

# Effects of Ethanol on Brain Injury: Role of 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

Traumatic Brain Injury (TBI) involves damage to the brain due to a hard blow or jolt. Alcohol consumption increases the risk of accidents and trauma, and data reveal that a significant percentage of TBI patients were intoxicated prior to the injury. The effects of ethanol on TBI's outcome, however, are unknown. A critical component of the brain's response to TBIs is the activation and migration of microglia cells, which are the primary innate immune cells of the brain. The objective of the present study was to determine the effects of ethanol on microglial migration. To this end, confluent mouse BV2 microglial cell layers were scratched with a pipette tip and then incubated in media in the absence and presence of ethanol for 6 hours. Reduction in the area of the scratch after 6 hours was less in the presence of ethanol. Our results indicate that ethanol inhibits microglial migration.

## Introduction

Alcohol consumption is associated with an increased risk of accidents and trauma as 35-81% of patients with TBIs were intoxicated and 42% of these patients were heavy drinkers prior to the injury<sup>c</sup>. Although ethanol is a causative agent, its effects on the severity and clinical outcomes of brain injury are unclear. Microglia, which are the primary innate immune cells of the brain, play a critical role in the response to TBI<sup>a</sup>. In the presence of extracellular mediators and cellular debris that are released upon brain injury, microglia transition from their resting state to an activated state, and migrate to the site of damage.

## Objective

The aim of this study was to determine ethanol's effects on migration of microglia cells.

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 microglial cells 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 6 hours in either DMEM only media or in DMEM media containing 100mM ethanol.
- 4) Each condition was measured in quadruplicate, and multiple images of the scratches were captured per well at t= 0 hours (immediately after the scratch) and at t= 6 hours, using an Olympus IX70 microscope fitted with a CCD camera.
- 5) Images were analyzed on the ImageJ software, using the 'MRI Wound Healing Tool' macro<sup>b</sup>. Presence of debris or abrasion of the substratum hampered analysis by the macro. Thus, unbiased manual analysis of those images was performed.

## Results

### Image Analysis: Macro Vs. Manual

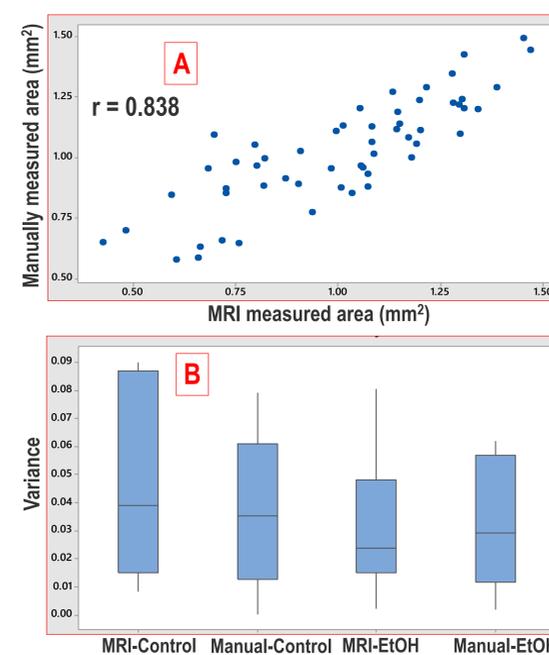


Fig.1. **A.** Scraped area measured at 6 hrs. using the MRI macro and by manual analysis. **B.** Intra-well variance in scraped area determination (N= 8 wells) at 6 hours. Differences between groups were not statistically significant (1-way ANOVA:  $F=0.56$ ;  $P=0.646$ ).

### BV2 Microglia Migration

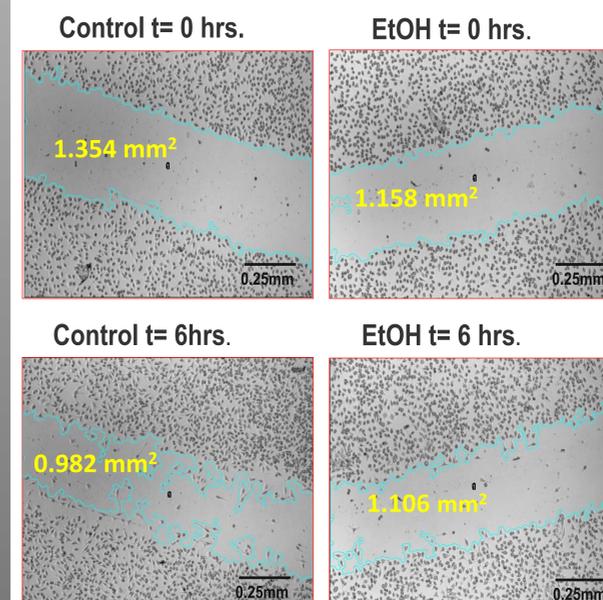


Fig.2 Representative images showing decrease in scraped area over a period of 6 hours in the presence and absence of 100mM ethanol.

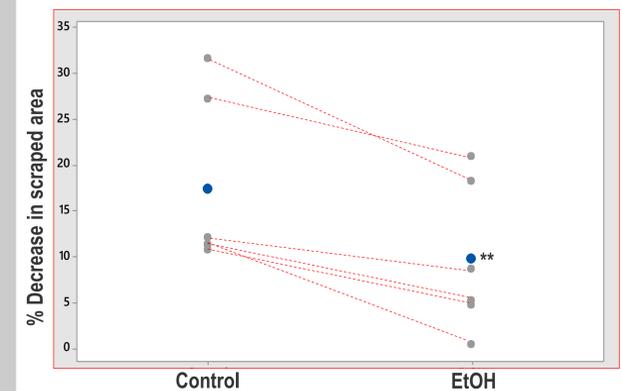


Fig.3 Percent decrease in scraped area after 6 hrs. in the presence and absence of 100mM ethanol. Large blue dots are mean values. Decrease in scraped area was significantly reduced in ethanol-treated cells (\*\*  $p = 0.01$ , paired t-test).

## Conclusions

- ❖ Comparable results were obtained with both macro and manually analyzed images.
- ❖ In the presence of ethanol, a smaller decrease in the area of the scratch was found indicating that ethanol inhibits the migration of BV2 microglia cells.

## References

- <sup>a</sup>Hernandez-Ontiveros DG, Tajiri N, Acosta S, Giunta B, Tan J, Borlongan CV. Microglia activation as a biomarker for traumatic brain injury. *Frontiers in Neurology* 4: 1-9, 2013.
- <sup>b</sup>MRI Wound Healing Tool macro. [http://dev.mri.cnrs.fr/projects/imagejmacro/s/wiki/Wound\\_Healing\\_Tool](http://dev.mri.cnrs.fr/projects/imagejmacro/s/wiki/Wound_Healing_Tool).
- <sup>c</sup>Opreanu RC, Kuhn D, Basson MD. Influence of ethanol on mortality in traumatic brain injury. *J Am Coll Surg* 210: 997-1007, 2010.

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