

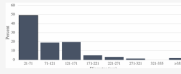
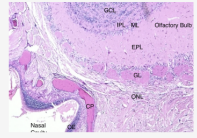
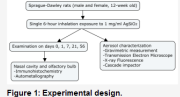
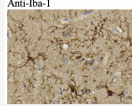




Microglial activation following nose-to-brain transport of silver silicate nanoparticles in the rodent



Microglial activation following nose-to-brain transport of silver silicate nanoparticles in the rodent
 Huong Huynh, Priya Upadhyay, Ching-Wen Wu, Kent E. Pinkerton
 University of California - Davis SVM



<p>Introduction</p> <ul style="list-style-type: none"> Silver engineered nanoparticles makes up ~50% of all nanomaterials in commercial use Inhaled nanoparticles may enter the brain through retrograde transport via the olfactory sensory neurons or by paracellular transport across the olfactory epithelium into lamina propria, which is connected to the subarachnoid space Nanoparticles in the brain could possibly trigger an immune response mediated by microglia, the resident macrophage of the central nervous system The mechanism of nanoparticle transport from the nose to the brain is still poorly understood <p style="text-align: center;">OPEN</p>	<p>Result: Aerosol Characterization</p> <table border="1" style="font-size: small;"> <thead> <tr> <th>Measurement</th> <th>Agilent</th> </tr> </thead> <tbody> <tr> <td>Mean aerodynamic diameter (µm)</td> <td>0.11 ± 0.01</td> </tr> <tr> <td>Geometric standard deviation</td> <td>1.11 ± 0.02</td> </tr> <tr> <td>Mass median diameter (µm)</td> <td>0.12 ± 0.01</td> </tr> <tr> <td>Mass median diameter (standard deviation)</td> <td>0.12 ± 0.01</td> </tr> </tbody> </table>  <p style="text-align: center;">OPEN</p>	Measurement	Agilent	Mean aerodynamic diameter (µm)	0.11 ± 0.01	Geometric standard deviation	1.11 ± 0.02	Mass median diameter (µm)	0.12 ± 0.01	Mass median diameter (standard deviation)	0.12 ± 0.01	<p>Result: Histopathology</p>  <p style="text-align: center;">OPEN</p>	<p>Conclusion & Future Plan</p> <p>Result</p> <ul style="list-style-type: none"> Aerosol characterization suggests that silver silicate in nanopure water are aerosolized and reached the inhalation chamber. The size distribution of silver silicate nanoparticle indicates these are small enough to be deposited in the nasal cavity olfactory region. <p>Future Plans</p> <ul style="list-style-type: none"> Microglial morphology characterization by morphometric analysis as well as skeleton and fractal analysis are still ongoing to identify the ratio of activated to resting <p style="text-align: center;">OPEN</p>
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<p>Methods</p>  <p>Figure 1: Experimental design.</p> <p style="text-align: center;">OPEN</p>	<p>Result: Skeleton and Fractal Analysis</p> <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>A</p> <p>Anti-Iba-1</p>  </div> <div style="text-align: center;"> <p>Binary</p>  </div> <div style="text-align: center;"> <p>Skeleton</p>  </div> </div> <p style="text-align: center;">OPEN</p>	<p>Acknowledgement</p> <p>Research Grant Support: NIEHS U01 E5027288 Student Support: University of California, Davis, School of Veterinary Medicine</p>											

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PRESENTED AT:



INTRODUCTION

- Silver engineered nanoparticles makes up ~50% of all nanomaterials in commercial use.
- Inhaled nanoparticles may enter the brain through retrograde transport via the olfactory sensory neurons or by paracellular transport across the olfactory epithelium into lamina propria, which is connected to the subarachnoid space.
- Nanoparticles in the brain could possibly trigger an immune response mediated by microglia, the resident macrophage of the central nervous system.
- The mechanism of nanoparticle transport from the nose to the brain is still poorly understood.
- We hypothesized that inhaled silver silicate nanoparticles are translocated to the brain through deposition in the nasal cavity olfactory region, followed uptake in the olfactory epithelium, retrograde transport to the olfactory bulb, and uptake into resident microglia.

