral loads were similar, after approximately 12 weeks the viral loads in the CSF and plasma in the alcohol-addicted animals was higher than in the control group. Like the previously described studies, this suggests that alcohol may accelerate disease progression in infected individuals. In addition, it also suggests that CNS disease in alcoholic patients may be similarly exacerbated.

Clinical Studies

Unlike animal studies and the few *ex vivo* studies that suggested that alcohol could increase viral replication, clinical studies have suggested that the relationship between alcohol use and disease progression is more complicated (reviewed in (Hahn and Samet, 2010). In one clinical study Samet and colleagues (Samet et al., 2007) examined CD4 cell counts and viral loads that were either treated or not treated with HAART. In patients that did not receive HAART there was an association between heavy alcohol use and lower CD4 counts. No correlations between viral load and CD4 counts were observed in the group receiving HAART or that were moderate users of alcohol. Subsequent studies have reported a significant association between alcohol use and patient non-adherence to ART (Hendershot

et al., 2009). A recent study also found a significant association between increased viral load and daily alcohol use (Wu et al., 2011). Taken together, it is clear that alcohol use presents a substantial obstacle to effective therapy for HIV-1 infection by affecting patient adherence as well as the biological parameters associated with the disease.

The effects of alcohol and HIV were examined using magnetic resonance imaging (MRI) with patients who were HIV+ and alcoholic (HIV-ALC) and the results compared with normal controls, patients who were alcoholics but not infected with HIV (ALC), HIV-infected patients who were not alcoholic (HIV), HIV-infected patients who had an AIDS-defining event (HIV-AIDS) and HIV patients who had an AIDS-defining event who were also alcoholic (HIV-AIDS-ALC) (Pfefferbaum et al., 2006). The ALC and HIV-ALC groups had larger ventricular volumes than the corresponding non-alcoholic groups, and the HIV-infected patients showed larger ventricular volumes than the corresponding uninfected groups. The effects on white matter, which may be associated with pathologies such as myelin pallor, gliosis, and multinucleated giant cells, were much greater for the HIV+AIDS +ALC group than any other group (i.e. HIV, ALC, HIV+ALC). This suggests that alcoholism exacerbates the neurological effects of HIV and AIDS.

Diffusion tensor imaging was used to examine the microstructure in the corpus callosum (Pfefferbaum et al., 2007). The parameters measured included fractional anisotropy, a reduction in which is associated with increased dementia. Levels of mean diffusivity, an increase in which has been associated with psychomotor deficits, were also measured. The ALC groups had lower levels of fractional anisotropy than the controls and the decrease in fractional anisotropy of the HIV+ALC group was greater than in the HIV+ only group. Increases in mean diffusivity levels were also apparent in the HIV+ALC groups, but not in the HIV only group. However, alcoholic patients with AIDS exhibited the most extreme abnormalities in measures of callosal fiber bundles. Furthermore, in the HIV+ALC group, abnormalities in callosal and fiber bundle measurements correlated with decreased motor performance. This provides additional support for a role in the exacerbation of HIV infection by alcohol use.

Another investigation utilized MRI in conjunction with neuropsychological tests to evaluate cortical volumes in patients who were alcoholic, HIV-infected, or both (Pfefferbaum et al., 2012). The greatest differences from the control group were seen in the ALC groups (ALC and ALC+HIV), whereas there were no significant differences between the HIV+ group and controls. In terms of white matter, the HIV+ALC group had volume deficits in the corpus callosum. The HIV+ALC+AIDS group had larger ventricular volumes compared to HIV +ALC without AIDS. Neuropsychological tests revealed increased deficits in the HIV+ALC group compared to HIV+, ALC, or control groups. This correlated with smaller volumes of occipital cortex and hippocampus/amygdale and larger ventricle volumes. Overall, the results showed that ALC +HIV increased the neurological damage caused by HIV alone.

Future Directions

Although the effects of alcohol have been studied in great detail, there remain many unanswered questions as to the impact of alcohol in HIV patients, especially with regard to

the potential effects on neuroAIDS. As detailed above, data from *in vitro* models, animal models, and clinical studies suggest that alcohol may increase viral replication and viral load in HIV patients who use alcohol. However, the effects of alcohol upon virus evolution, particularly on the development of antiretroviral drug resistance, are unknown. This is especially important because selection pressures in the absence of alcohol seem to be substantially different in the CNS and the periphery. It is also critical to understand the impact of alcohol on the metabolism and disposition of antiretrovirals. At present, there are only a limited number of antiretrovirals that achieve therapeutic concentrations in the CNS, and understanding the impact of alcohol use upon their disposition is of utmost importance in treating these patients. Furthermore, greater understanding of interactions between alcohol and HIV, especially with respect to pathways leading to the induction of inflammatory mediators may lead to identification of potential therapeutic targets for HAND. Since such a high percentage of HIV+ patients use alcohol, it is imperative to understand the interactions between HIV-1 infection and alcohol in the CNS. Clearly, this is an area of research which will see significant growth in the next several years.

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Figure 1.

This is a representation of some of the pathways leading to oxidative stress and neuroinflammation that are activated in cells of the CNS that are infected by HIV-1 and exposed to ethanol. Ethanol exerts its effects through at least three major pathways. One pathway involves its effects on NMDAR and the eventual generation of ROS. As described in the text, ethanol can also interact with CYP2E1 which will also lead to increased ROS generation, or it can interact with TLR4 to increase production of pro-inflammatory cytokines. HIV-1 and its associated proteins have also been demonstrated to interact with NMDAR and result in oxidative stress, and it can also activate the NF-κB pathway leading to increased production of pro-inflammatory cytokines.