The Impact of Interferons on Microglial Activation in the Context of Alcohol Withdrawal

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Introduction

1. Alcohol Use Disorder (AUD) affects nearly 29.5 million individuals in the United States alone.[1]
2. While it is unknown how to treat the negative affects during withdrawal/abstinence, it is known that alcohol use disorder causes cognitive dysfunction and neuroinflammation due to the release of pro-inflammatory cytokines.[2]
3. Of these proteins, interferons, have an induced proinflammatory signaling pathway due to alcohol use.[3]
4. Chronic interferon signaling is known to cause negative affect, including depression and suicidal ideations.[4]
5. Interferon-α/β Receptor Knockout (IFNAR KO) mice lack the antigen receptor, which is composed of two subunits, IFNAR1 and IFNAR2, and are protected from the negative affect behaviors caused by alcohol use.[5]

Objectives

Examine the prevalence of microglial activation in various areas of the brain to determine the degree to which interferons play a role in proinflammatory responses as a result of alcohol withdrawal.

Methods

Mice Treatment and Sacrifice

The mice were treated with ethanol for five weeks and received five grams of alcohol per kilogram via oral gavage. The mice received the ethanol treatments for five days, followed by two days of no treatment. This method mimics the cyclic nature of alcohol use disorder, which involves periods of binge use followed by periods of withdrawal.

Figure 1. The treatment process for ethanol-treated mice involves receiving 5 grams of ethanol per kilogram of weight for 5 days, followed by 2 days of no treatment. The mice received treatment via oral gavage for 5 weeks and were sacrificed 24 hours after their last treatment.

As a control, two treatment groups received water via oral gavage and followed the same treatment process. All treatment groups of mice were sacrificed twenty-four hours after five weeks of treatment.

Immunofluorescence

Immunohistochemistry was performed using the Rabbit monoclonal anti-Iba1 antibody (1:1000; ab184938) in the cortex (infralimbic-IL), bed nucleus of the stria terminalis (vBNST), dentate gyrus (DG), and central nucleus of the amygdala (CeA). Images were taken on a BZ-X810 microscope and quantified with BZ-X800 analysis software. The analysis software was utilized to measure the total Iba-1-stained area. The area of microglial activation of the brain region of interest was divided by the total area. This area was averaged per test group to standardize the data.

