

# Does Ethanol Affect Chemokine-Induced Microglial Migration?

Sushobhna Batra<sup>1,2,3</sup>, Richard A. Rabin<sup>3</sup>

<sup>1</sup>Department of Biological Sciences, <sup>2</sup>Department of Biochemistry, <sup>3</sup>Department of Pharmacology and Toxicology; University at Buffalo

## Abstract

Ethanol abuse is a major health concern in the U.S. where 86.8% of people 18yrs. or older have drunk alcohol in their lifetime. Alcohol consumption increases the risk of accidents and brain injury. A critical component of brain's response to injury is the activation and migration of microglia cells, the innate immune cells of the brain. The objective of this study was to determine if ethanol alters microglial migration stimulated by fractalkine, a chemokine released by injured neurons. Our results show that ethanol inhibits both basal and fractalkine-stimulated migration of microglia.

## Introduction

Alcohol consumption is associated with an increased risk of accidents and trauma as 35-81% of patients with traumatic brain injuries were intoxicated and 42% of these patients were heavy drinkers prior to the injury. Although ethanol is a causative agent, its effects on the severity and clinical outcomes of brain injury are unclear. In the event of a brain injury, microglia, the primary innate immune cells of the brain, transition from their resting state to an activated state, and migrate to the site of damage where they can phagocytize the cellular debris. Furthermore, microglia have the potential to exert either neuroprotective or pro-inflammatory effects at the site of injury.

Fractalkine/CX3CL1 is a chemokine ligand that is released during cellular injury and it plays a major role in the inflammatory response. In the brain, only microglia express the CX3CR1 receptor for fractalkine, and stimulation of this receptor increases microglial migration.

## Objective

The aim of this study was to determine ethanol's effects on migration of microglia cells in response to the chemokine fractalkine.

Approach: Mouse BV2 microglia cell line was used as a model system, and cell migration was studied using the wound healing assay.

## Methods

- 1) BV2 microglia cells, maintained in complete DMEM media containing 10% fetal bovine serum and an antibiotic- antimycotic solution (100 units penicillin, 0.10mg streptomycin, and 250ng amphotericin B per mL), were plated (0.5M cells/well) onto a poly-lysine treated 12-well plate.
- 2) To perform the wound healing assay, a cross was placed on each well by scraping the confluent microglial cell layer using a 200µL pipette tip.
- 3) Cells were incubated at 37°C for 16 hours in DMEM only media, DMEM media containing 100mM ethanol, DMEM media containing 3nM fractalkine, or in DMEM media containing a combination of 100mM ethanol and 3nM fractalkine.
- 4) Each condition was measured in quadruplicate, and multiple images of the scratches were captured per well at t= 0 hour (immediately after the scratch) and at t= 16 hours, using an Olympus IX70 microscope fitted with a CCD camera.
- 5) Images were analyzed on the Fiji software, and cell coverage in the scratched area at the two time points was calculated.

