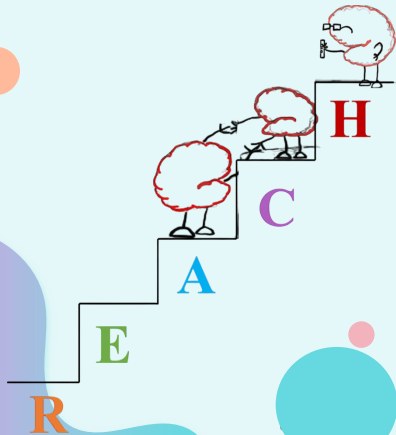


Autoimmunity and ASD: The Microglial-Synaptic Connection



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01

Goals and Central Hypothesis

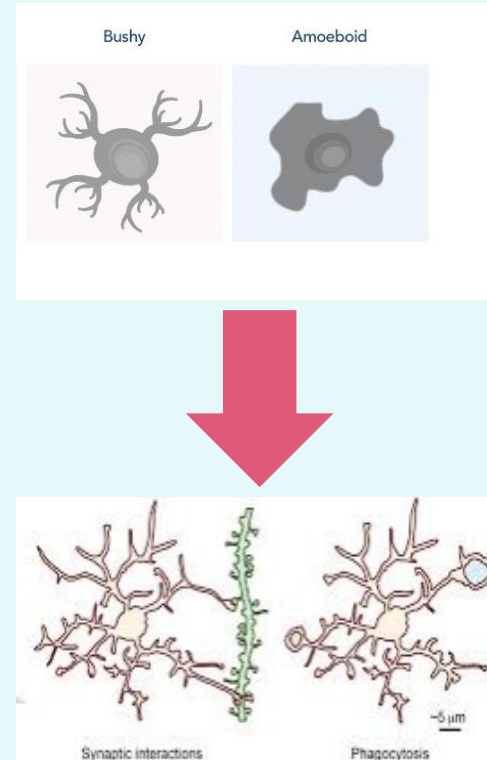
Goal

- To use a mouse model of systemic lupus to investigate the connection between autoimmune disease, neuroinflammation and the changes in neuronal connectivity that result in ASD



Hypothesis

- Immune activation in the lupus mouse will have neuroinflammation (activated microglia) leading to an increase in the number excitatory synaptic connections (PSD- 95 proteins) due to a decrease synaptic pruning by activated microglia.



- 12 week old NZBWF!/J (Lupus)
- 12 week old C57BL/1 (control)
- Hippocampus



02

Specific Aims

Specific Aims

1

To compare and contrast the morphology (size and shape) of the microglia

2

Compare the quantity of PSD-95 protein in the lupus mouse vs our control mouse

Working Hypothesis

1

The lupus mouse will have activated microglia resulting in amoeboid microglial cells

2

There will be more PSD-95 proteins identified in the lupus mouse



03

Methods

Methods

Technique: IHC (Immunohistochemistry)

Primary antibodies in use:

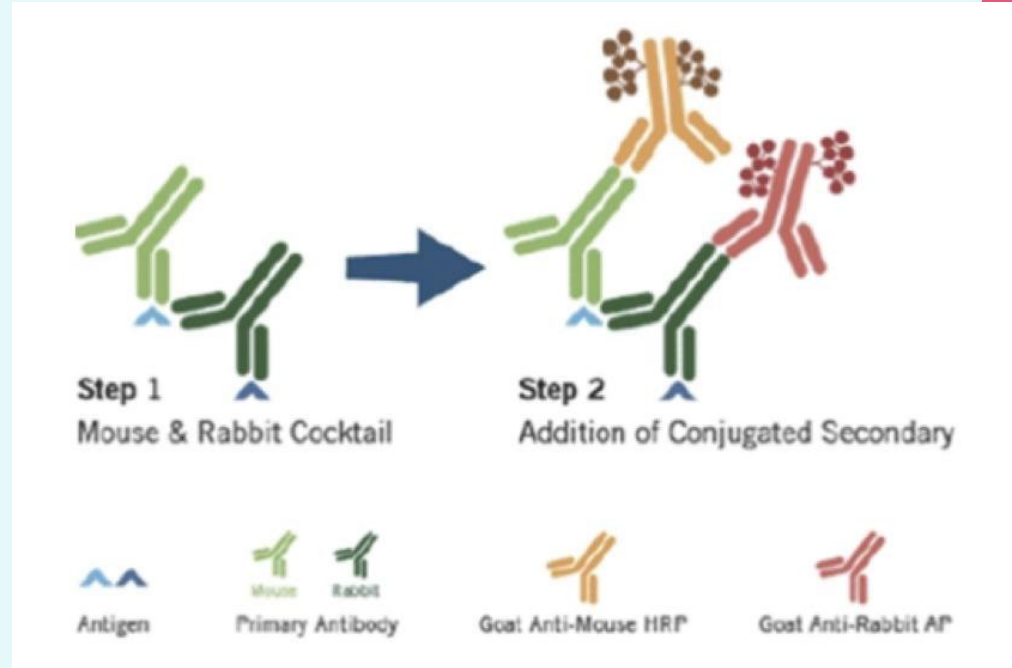
- Mouse monoclonal anti IBA- 1
(**Microglia marker**)
- Rabbit polyclonal Anti- PSD95
(**Synaptic Marker**)

