Astrocyte Morphology Analysis: Detecting Morphological Changes in Human Cortical Astrocytes

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Abstract

Astrocytes, the most abundant glial cells in the central nervous system, have emerged as active participants in brain function, far beyond their initial classification as passive support cells. This paper reviews the evolution of astrocyte research, focusing on their morphology, diverse roles, and responses to injury and stress. This paper also produces a comparison between current computational models being employed to conduct morphological analyses.

Later detailed in this paper is a proposal for the implementation of a model that uses a publicly available annotated dataset of human cortical astrocytes, for the analysis of their morphological changes. The dataset, which provides comprehensive annotations of human astrocyte structures, serves as the basis for this project. Through the development of this model, the paper aims to refine current methodologies and enable more accurate analysis of astrocyte morphological changes in the human brain, offering a path forward for advancing astrocyte research.

☐ **I have not used any GenAI tools in preparing this assessment.**

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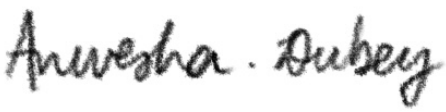
Signature:  _____

1 Introduction

Unequivocally, the brain stands as the most powerful and complex organ of most living organisms, driving their very existence and functioning. It holds the distinction of being the most crucial organ, commanding its own dedicated field of study. Serving as the command centre of the nervous system, the brain orchestrates every aspect of an organism's life—processing sensory input, regulating physiological functions, governing movement, and enabling thought, emotion, and memory.

A vast, intricate labyrinth of cells, the brain can be largely classified into being composed of neurons, and non-neuron cells called glial cells. Previously understated as mere "supporter" cells, these glial cells play an indispensable role in maintaining and enhancing nervous system function[1]. Beyond providing structural support for neurons, glial cells regulate homeostasis, facilitate repair, and form myelin, a critical component for efficient neural signalling[1]. Glial cells can be principally catalogued as astrocytes, ependymal cells, microglia, oligodendrocytes, and Schwann cells.

Astrocytes, the most prolific glial cells in the brain, are highly specialised and integral to maintaining homeostasis and supporting neuronal activity. Tiling the central nervous system (CNS), they communicate through gap junctions and play a key role in neurotransmitter regulation by releasing and absorbing chemicals at synapses[1]. With processes containing glial fibrillary acidic protein (GFAP), astrocytes respond to injuries through gliosis, a protective process aimed at preserving neural tissue integrity. Moreover, they provide substrates necessary for neuronal ATP(Adenosine Triphosphate) production and are crucial in potassium ion metabolism and water uptake, ensuring the brain's ionic and osmotic balance[1][2].

These star-shaped, spongy cells also contribute significantly to the structure and function of the blood-brain barrier. Their foot processes extend to surround capillaries, forming a dynamic interface that regulates the exchange of substances between the blood and neural tissue. This multifaceted functionality underscores the imperative role astrocytes play in both the healthy and diseased brain[1][2].

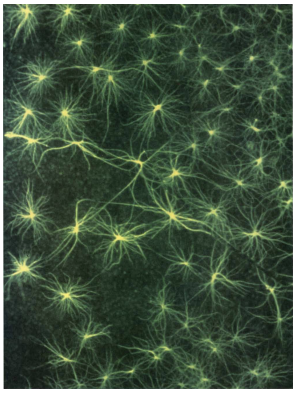


Figure 1: **Fluorescent dyed astrocytes as shown in tissue from the retina of a cat[3].**

Astrocytes are not static entities; their morphology adapts dynamically under various physiological and pathological conditions. Changes in astrocyte structure—whether in their branching patterns, cellular hypertrophy, or interactions with the blood-brain barrier—provide critical insights into the brain's responses to external stressors, traumatic injury, and disease(usually neurodegenerative). However, much of the current understanding of astrocyte morphology has been derived from rodent models, which, while foundational, might fail to fully represent the unique complexities of human astrocytes.

This paper explores current advanced methods used to analyse astrocyte morphology, surveying different computational tools and their applications in rodent studies. It critiques the limitations of these methods, particularly their reliance on rodent data, and highlights the need for more human-centric approaches. By utilising an annotated dataset of human astrocytes[4], this work proposes a new model to address these gaps, providing a framework for advancing astrocyte morphology analysis and offering new insights into their diverse roles in the human brain.

