AI-Based Segmentation and Morphometric Analysis Reveal Region-Specific Astrocyte Responses After Diffuse Traumatic Brain Injury

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Introduction

Traumatic brain injuries (TBIs) represent a critical public health issue. Astrocyte reactivity is a key marker of this response, commonly assessed using GFAP staining, and remains elevated for extended periods after injury. Most studies characterize GFAP changes qualitatively or with manual threshold-based measures on limited brain sections. This constrains throughput and region-specific analysis. To address this gap, we used a ferret model of diffuse TBI, leveraging its gyrencephalic cortex, human-like regional brain anatomy and fractional volumes (Fig. 1), and astrocyte features that more closely resemble the human brain than rodent models. We applied our recently developed AI-driven segmentation and atlas-based mapping for whole-brain, region-resolved quantification of astrocyte reactivity and morphological remodeling, **aiming to reveal region-specific patterns of astrocyte response following diffuse TBI** and support future targeted, region-specific therapeutic strategies.

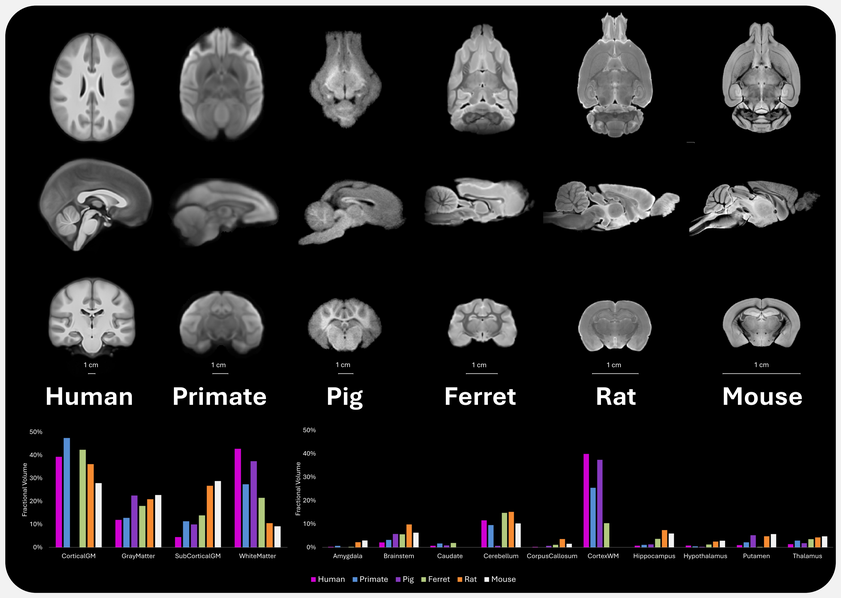

Figure 1. Top: MRI templates comparing brain anatomy across human, non-human primate, pig, ferret, rat, and mouse. Bottom: Regional fractional brain volumes across species.

Methods

Ten male ferrets (~6 months old) were randomly assigned to TBI (n=5) or sham (n=5) groups under an approved IACUC. Under isoflurane anesthesia, TBI animals received a single rotational head impact (~16 m/s, 25.6 J) using the CHIMERA device (Fig. 2A), inducing diffuse axonal injury without direct skull contact. Sham animals underwent identical handling without impact. Animals were euthanized 7 days post-injury, brains were extracted, post-fixed, and sectioned coronally at 2-mm intervals, then paraffin-embedded. Sections (6-10 μm) were immunostained for GFAP and imaged at 20x on a Keyence BZ-X800 fluorescence microscope, yielding an average of 10 slices per animal.

A validated deep-learning model segmented GFAP-positive astrocytes from pathology images. Segmented images were co-registered to a 3D MRI-based ferret brain atlas (Fig. 2C-E) using centroid-based alignment and some manual slice matching steps, assigning each astrocyte to major regions (white matter, gray matter, cortical and subcortical gray matter, sulcus) and their defined 14 subregions. Astrocyte masks were exported to a custom SMorph-based pipeline (Fig. 2B) for single-cell morphometric analysis, extracting convex hull area, branch counts, and branch lengths across primary through quaternary orders (2,031,815 cells analyzed total). Regional and whole-brain reactivity (percent GFAP-positive area) were compared between groups using Mann-Whitney U tests. Further, morphometric parameters were compared between TBI and sham groups at the single-cell level using linear mixed-effects models with group as a fixed effect and animal ID as a random intercept. Significance was set at $p<0.05$, with $p<0.10$ considered marginal.