RESULT: AEROSOL CHARACTERIZATION

Measurement	AgSiO ₂
Mass concentration-gravimetric (mg/m ³)	4.9 +/- 2.3
Mass concentration-XRF (mg/m ³)	5.6 +/- 2.0
Mass median aerodynamic diameter (cascade impactor) (μm)	1.9 +/- 0.3

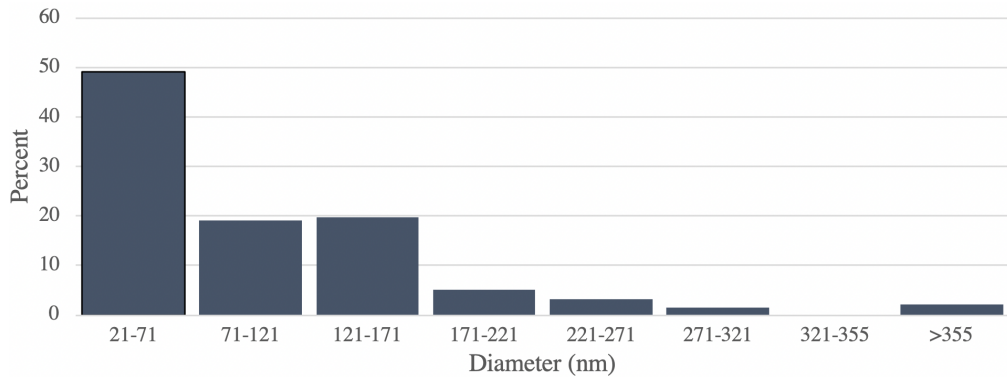


Figure 3: Size distribution of AgSiO₂ obtained by the cascade impactor.