2 The Evolution of Astrocyte Research

Astrocytes, long regarded as simple support cells, have gradually emerged as dynamic contributors to the brain's intricate functionality. The foundation for their study was laid by Rudolf Virchow, who coined the term "neuroglia" in 1846 to describe the "glue" between neurons. This idea evolved with the pioneering work of Camillo Golgi in the 1870s, whose staining techniques enabled the identification of glial cells as distinct entities. Santiago Ramón y Cajal, along with his student Pío del Río Hortega, later developed a classification system for glial cells that remains largely unchanged. Despite these foundational advances, astrocytes were overshadowed by neurons throughout much of the 20th century, with their significance only hinted at by researchers like Alexander von Lenhossék and Alois Alzheimer[3].

A resurgence of interest in astrocytes occurred in the mid-20th century, catalysed by the discovery of glial fibrillary acidic protein (GFAP) by Lawrence F. Eng and Amico Bignami in the 1970s. GFAP provided a reliable marker for studying astrocytes, enabling breakthroughs in understanding their active roles in homeostasis, synaptic activity, and neural responses to injury. Far from being mere support cells, astrocytes are now recognised as vital regulators of neurotransmitters such as glutamate and gamma aminobutyric acid (GABA), crucial for maintaining synaptic function[3].

3 Current Approaches to Astrocyte Research

3.1 Rodents in Research

Rodents have long been central to medical research due to their genetic similarity to humans, along with low cost, and ease of housing. Their shorter lifespans and rapid reproductive cycles also make them ideal for longitudinal studies[5].

However, with respect to astrocytic morphology and subtypes, rodent models face significant limitations. Their astrocytes are smaller, less complex, and differ in subtypes and functions compared to human astrocytes. For instance, unique astrocyte subtypes, such as inter-laminar and polarised astrocytes, are absent in rodents. These differences raise questions about the translational validity of rodent-derived findings to human biology[6]. These limitations highlight the critical need for human-centric studies, as further detailed in the subsequent sections.

3.2 Astrocyte Diversity and Knowledge Gaps

Comparative research has revealed significant differences between rodent and human astrocytes, highlighting the unique complexities of the human brain. Rodent (protoplasmic) astrocytes, while instrumental in advancing our understanding of glial cell function, are smaller and less morphologically complex compared to their human counterparts. Human protoplasmic astrocytes, which dominate the gray matter, are substantially larger, with diameters threefold greater than those of rodent astrocytes[6]. Moreover, human astrocytes possess up to tenfold more primary processes, enabling them to interact extensively with neurons and blood vessels[6].

This increased complexity is not merely structural but also functional. The expansive processes of human astrocytes facilitate connections with a larger number of synapses, enhancing their ability to regulate neurotransmitter levels, modulate synaptic activity, and maintain the ionic balance necessary for neural function[6]. Such intricate networks suggest a pivotal role for astrocytes in the higher-order cognitive processes characteristic of the human brain. In contrast, rodent astrocytes, with their simpler morphology, may reflect the less complex neural circuitry of their brains.

Additionally, humans exhibit unique astrocyte subtypes not found in rodents. Primate-specific **inter-laminar astrocytes**, located in cortical layer 1, extend exceptionally long fibres that span millimetres across cortical layers, a feature entirely absent in rodent brains[7]. These fibres are hypothesised to facilitate long-distance communication between cortical regions. Similarly, **polarized**

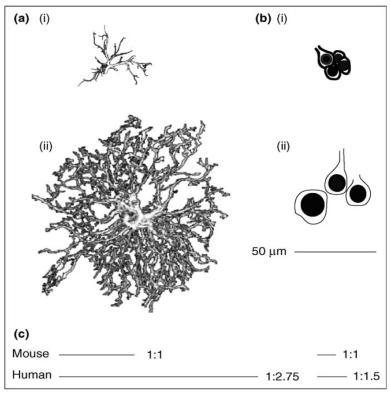


Figure 2: **Rodent vs. Human Protoplasmic Astrocytes (a) Graphical representation and GFAP immunostaining of mouse (i) and human (ii) cortical astrocytes. (b) Graphical representation and MAP2 immunostaining of mouse (i) and human (ii) cortical neurons. (c) Bars illustrating the sizes of human astrocytes (left) and neurons (right) relative to the sizes of these cells in mice.[6].**

(or varicose projection) astrocytes, found in layers 5 and 6, exhibit distinct long processes that contribute to specialised interactions with surrounding neural elements, and coordinating blood flow[7].