Results

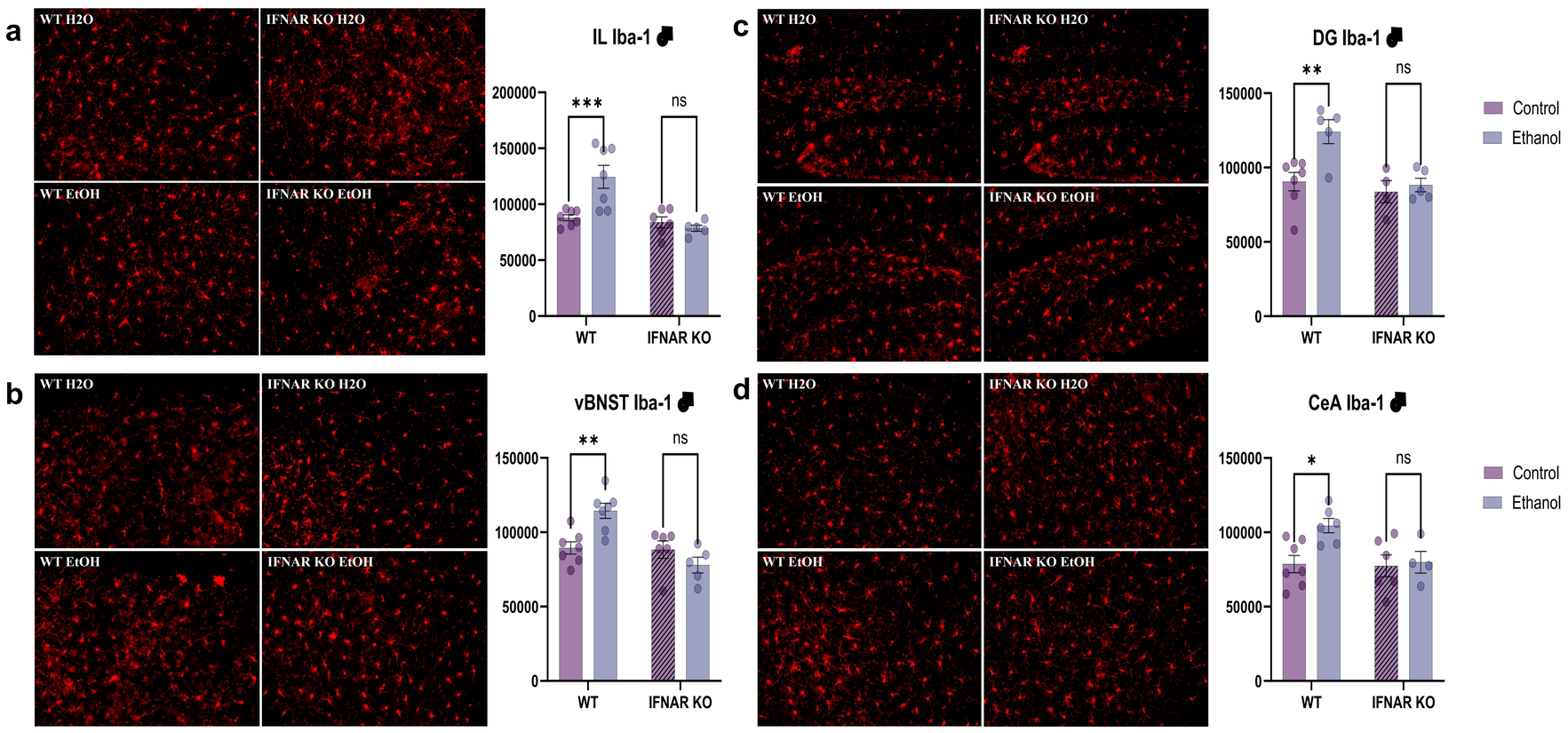

Figure 2. Images representative of the microglial activation (Iba-1) of Wild Type (WT) and Interferon-α/β Receptor Knockout (INFAR KO) mice treated with water (H2O) or ethanol (EtOH). (**a**) Immunofluorescence shows ethanol significantly increases microglial activation in the infralimbic cortex in wild-type mice ($p = 0.0008$), and there was no change in activation in interferon-α/β receptor knockout mice ($p = 0.8328$). (**b**) Immunofluorescence shows ethanol significantly increases microglial activation in the stria terminalis in wild-type mice ($p = 0.0027$), and there was no change in activation in interferon-α/β receptor knockout mice ($p = 0.3403$). (**c**) Immunofluorescence shows ethanol significantly increases microglial activation in the dentate gyrus in wild-type mice ($p = 0.0032$), and there was no change in activation in interferon-α/β receptor knockout mice ($p = 0.8857$). (**d**) Immunofluorescence shows ethanol significantly increases microglial activation in the amygdala in wild-type mice ($p = 0.0127$), and there was no change in microglial activation in the amygdala in interferon-α/β receptor knockout mice ($p = 0.9625$).

Conclusions

1. By utilizing immunofluorescence on brain regions from both wild-type and interferon-α/β receptor knockout mice, it is evident that interferons are involved in the proinflammatory response of alcohol use.
2. This phenomenon is evident in the infralimbic cortex (IL), bed nucleus of the stria terminalis (vBNST), dentate gyrus (DG), and central nucleus of the amygdala (CeA). Therefore, targeting the proinflammatory pathway of AUD and the release of interferons is a viable course of future study.
3. To expand on the findings of this experiment, future studies can investigate the activation of astrocytes due to alcohol use. Astrocytes are another example of microglial cells that mediate proinflammatory responses. This study can utilize a similar approach, using immunofluorescence to reveal astrocyte activation in brain regions of interest.

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