RESULT: HISTOPATHOLOGY

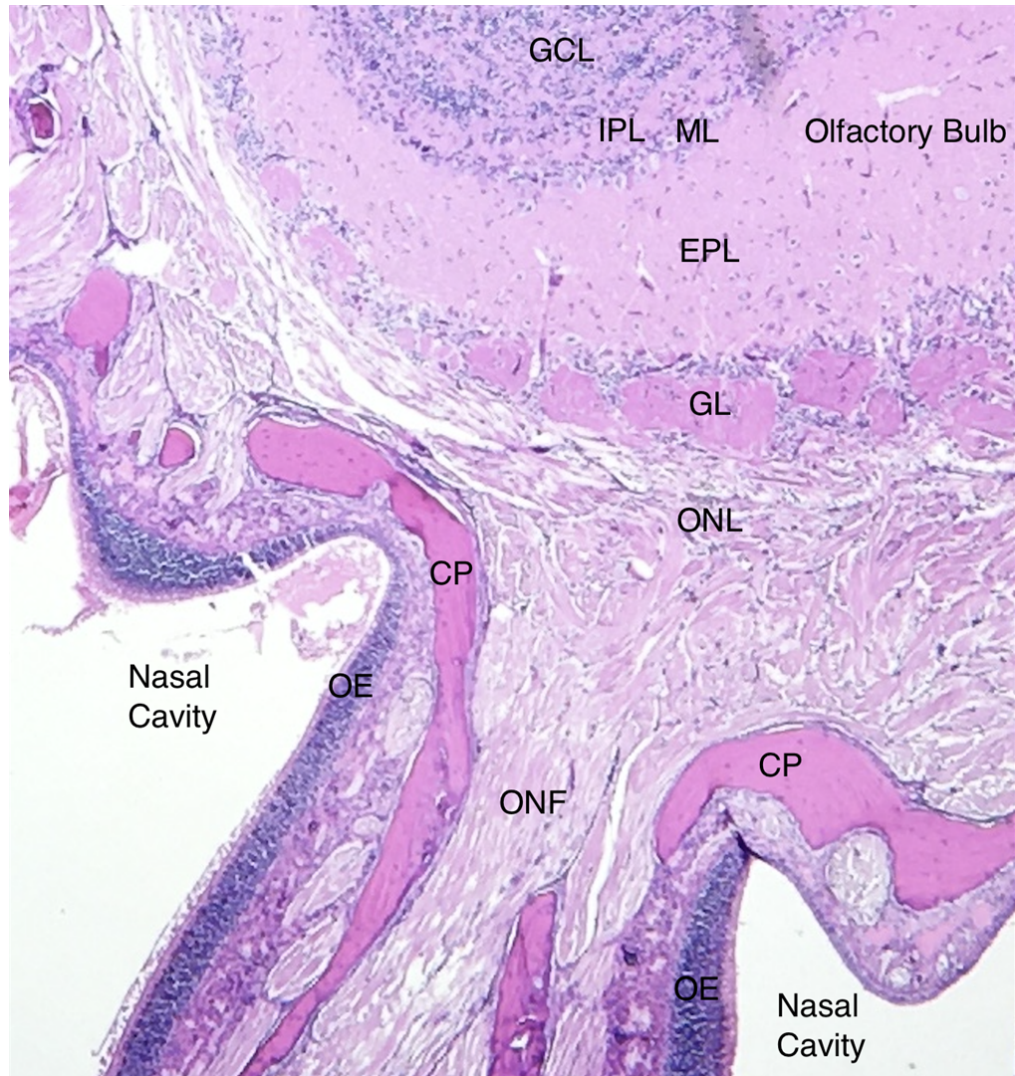


Figure 4: Visualization of the nose-to-brain pathway from the nasal cavity to the olfactory bulb. The olfactory sensory neuron, located in the olfactory epithelium, has nerve fascicles passing through the cribriform plate to enter the olfactory bulb, which is part of the central nervous system. CP=cribriform plate; EPL=external plexiform layer; GL=glomerular layer; GCL=granule cell layer; IPL=internal plexiform layer; ML=mitral cell layer; OE=olfactory epithelium; ONF=olfactory nerve fascicle; ONL=olfactory nerve layer.

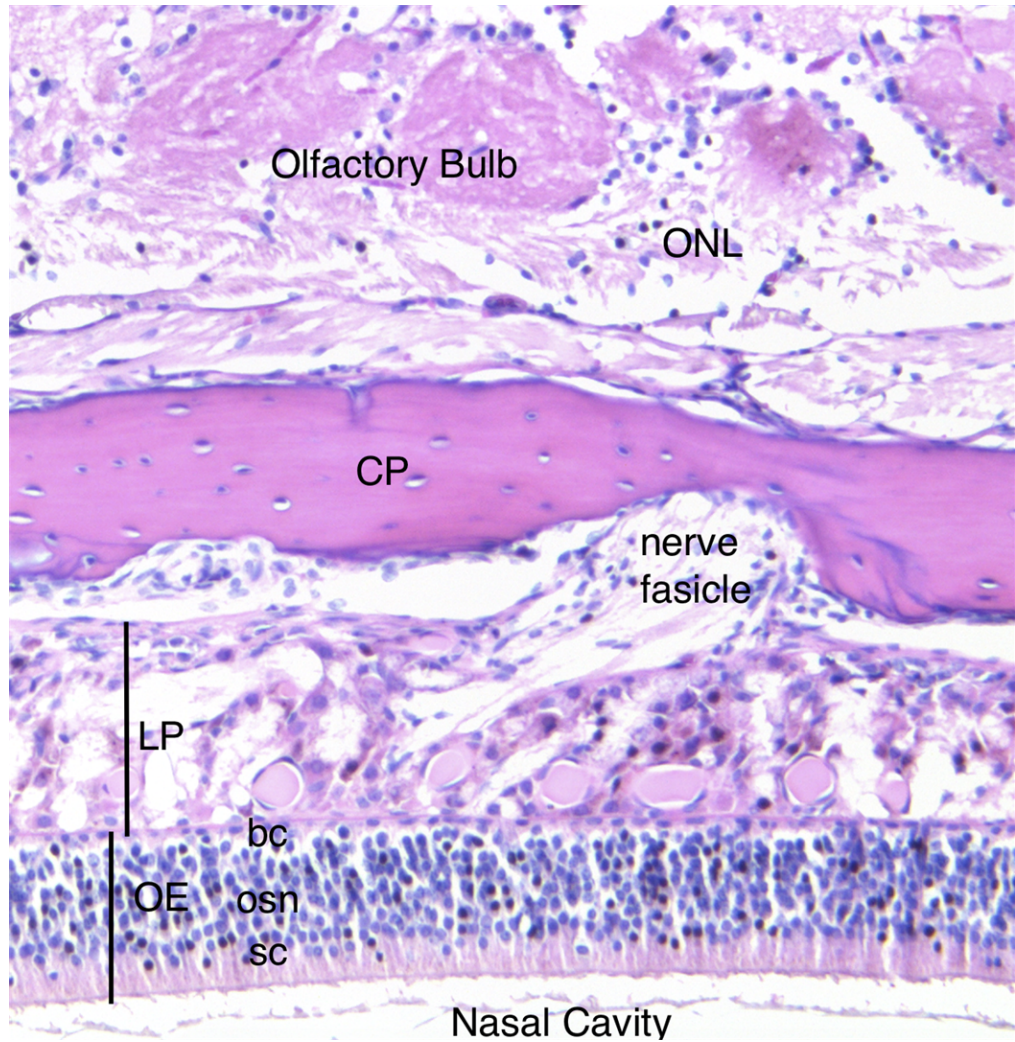


Figure 5: Neural pathway from the olfactory sensory neuron, located in the olfactory epithelium, has nerve fascicles passing through the cribriform plate to enter the olfactory bulb. bc=basal cell; CP=cribriform plate; ONL=olfactory nerve layer; osn=olfactory sensory neuron; sc=sustentacular cell.