Like inter-laminar astrocytes, varicose projection astrocytes are not present in rodents, further emphasising the evolutionary divergence in astrocytic structure and function[7][6]. These differences underscore the evolutionary adaptations of human astrocytes, suggesting that their structural complexity and diversity play a critical role in supporting advanced cognitive functions.

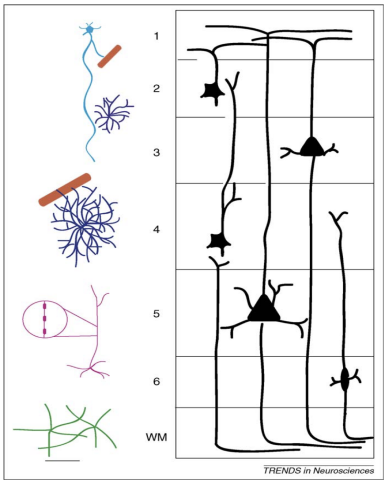


Figure 3: **Primate-specific inter-laminar astrocytes (light blue, layers 1-4). Protoplasmic astrocytes (dark blue, layers 2–6). Protoplasmic astrocytes(red). Polarized astrocytes (pink, layers 5–6). Fibrous astrocytes (green).[6].**

4 Astrocytic Responses to Diseases, Stressors and Injury

Astrocytes are uniquely positioned within the central nervous system to respond dynamically to stressors, diseases, and injuries. Their highly adaptable morphology and active roles in neurophysiological processes, including maintaining homeostasis, render them critical players in the brain's reaction to pathological conditions. However, these same characteristics make astrocytes susceptible to dysfunction, which can exacerbate neurological conditions.

4.1 Astrocytic Morphology and Huntington's Disease

Astrocytes have long been noted for their ability to change morphology under pathological conditions; undergoing hypertrophy, proliferation, process thickening and glial scar formation[8]. These changes can manifest as correlative responses to disease progression, reactive adaptations, or cell-autonomous mechanisms stemming from the disease's underlying causes. Each mechanism highlights the profound interplay between astrocyte structure and function, with these changes contributing variably to disease pathophysiology[9].

In Huntington's disease (HD), astrocytes exhibit distinct structural alterations that correlate with disease progression. While astrocyte somata size remains unchanged, their domain territories and process coverage significantly decrease. This reduction affects their interactions with cortical and thalamic synapses, potentially triggering synapse dysfunction. Such changes precede neuronal loss, implicating astrocytic morphology as a precursor to HD-associated dysfunction[9][10].

4.2 Responses to Brain Injury

Astrocytes react swiftly to brain injuries such as ischaemic stroke (blocked blood vessel restricting blood flow) or traumatic brain injury (TBI). Reactive Astrogliosis, characterised by upregulated glial fibrillary acidic protein (GFAP) expression and hypertrophic processes, is a hallmark of astrocytic responses. In ischaemic stroke, astrocytes in the ischaemic core show marked hypertrophy and larger calcium signals compared to those in the surrounding penumbra[11]. These reactive astrocytes release neuroprotective mediators, cytokines, and chemokines, contributing to both tissue repair and chronic inflammation. Similarly, in TBI, astrocytes form glial scars that isolate damaged areas, but these scars can impede axonal regeneration, posing challenges for recovery[10][11].

4.3 Psychiatric and Neurodegenerative Disorders

Astrocytic dysfunction plays a significant role in psychiatric conditions such as obsessive-compulsive disorder (OCD) and depression. In OCD, astrocytes exhibit reduced territory sizes and Actin Cytoskeletal disruptions. These changes, driven by SAPAP3 gene deficiency, lead to morphological deficits that contribute to compulsive behaviours. Similarly, depression has been linked to subtle alterations in astrocytic processes in specific brain regions, highlighting their involvement in mood regulation[10].

In neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD), astrocytes undergo a complex sequence of changes. Early stages of AD are characterised by astrocyte atrophy, with reduced GFAP expression and diminished interactions with the neurovascular unit[10]. As the disease progresses, astrocytes transition to a hypertrophic phenotype, actively degrading amyloid plaques (beta-amyloid protein build-up) but also contributing to inflammatory cascades. In PD, astrocyte processes expand to increase synaptic coverage, potentially altering synaptic strength and contributing to excitotoxicity[10].

4.4 Astrocytes in Epilepsy

Astrocytes play a crucial role in keeping the brain's electrical activity balanced. However, this can prove difficult when suffering from epileptic seizures where the brain experiences sudden bursts of abnormal electrical activity[13]. One of the main jobs for astrocytes is to clear excess glutamate, a chemical that excites neurons, from the spaces between brain cells. When this process is disrupted, as it often is in epilepsy, glutamate builds up and makes neurons overly active, increasing the likelihood of seizures[13].

Astrocytic cells also help regulate potassium levels, another key factor in controlling electrical signals. In epilepsy, this regulation can break down, allowing potassium levels to rise and further fuelling the overactivity of neurons. Together, these disruptions in glutamate and potassium balance

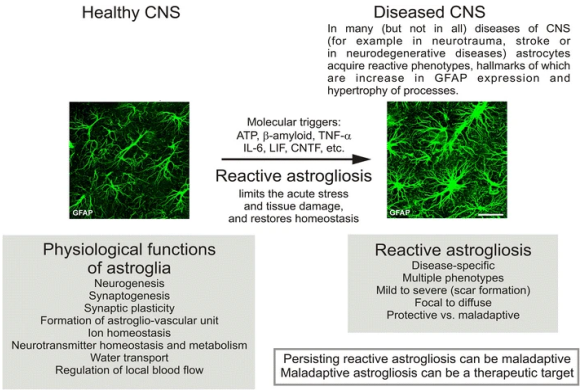


Figure 4: **Overview of Astrocyte Functions and Reactive Astrogliosis[12].**

create a feedback loop that makes the brain more prone to seizures[13]. This highlights how astrocyte dysfunction contributes to the development and severity of epilepsy.

Astrocytes are paramount to the brain's adaptive responses, yet their anatomical and functional changes in disease and injury often carry dual roles. While reactive astrocytes can protect and repair neural tissue, prolonged or excessive reactivity may lead to detrimental effects such as glial scarring and inflammation. Understanding the mechanisms underlying astrocytic changes is crucial for developing therapies aimed at preserving their protective roles while mitigating pathological contributions.

5 Current Computational Models for Astrocyte Morphology Analysis Research

Astrocyte research has increasingly relied on computational models and advanced imaging techniques to understand their complex morphology and functional roles. These models enable researchers to segment, classify, and simulate astrocytic behaviour across diverse conditions, offering valuable insights into their adaptability and responses. However, the existing models face several limitations in their design and applicability. This section reviews three key approaches: deep-learning segmentation, functional simulations, and imaging software tools.

Deep Learning for Astrocyte Segmentation Deep-learning techniques have revolutionised the study of astrocytes by automating the segmentation and analysis of complex cellular structures. Vuorimaa et al.[14] introduced a convolutional neural network (CNN)-based model designed to identify reactive astrocytes in the rat hippocampus. The model effectively distinguished astrocyte territories with high accuracy and sensitivity, particularly under conditions such as traumatic brain injury (TBI). A standout feature of this model was its ability to differentiate subtle morphological variations in astrocytes during reactive states, such as hypertrophy or process retraction. By utilising multiple layers of convolutional filters, the model captured nuanced structural changes, providing a robust platform for astrocyte analysis[14]. However, this approach relies heavily on well-annotated datasets and lacks validation in more diverse datasets that include astrocyte subtypes beyond those found in rodents.