## Results

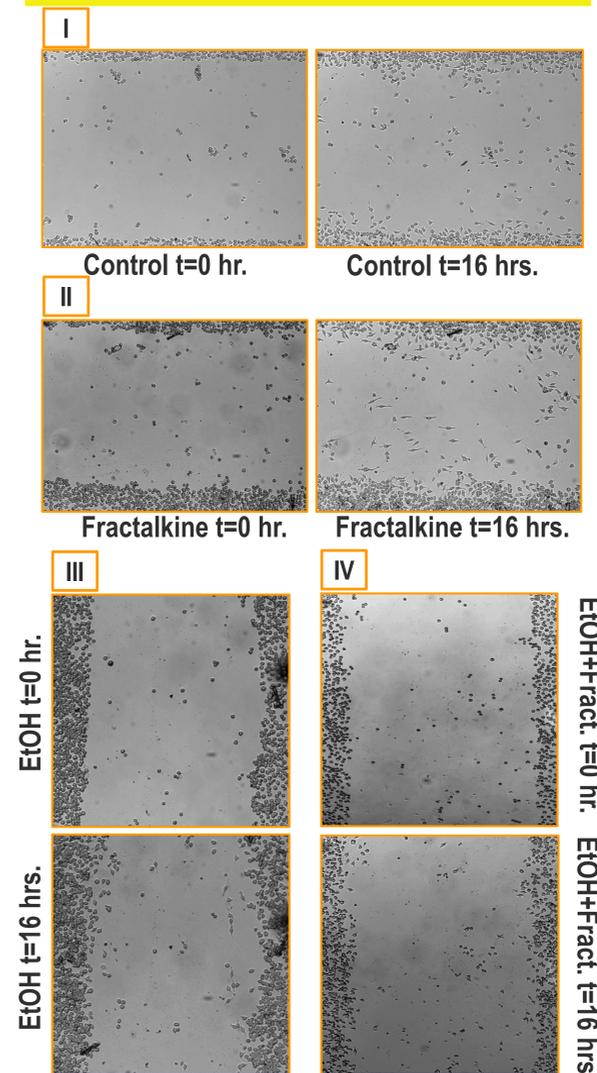
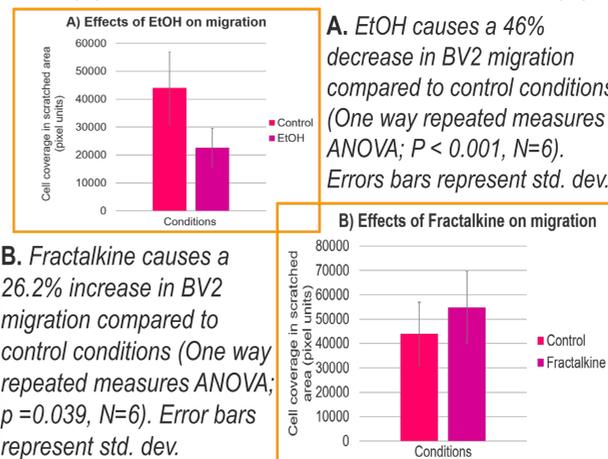


Fig.1 Images captured at t= 0 and t=16 hrs., showing the extent of migration in the scratched area in the presence of DMEM alone (basal migration) (I); 3nM Fractalkine alone (II); 100mM EtOH alone (III); combination of 3nM Fractalkine+100mM EtOH (IV).



**B. Fractalkine causes a 26.2% increase in BV2 migration compared to control conditions (One way repeated measures ANOVA;  $p=0.039$ ,  $N=6$ ). Error bars represent std. dev.**

**A. EtOH causes a 46% decrease in BV2 migration compared to control conditions. (One way repeated measures ANOVA;  $P < 0.001$ ,  $N=6$ ). Errors bars represent std. dev.**

**C. EtOH inhibits fractalkine-stimulated migration by 57.6% (One way repeated measures ANOVA;  $p < 0.001$ ,  $N=6$ ). Error bars represent std. dev.**

There is no significant difference in the inhibitory effects of EtOH on BV2 migration in the absence ( $46\% \pm 6.85\%$ ) or presence of fractalkine ( $57.6\% \pm 5.87\%$ ) (One way repeated measures ANOVA;  $p = 0.972$ ,  $N=6$ ).

## Conclusions

- ❖ Ethanol inhibited basal (absence of any modulators) BV2 microglial migration.
- ❖ Fractalkine alone increased the migration of BV2 cells.
- ❖ In the presence of ethanol, fractalkine-stimulated migration of BV2 cells was inhibited.
- ❖ Inhibitory effects of ethanol on basal and fractalkine-stimulated migration were similar.

## References

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## Acknowledgements

This research was funded by CURCA. Special thanks to Dr. Fraser Sim for allowing the use of his Olympus IX70 microscope setup.