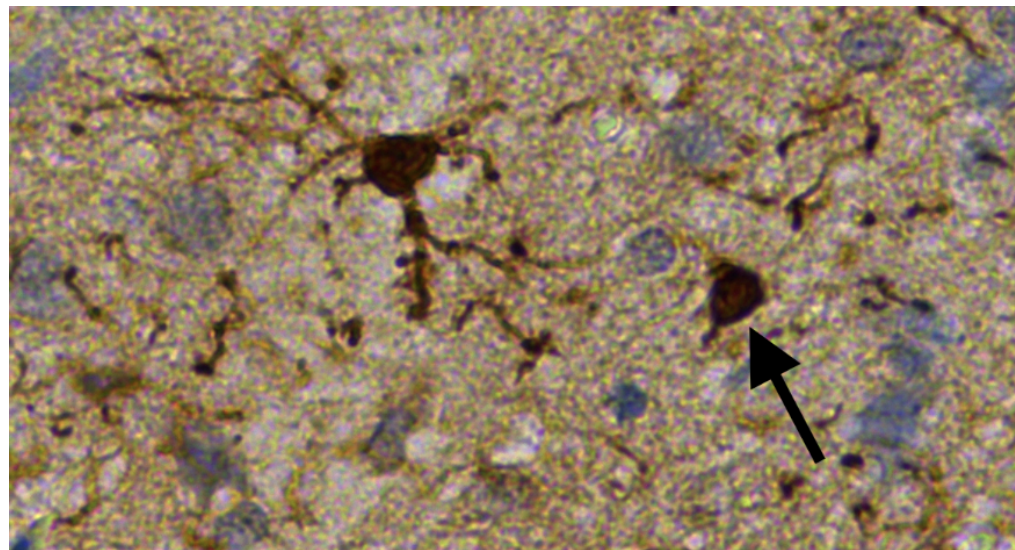


Figure 6: Brightfield microscopic images of IHC (anti-Iba-1) stained tissue sections from a rat's olfactory bulb. Resting microglia is on the left, characterized by a small cell body with ramified processes. Activated microglia is on the right

(arrow), characterized by retracted processes.

CONCLUSION & FUTURE PLAN

Result

- Aerosol characterization suggests that silver silicate in nanopure water are aerosolized and reached the inhalation chamber. The size distribution of silver silicate nanoparticle indicates these are small enough to be deposited in the nasal cavity olfactory region.

Future Plans

- Microglial morphology characterization by morphometric analysis as well as skeleton and fractal analysis are still ongoing to identify the ratio of activated to resting microglia in rats exposed to silver silicate as opposed to filtered air.
- Silver visualization by autometallography is still ongoing for detection of silver in the olfactory epithelium, olfactory nerve fascicles and microglial cells in the olfactory bulb.

METHODS

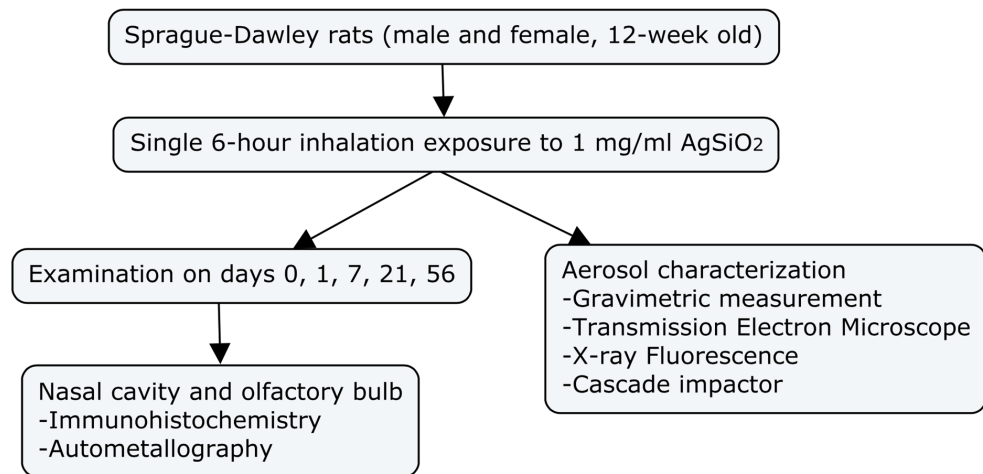


Figure 1: Experimental design.