Simulation-Based Modelling of Astrocytic Functions Functional models have also advanced significantly, offering simulations of astrocytic calcium dynamics and their role in neural networks[15]. These simulations captured the temporal and spatial patterns of calcium waves, elucidating how astrocytes respond to neuronal inputs and external stressors[15]. While these models are invaluable for understanding astrocytic contributions to synaptic regulation and neurotransmitter cycling, they often oversimplify astrocytic morphologies by relying on fixed parameters that fail to account for dynamic process branching and interactions with neighbouring cells[16][15].

Imaging Software Tools: ImageJ Plug-in Advanced imaging tools such as ImageJ and its custom plug-ins are frequently employed to study astrocytic morphology. One such plug-in (Simple Neurite Tracer - SNT), designed to quantify astrocytic processes and branching patterns, has become a staple for morphometric analysis[17]. This tool uses fluorescent images to measure parameters such as process length, branching density, and territory volume. The plug-in's automated measurements ensure reproducibility and reduce the subjective bias of manual tracing methods. Despite its effective utility, the ImageJ plug-in struggles with high-resolution, 3D datasets and often fails to capture the intricate interactions between astrocyte processes and synaptic structures. These limitations restrict its applicability to broader, more complex datasets[18].

Challenges and Future Improvements While current models have advanced astrocyte research, they face several limitations:

1. **Limited Knowledge of Astrocyte Subtypes**: Models mainly focus on protoplasmic astrocytes, neglecting other subtypes like fibrous, inter-laminar, and polarized astrocytes due to scarce data and knowledge about these other subtypes. This gap hinders a comprehensive understanding of astrocyte diversity, functionality and interactivity.

2. **Integration of Morphology and Function**: Current models often separate structural and functional analyses. Linking morphological changes with functions like calcium signalling could offer a holistic view of astrocyte activity.

3. **Human-Centric Models**: The disproportionate focus on rodent data limits applicability to human astrocytes, which are larger and more complex. Developing models validated with human data is crucial for accurate insights into human brain physiology.

4. **Enhanced Imaging and Analysis Tools**: Existing software struggles with high-resolution 3D datasets and complex interactions. Advancements in imaging tools to automate segmentation and analysis in 3D would improve accuracy and scalability.

Addressing these challenges by expanding data on various astrocyte subtypes, integrating 3D imaging, and focusing on human-specific models will significantly enhance our understanding of astrocyte roles in physiological and pathological states.

6 Annotated Dataset of Human Cortical Astrocytes

This project will utilise an annotated dataset of human astrocytes that addresses the limitations of rodent-centric research and provides a valuable resource for advancing astrocyte morphology analysis[4]. Derived from post-mortem human brain tissue, the dataset includes 8730 high-resolution image patches (500×500 pixels) collected from 16 slides across 8 patients. The image patches are further categorized by pixel size - 0.5019 μm/pixel and 0.3557 μm/pixel. The tissue was stained using GFAP and ALDH1L1, markers that specifically highlight astrocyte bodies and processes, enabling detailed morphological assessments[4].

Key Features of the Dataset

1. **Annotations**: The glial cell bodies are annotated using bounding boxes with a modified COCO-annotator tool, incorporating variability across annotators. This method ensures robust data that reflects real-world discrepancies in cell identification[4].

2. **Diversity**: The dataset captures variations in astrocyte morphology across different brain regions, providing a comprehensive view of astrocytic structures and densities[4].

3. **Machine Learning Integration**: Designed for deep-learning applications, baseline models trained on this dataset, such as Faster R-CNN with a ResNet-50 backbone, achieved strong performance metrics, demonstrating the dataset's utility in automated astrocyte detection[4].

Unique Strengths This dataset stands out due to its scale, human specificity, and dual-staining approach, which enables a nuanced exploration of astrocytes and their interactions with neural structures. It bridges the gap in existing datasets by offering human-centric data, critical for understanding the unique anatomy of human astrocytes compared to rodents.

7 Ethical Considerations and Risk Assessment

7.1 Ethical Considerations

This project uses an annotated dataset derived from post-mortem human brain tissue. The dataset is anonymised, and ethical guidelines for the use of human-derived data have been adhered to. However, specific considerations include:

- **Data Privacy**: Ensuring that the dataset remains anonymised and securely stored.
- **Informed Consent**: Verification that the tissue donors or their families provided informed consent for the use of these samples in research.
- **Respect for Human Tissue**: Acknowledging the ethical responsibility in handling human-derived samples with respect and transparency.