Methods

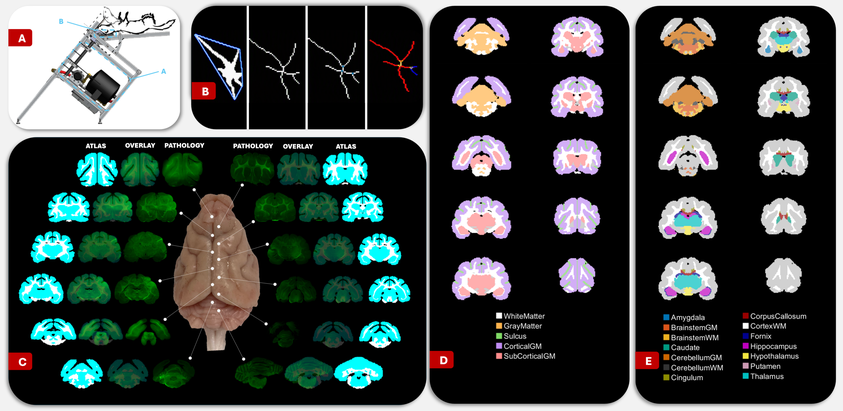

Figure 2. Experimental and analytical pipeline. (A) CHIMERA device for diffuse TBI induction. (B) SMorph-based astrocyte morphometric analysis: convex hull, cell skeleton, branch detection, and branch length. (C) Representative pathology-atlas slice alignment and overlay. (D)Ferret brain atlas showing major regions across anterior-posterior slices. (E) Atlas subregions used for regional quantification.

Results & Discussion

Visualizations of reactivity in key sub-regions illustrate the region-specific activation patterns (Fig. 3). Further, heatmaps of spatial distribution across 10 slices per animal corroborated these regional patterns, showing diffuse non-uniform astroglial responses rather than uniform whole-brain elevation (Fig. 4). This nonuniform distribution is biologically meaningful. Diffuse, rotation-driven TBI generates widespread but spatially heterogeneous mechanical strain rather than the localized injury of focal models.

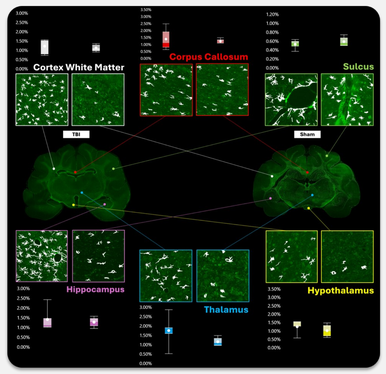

Fig 3. Astrocyte reactivity in key sub-regions.

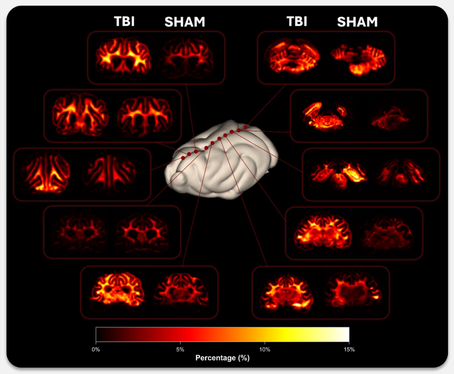

Fig 4. Spatial heatmaps of astrocyte reactivity in sham vs. TBI.

Whole-brain quantification revealed greater astrocyte reactivity in the TBI group than in the sham group (1.16% vs. 0.95%), indicating a robust astroglial response to diffuse injury (Fig. 5). Among the major tissue regions, gray matter (GM) showed the largest and most statistically significant increase (1.25% vs. 2.86%), whereas white matter (WM) showed an approximately twofold increase, but statistically nonsignificant. The pronounced GM response may reflect its high synaptic density, elevated strain at GM–WM interfaces, and predominance of protoplasmic astrocytes, which are closely associated with neurons, synapses, and capillaries and respond strongly to metabolic disturbances. The increased WM reactivity may be related to fibrous astrocytes located along myelinated tracts, where axonal stretching is a primary injury mechanism. The absence of significant differences in the sulci, subcortical GM, and cortical GM may reflect regional variations in effective strain, injury loading, or astrocyte response phenotypes.