Secondary antibodies in use:

- Goat Anti-rabbit IgG & Goat Anti-Mouse IgG

DNA stain in use:

- **DAPI**



Methods & Timeline



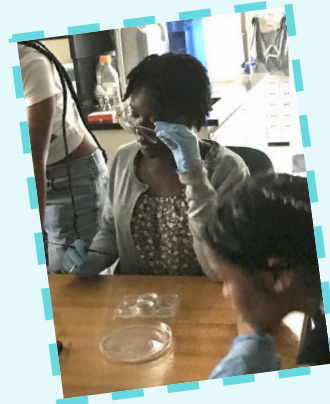
Day 1

Primary Antibodies



Day 3

Imaging on the Confocal
Microscope



Day 2

Secondary antibodies
Mounting

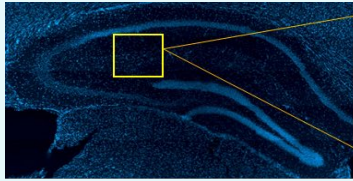


Day 4

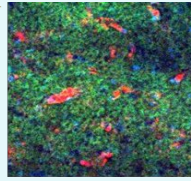
Data Analysis Using
Image J



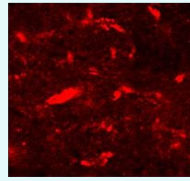
DATA ACQUISITION AND ANALYSIS



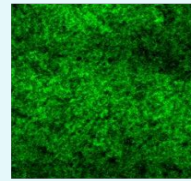
Control (Left) 10X [DAPI]



DAP1
Alexafluor 647
Alexafluor 488

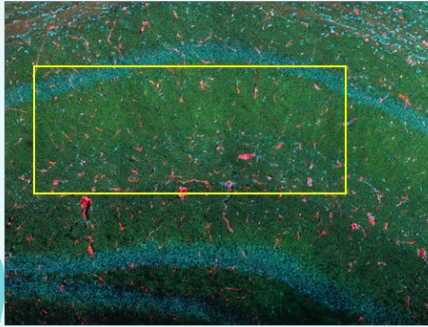


647 anti-Iba1
[Microglia]

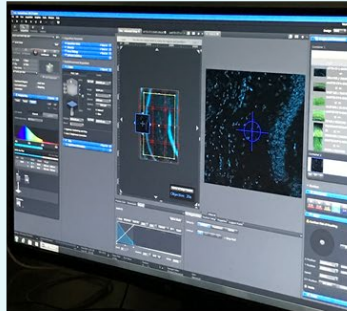


488 anti-PSD-95
[Synapses]

Olympus Confocal Microscope:
Each hippocampus (Left & Right) was imaged at 10X. At least four different ROIs from the CA1 region were captured for analysis.



Control (Right) 20X [All channels]



Using the **Zeiss Confocal**, the whole hippocampus was scanned, Z stacks were made, and a single ROI containing the CA1 region was analyzed.