As no new human data will be collected during the course of this project, the need for additional ethical approval is minimal. However, compliance with university ethical standards and regular reviews of ethical practices will be maintained.

7.2 Risk Assessment

Risk	Likelihood	Impact	Mitigation
Dataset Issues	Moderate	High	Conduct a preliminary data quality assessment; use alternative annotated datasets if critical gaps are identified.
Technical Errors	High	Moderate	Use a modular development approach with regular debugging. Employ cloud-based systems to handle computationally intensive processes.
Overambitious Goals	Moderate	High	Regularly review the project's scope and adjust objectives to ensure feasibility within the given time frame.
Software Failures	Low	High	Use version control (ex. Git) to ensure a backup of code and data. Maintain a log of system dependencies for easy recovery.

8 Conclusion

Over the course of this paper, I have explored the dynamic role of astrocytes in brain function and their response to pathological conditions. Current methods for analysing astrocyte morphology—ranging from deep-learning segmentation tools to imaging software—have provided significant insights but also revealed gaps in understanding human-specific astrocyte characteristics. By utilising an annotated dataset of human cortical astrocytes[4], this project aims to address these gaps and develop a model tailored to analysing morphological changes in human astrocytes, in hopes to advance and provide new, valuable insights in astrocyte research.

9 Project Proposal

Problem Definition: The project aims to develop a machine-learning-based model to detect and quantify morphological changes in human cortical astrocytes. Morphological characteristics such as domain size, process branching, and density are critical indicators of astrocytic responses to pathological conditions. The model will focus on identifying these changes using the annotated dataset without delving into the segmentation of specific astrocyte subtypes.

Pipeline for Morphological Analysis

1. **Data Pre-processing**:
   - Standardise image patches for consistent brightness and contrast.
   - Apply data augmentation techniques to improve model generalisation.
   - Ensure uniform patch sizing for computational efficiency.
2. **Feature Extraction**:
   - Use a **ResNet-50 backbone** to identify structural features such as process extensions and domain boundaries.
   - Incorporate an **attention mechanism** to highlight intricate astrocytic structures.
3. **Morphology Quantification**:
   - Measure process branching, astrocytic domain area, and overlap with neighbouring astrocytes.
   - Validate these metrics using annotated bounding boxes from the dataset.
4. **Model Training and Validation**:
   - Train a Faster R-CNN model, using pre-trained weights for efficient convergence.
   - Evaluate performance using IoU (Intersection over Union) for segmentation accuracy and FROC curves to assess sensitivity and false positives.

Expected Outcomes

1. Accurate detection of morphological changes such as hypertrophy, process retraction, and domain overlap.
2. Quantitative metrics for astrocyte morphology that can be applied to study their responses to stressors, injuries, or diseases.
3. A validated pipeline for analysing astrocytic morphology using human-specific data, addressing the limitations of rodent-based research.

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References

[1] D. J. Ludwig PE, "Histology, glial cells," *National Library of Medecine*, 2024. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK441945/

[2] S. N. Dotiwala AK, McCausland C, "Anatomy, head and neck: Blood brain barrier," *National Library fo Medecine*, April 4, 2023.

[3] H. K. Kimelberg and M. D. Norenberg, "Astrocytes," *Scientific American*, vol. 260, no. 4, pp. 66–77, 1989. [Online]. Available: http://www.jstor.org/stable/24987213

[4] A. Olar, T. Tyler, P. Hoppa, E. Frank, I. Csabai, I. Adorjan, and P. Pollner, "Annotated dataset for training deep learning models to detect astrocytes in human brain tissue," *Sci. Data*, vol. 11, no. 1, p. 96, Jan. 2024.

[5] T. J. Laboratory, "Why are mice considered excellent models for humans?" [Online]. Available: https://www.jax.org/why-the-mouse/excellent-models

[6] S. G. M. N. Nancy Ann Oberheim, Xiaohai Wang, "Astrocytic complexity distinguishes the human brain," *Trends in Neurosciences*, vol. 29, no. 10, pp. 547–553, Oct. 2006.

