A Semi-Automated and Unbiased Microglia Morphology Analysis in a Rodent Model of Mild Traumatic Brain Injury

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ABSTRACT

Mild traumatic brain injury (mTBI) is the most common form of TBI, accounting for over 85% of TBI cases in the U.S. Armed Forces in 2025.[1]

One of the hallmarks of mTBI is neuroinflammation which includes the activation of microglia, the brain's resident immune cells. Microglia exhibit distinct morphological changes based on their activation states, ranging from surveilling/ramified (resting) to hypertrophic, ameboid, and rod-like (activated) forms.

We investigated the effects of repeated mTBI on microglial morphology in rats using CHIMERA (Closed-Head Impact Model of Engineered Rotational Acceleration). We utilized the MicrogliaMorphology analysis pipeline, a semi-automated and unbiased ImageJ macro and R statistical language-based package, to quantify 27 morphological features from individual cells. Using multi-dimensional data reduction (PCA) and k-means clustering algorithms, we classified microglia into four clusters: ramified, rod-like, ameboid, and hypertrophic.

The current mTBI paradigm significantly shifted microglial profiles in the cortical injury sites, characterized by an increase in hypertrophic, activated cells and a decrease in ramified, inactive cells as compared to sham controls.

Further optimization of this unbiased and semi-automated analysis pipeline offers a valuable tool for characterizing neuroinflammation and mTBI pathophysiology.

METHOD

The Closed-Head Impact Model of Engineered Rotational Acceleration (CHIMERA) is a recent technique that replicates the biomechanics of an impact TBI in humans.[2]

Animals received either a sham or CHIMERA injury under isoflurane anesthesia (1.5 J, 5-10 seconds between 3 impacts).[3] Brain tissue samples were then collected at post-injury day four (PID-4) for analysis of microglia (Iba-1) on cortical injury sites of rats.

Microglia analysis was conducted using MicrogliaMorphology ImageJ macro and the MicrogliaMorphologyR R package.[4]

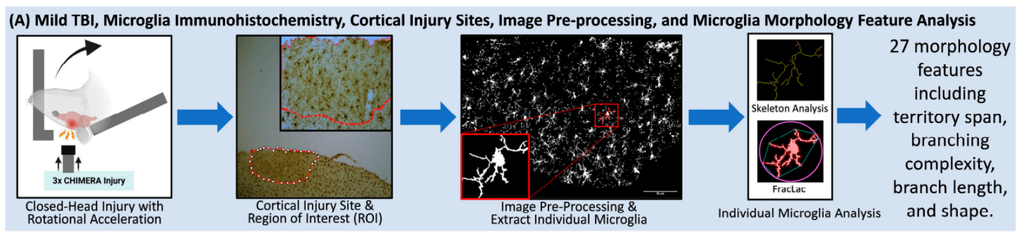

Figure 1A Study design.[5] CHIMERA injury and microglia morphology data acquisition. Brain tissue of rats sustaining a within-session repeated head impacts were collected, stained for microglia using Iba-1 immunohistochemistry, and cortical injury sites were identified at 4× and 10× magnification. Images (20×) were inverted, then processed and analyzed for 27 morphological features.

RESULTS

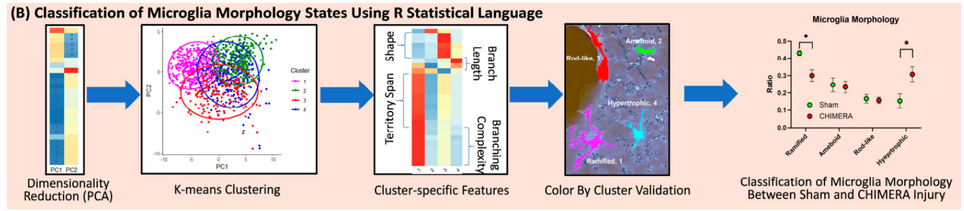

Figure 1B Data analysis. Microglia data were analyzed with principal component analysis (PCA) via dimensionality reduction, k-means clustering, cluster-specific features, and validated using the ColorByCluster function. Morphological features and clusters were compared between the sham and CHIMERA groups, * $p < 0.05$.

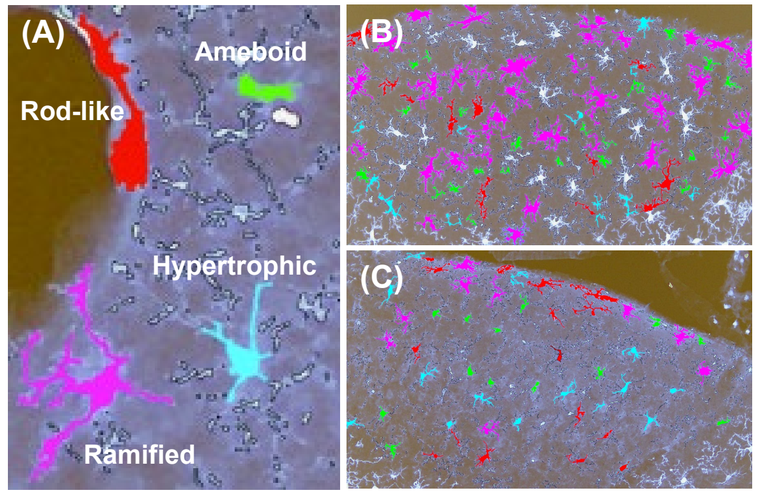

Figure 2 ColorByCluster. Individual microglia in the photomicrograph are color-coded based on their cluster membership from the k-means clustering. (A) Representative image of the four microglial subtypes. (B) Sham brain section. (C) CHIMERA brain section.

CONCLUSIONS

The within-session repeated CHIMERA paradigm produced mild TBI effects without causing any gross brain tissue damage on the injury sites of rats.

Specifically, the CHIMERA group had more activated microglia morphology and features compared to the sham group.

To our knowledge, this is the first study demonstrating microglial activation and density changes in the cortical injury sites following mild, closed-head injury using an automated and high-throughput analysis of 27 microglial morphology features in rats.

This method of analyzing microglia morphology offers greater objectivity, efficiency, and potential for implementing machine learning algorithms. This approach presents a new way forward in analyzing microglia morphology in the post-mortem brain tissue.

Figure 3 K-means Clustering. Microglia were clustered into four morphological states according to 27 morphological features. Left to right: (1) Ameboid, with high circularity and density of foreground pixels and low territory span; (2) Ramified, with high territory span; (3) Hypertrophic, with high average branch length; and (4) Rod-like, with high oblongness. COM = center of mass.

REFERENCES

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