Aerosolized silver silicate nanoparticles were generated with an aerosol nebulization system, consisting of a BGI 6-jet Collision nebulizer, 2 diffusion dryers, and a Krypton-85 charge neutralizer, all connected by steel piping to the nose-only exposure chambers. Aerosol characterization was performed on air samples collected during the 6-hour exposure period by gravimetric measurement, transmission electron microscope, X-ray fluorescence, and cascade impactor. Sprague-Dawley rats underwent a single 6-hour exposure to either filtered air or 1% silver silicate at a concentration of 1 mg/ml.

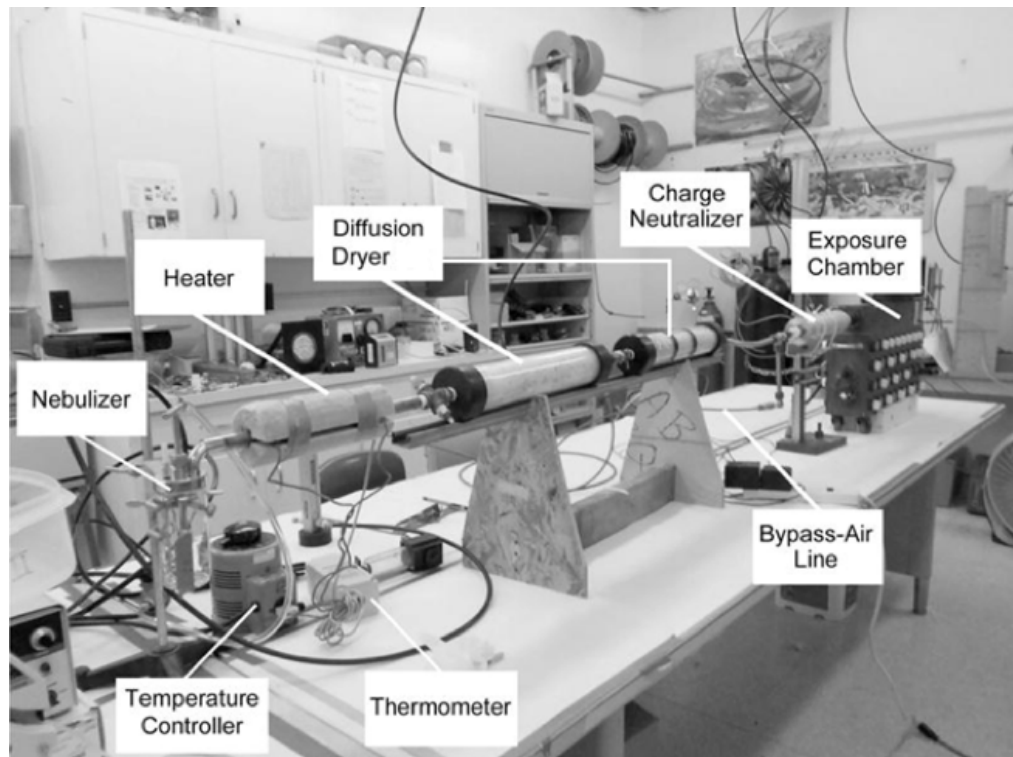


Figure 2: Components of the aerosol nebulization system used to aerosolize silver silicate nanoparticles.

Microglia in the olfactory bulb were visualized via immunohistochemistry with anti-Iba-1 antibody. Microglial morphology was

assessed using a systematic approach of morphometric analysis along with the quantitative techniques of skeleton analysis and fractal analysis using ImageJ.