[7] N. A. Oberheim, T. Takano, X. Han, W. He, J. H. C. Lin, F. Wang, Q. Xu, J. D. Wyatt, W. Pilcher, J. G. Ojemann, B. R. Ransom, S. A. Goldman, and M. Nedergaard, "Uniquely hominid features of adult human astrocytes," *Journal of Neuroscience*, vol. 29, no. 10, pp. 3276–3287, 2009. [Online]. Available: https://www.jneurosci.org/content/29/10/3276

[8] A. S. Şovrea and A. B. Boşca, "Astrocytes reassessment - an evolving concept part one: embryology, biology, morphology and reactivity," vol. 1, no. 1, p. 18, Oct. 2013.

[9] M. K. K. K. B. S. Baldwin, Katherine T., "Astrocyte morphology," *Trends in Cell Biology*, vol. 34, no. 7, pp. 547–565, Jul. 2024.

[10] B. Zhou, Y.-X. Zuo, and R.-T. Jiang, "Astrocyte morphology: Diversity, plasticity, and role in neurological diseases," *CNS Neuroscience & Therapeutics*, vol. 25, no. 6, pp. 665–673, 2019. [Online]. Available: https://onlinelibrary.wiley.com/doi/abs/10.1111/cns.13123

[11] X. S. L. S. Z. G. Y. L. Xiaoyun Cheng, Jin Wang, "Morphological and functional alterations of astrocytes responding to traumatic brain injury," *JIN*, vol. 18, no. 2, pp. 203–215, 2019.

[12] M. Pekny, M. Pekna, A. Messing, C. Steinhäuser, J.-M. Lee, V. Parpura, E. M. Hol, M. V. Sofroniew, and A. Verkhratsky, "Astrocytes: a central element in neurological diseases," *Acta Neuropathol.*, vol. 131, no. 3, pp. 323–345, Mar. 2016.

[13] R. Siracusa, R. Fusco, and S. Cuzzocrea, "Astrocytes: Role and functions in brain pathologies," *Frontiers in Pharmacology*, vol. 10, 2019. [Online]. Available: https://www.frontiersin.org/journals/pharmacology/articles/10.3389/fphar.2019.01114

[14] M. Vuorimaa, I. Kareinen, P. Toivanen, S. Karlsson, and S. Ruohonen, "Deep learning–based segmentation of morphologically distinct rat hippocampal reactive astrocytes after trimethyltin exposure," *Toxicologic Pathology*, vol. 50, no. 6, pp. 754–762, 2022. [Online]. Available: https://doi.org/10.1177/01926233221124497

[15] T. A. Syed, M. Youssef, A. L. Schober, Y. Kubota, K. K. Murai, and C. K. Salmon, "Beyond neurons: computer vision methods for analysis of morphologically complex astrocytes," *Frontiers in Computer Science*, vol. 6, 2024. [Online]. Available: https://www.frontiersin.org/journals/computer-science/articles/10.3389/fcomp.2024.1156204

[16] E. V. Yakovlev, I. V. Simkin, A. A. Shirokova, N. A. Kolotieva, S. V. Novikova, A. D. Nasyrov, I. R. Denisenko, K. D. Gursky, I. N. Shishkov, D. E. Narzaeva, A. B. Salmina, S. O. Yurchenko, and N. P. Kryuchkov, "Machine learning approach for recognition and morphological analysis of isolated astrocytes in phase contrast microscopy," *Sci. Rep.*, vol. 14, no. 1, p. 9846, Apr. 2024.

[17] G. Tavares, M. Martins, J. S. Correia, V. M. Sardinha, S. Guerra-Gomes, S. P. das Neves, F. Marques, N. Sousa, and J. F. Oliveira, "Employing an open-source tool to assess astrocyte tridimensional structure," *Brain Struct. Funct.*, vol. 222, no. 4, pp. 1989–1999, May 2017.

[18] I. Wiki, "Simple neurite tracer (snt): Step-by-step instructions," https://imagej.net/plugins/snt/step-by-step-instructions, 2024.