Alteration in Neuronal and Inflammatory Gene Expression in the Ferret Retina Following Blast Exposure

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Background

Blast-induced ocular injury (bOI) poses a significant risk of vision loss, particularly among military personnel exposed to improvised explosive devices and other high-impact blasts. Despite accounting for only 0.1% of total body surface area, the eyes sustain 6–13% of all battlefield blast injuries, underscoring their extreme vulnerability. The retina, the innermost and delicate layer of eyes, is highly susceptible to the blast injuries. Although the structural and clinical consequences of bOI have been largely studied, the underlying molecular mechanisms involving neuronal damage and inflammatory responses in the retina remain unexplored.

Objectives: (1)To evaluate the temporal effects of blast exposure on retinal integrity. (2) Analyze the temporal changes in neuronal and inflammatory gene expression in the ferret retina following blast exposure, focusing on key markers such as NFL, NFM, NFH, NeuN, GFAP, Iba1, and UCHL-1. (3) Identify future research directions aimed at elucidating the cellular and molecular mechanisms underlying retinal damage after blast exposure and discuss potential strategies for therapeutic intervention.

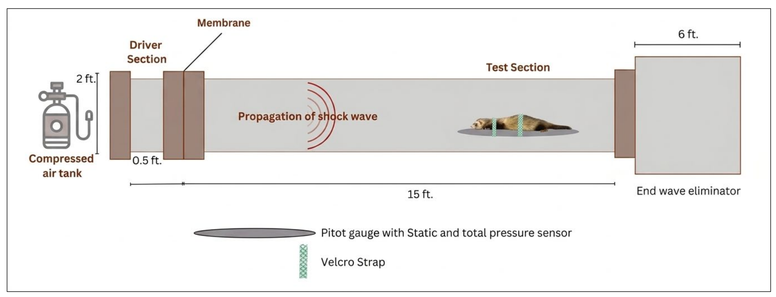
Methods

Adult male ferrets (14-15 weeks) were anesthetized with isoflurane and exposed to tightly coupled blast overpressure waves (two 19 psi blasts) using an advanced blast simulator

Ferrets were euthanized under anesthesia at 4 hours, 24 hours, 7 days or 30 days post blast

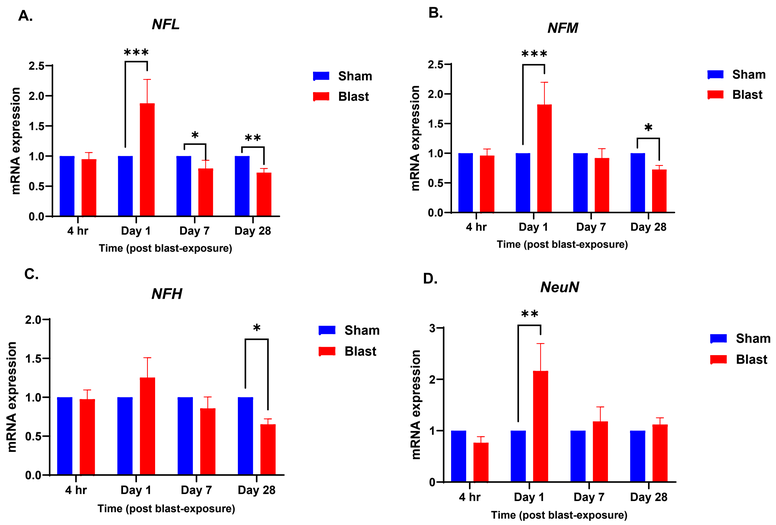
Sample collection: Retinas from both eyes were dissected and pooled

RNA extracted from the retina, cDNA was synthesized, and qPCR was performed using QuantStudio™ 6 Flex Real-Time PCR Systems



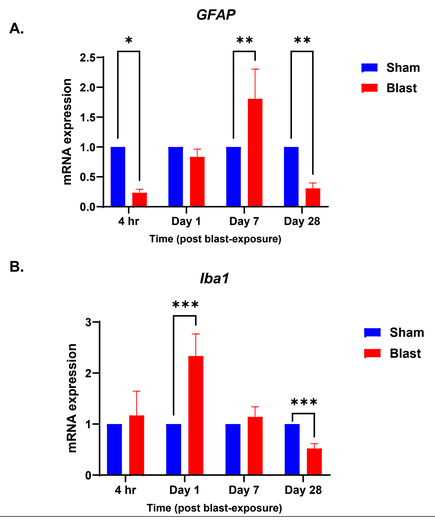
Results

1. Differential gene expressions in neuronal markers in the retina following blast exposure.



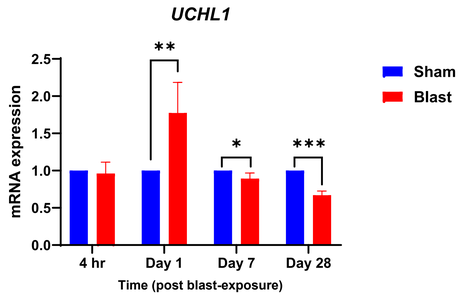
Fold change expression of neurofilament and NeuN genes in the retina at 4 hrs, 24 hrs, 7 days and 28 days post-blast injury. Changes in gene expression of (A) *NFL*, (B) *NFM*, (C) *NFH*, and (D) *NeuN* at four different time points post-blast injury. Results are expressed as mean ± SEM (n=6), ***p<0.001, **p<0.01 and *p<0.05 for sham vs. repeated blast group.

2. Differential gene expressions in neuroglial protein markers in the retina following blast exposure.



Fold change expression of neuroglial markers in the retina at 4 hrs, 24 hrs, 7 days and 28 days post-blast injury. Changes in gene expression of (A) *GFAP*, and (B) *Iba1* at four different time points post-blast injury. Results are expressed as mean ± SEM (n=6), ***p<0.001, **p<0.01 and *p<0.05 for sham vs. repeated blast group.

3. Temporal gene expression changes in neuronal protein homeostasis marker in the retina following blast exposure



Fold change expression of UCHL1 gene in the retina at 4 hrs, 24 hrs, 7 days and 28 days post-blast injury. Changes in gene expression of *UCHL1* at four different time points post-blast injury. Results are expressed as mean±SEM (n=6), ***p<0.001, **p<0.01 and *p<0.05 for sham vs. repeated blast group.

Conclusions

- This study demonstrated a dynamic, time-dependent molecular response in the retina following blast-induced traumatic ocular injury (bOI), characterized by distinct patterns in gene expressions of neuronal and glial markers.
- The data highlight a clear sequence in retinal response to blast injury: an acute phase marked by neuronal stress and microglial activation and the chronic phase is characterized by widespread downregulation of both neuronal and glial markers, possibly reflecting tissue degeneration or failure to repair.
- These findings underscore the importance of examining multiple markers over time and considering the cellular context of each gene when interpreting injury responses.
- Mapping this molecular timeline of retinal injury offers critical insight into the temporal phases of neuronal and glial involvement in the retina following blast trauma and may help identify optimal therapeutic windows to mitigate long-term visual dysfunction.

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Disclaimer:

The experiments reported herein were conducted in compliance with the Animal Welfare Act and per the principles set forth in the "Guide for Care and Use of Laboratory Animals," Institute of Laboratory Animals Resources, National Research Council, National Academy Press, 2011.

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