RESULT: SKELETON AND FRACTAL ANALYSIS

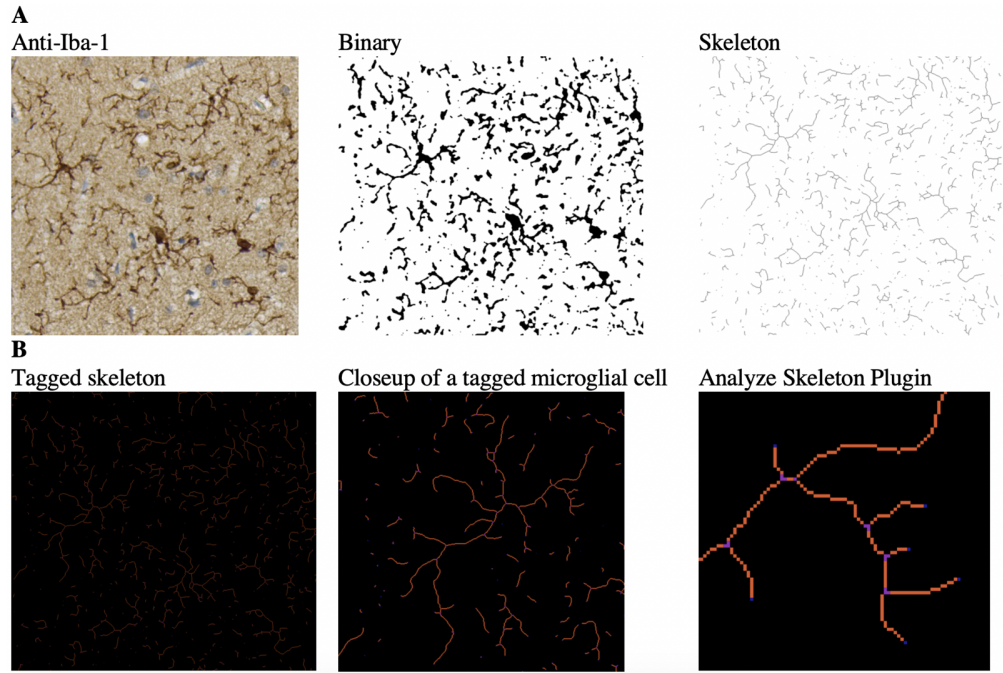


Figure 7: Skeleton of brightfield microscopic images of IHC (anti-Iba-1) stained tissue sections from rats. (A) The process of preparing a brightfield microscopic image of IHC (Iba-1) stained tissue from rat for skeleton analysis in ImageJ. (B) Skeletonized image is analyzed with the plugin AnalyzeSkeleton for ImageJ; orange denotes process length, blue denotes endpoint, purple denotes junction.

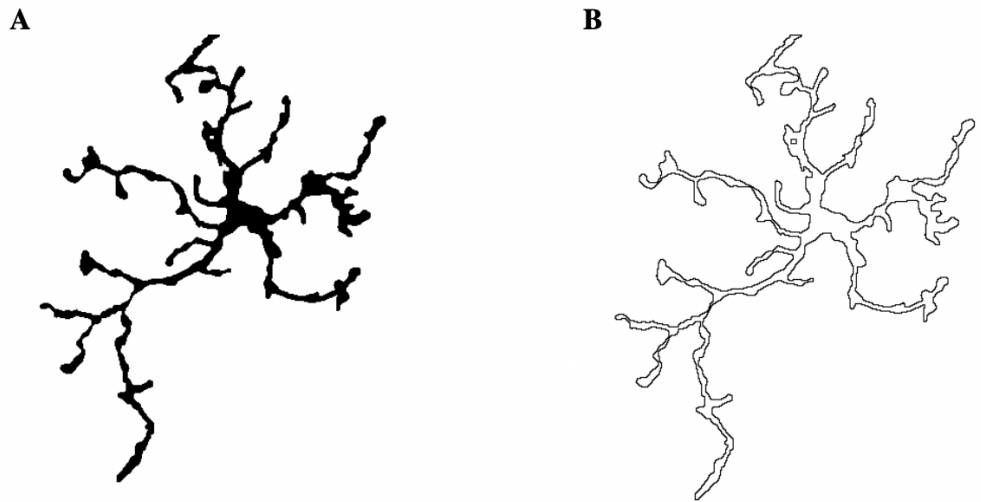


Figure 8: Fractal analysis of brightfield microscopic images of IHC (Iba-1) stained tissue sections from rats. (A) Binary image of an isolated microglial cell. (B) Outlined image of an isolated microglial cell. The outlined image is analyzed with the plugin FracLac for ImageJ to obtain fractal dimension, perimeter, density values.

ACKNOWLEDGEMENT

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