Sub-regional analysis identified the largest increases in astrocyte reactivity in cerebellar WM and the brainstem (TBI: 2.82% & 1.82% vs. sham: 0.96% & 0.58%, respectively), with a marginal increase in cerebellar GM (Fig. 5). In contrast, more centrally located structures, including the thalamus, hypothalamus, and putamen, showed only modest differences. These findings may reflect the distribution of tissue and tract-oriented deformation during sagittal head rotation, which is expected to preferentially load the brainstem–cerebellar pathways. Overall, the observed pattern supports a potential association between regional mechanical loading and astrocyte activation, although confirmation will require detailed finite-element strain mapping.

Results & Discussion

Morphometric analysis (Fig. 6) reinforced and extended these findings. TBI astrocytes showed significantly greater convex hull area, and secondary branch length at the whole-brain level, consistent with hypertrophic remodeling rather than new branch proliferation. This elongation-dominant pattern, more consistent than changes in branch count, is characteristic of the mild-to-moderate hypertrophic astrogliosis seen in diffuse TBI, in which astrocytes expand existing territory rather than proliferate. Regions with the strongest GFAP signal, brainstem, cerebellum GM/WM, and GM overall, also showed the most extensive morphological remodeling, reinforcing that reactivity and structural change co-localize where mechanical and metabolic demand are highest. A key exception was the hippocampus: despite lacking a statistically significant increase in GFAP-positive area, it was among the most morphologically remodeled subregions, with significant increases in convex hull and astrocyte area. This dissociation indicates that structural remodeling is a partially independent dimension of the astroglial response, not fully captured by GFAP intensity alone. Given the hippocampus's central role in memory and spatial navigation, and its established vulnerability to TBI, this finding argues for pairing molecular markers with morphometric analysis when characterizing astrocyte pathology, particularly in regions where functional consequences are behaviorally significant.

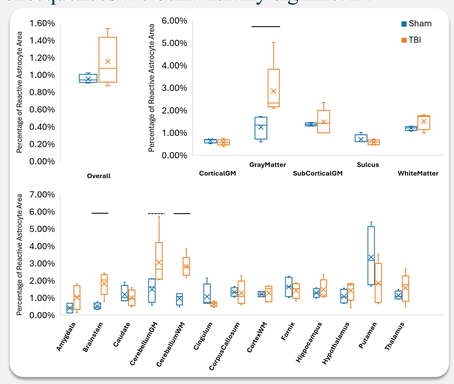

Figure 3. Regional astrocyte reactivity and morphology following diffuse TBI (solid lines: $p<0.05$; dashed: $p<0.1$).

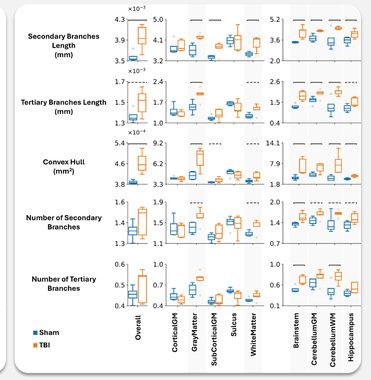

Figure 4. Astrocyte morphometric comparisons across overall, major-regions, and sub-regions.

In summary, this study establishes the ferret CHIMERA model, combined with AI-based segmentation and atlas-guided mapping, as a practical and translationally relevant platform for whole-brain assessment of astrocyte pathology after diffuse rotational TBI. **The ferret's gyrencephalic cortex, human-like regional anatomy and fractional brain volumes, and astrocyte features that more closely resemble those of humans than rodents strengthen its translational relevance. Astrocyte reactivity and hypertrophic remodeling were most pronounced in gray matter, the brainstem, and cerebellar white and gray matter, potentially reflecting preferential loading of brainstem–cerebellar pathways during sagittal rotation, while the hippocampus showed substantial morphological remodeling despite limited GFAP-area changes.** These findings support future finite-element/biomechanical and multimodal studies of region-specific vulnerability and targeted interventions.

References

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OUR RELATED WORK

Whole-Brain, Region-Specific Astrocyte Reactivity and Morphological Remodeling After Diffuse Traumatic Brain Injury in A Gyrencephalic Ferret Model

Comprehensive benchmarking of deep learning approaches for automated astrocyte segmentation in traumatic brain injury

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