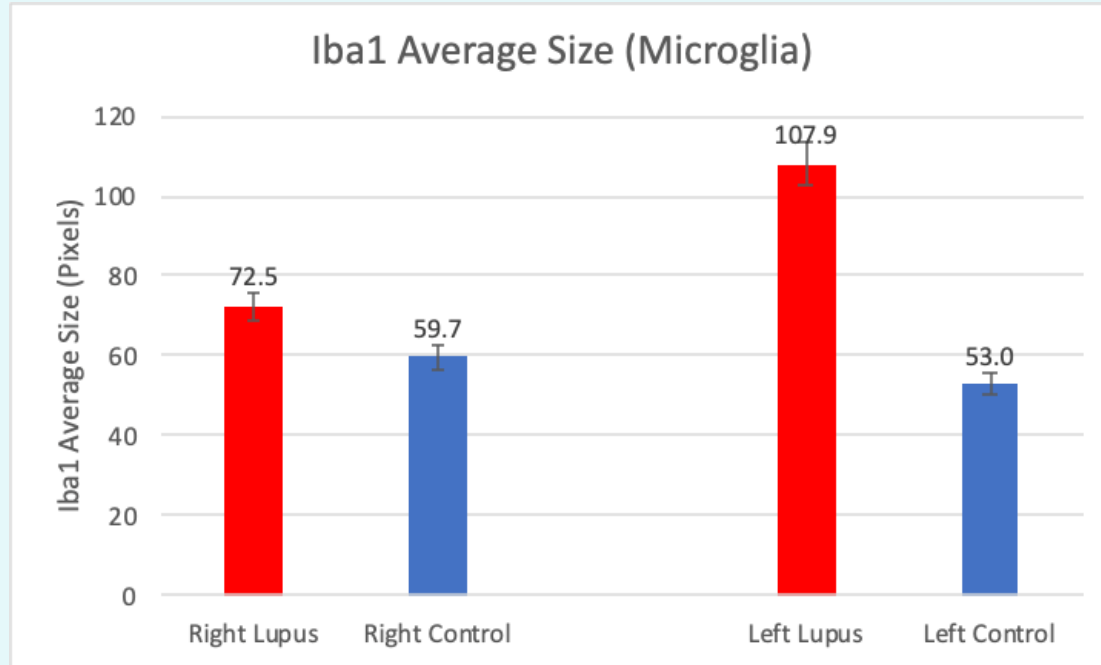
ANALYSIS: Using **ImageJ** software, red particles (Iba1) were assessed for number and size. Green particles (PSD-95 synaptic markers) were counted.



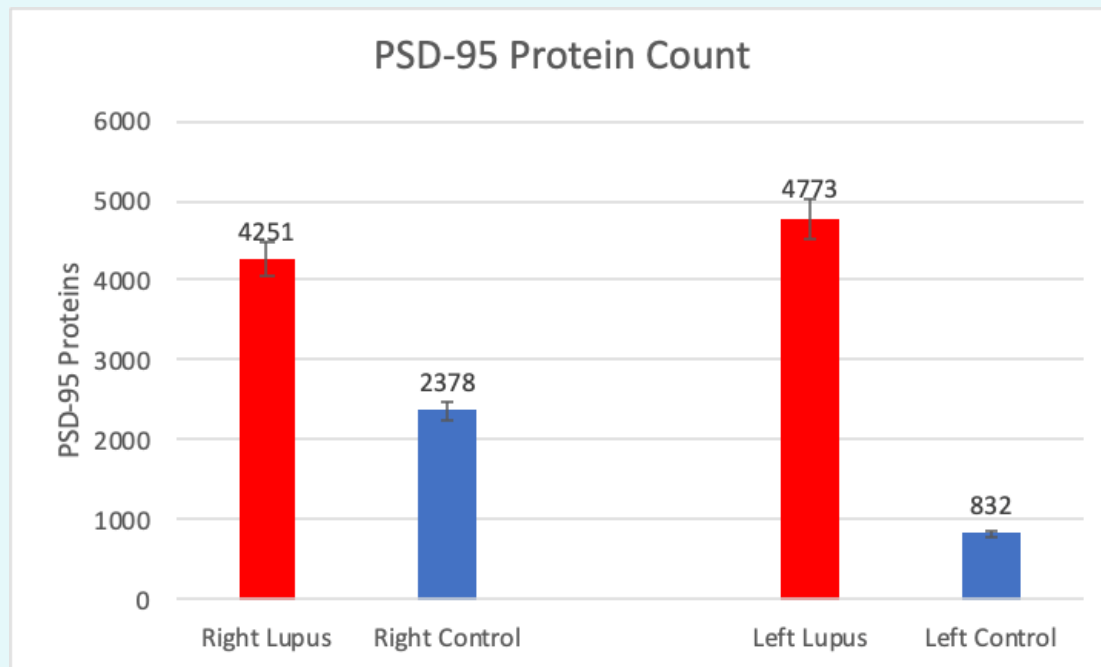
04

Results

Results: IBA-1 (Microglia)



Results: PSD-95



05

Interpretations & Limitations

Interpretations

IBA-1 Average Size

- An increase in the average size of IBA-1 particles in the lupus mouse hippocampus than the control
- This indicates that the microglia are activated

PSD-95 Proteins

- There were more PSD-95 proteins in the lupus mouse than the control mouse
- This indicates that there are more synaptic connections in the lupus mouse

Limitations

- **LIMITED :**
 - Small Sample size (One mouse per group)
- **MORPHOLOGY :**
 - Could not use to identify microglia in the focal plane using the Olympus microscope.
- **IMAGE ANALYSIS :**
 - Trouble establishing thresholds
 - Needed Z- stacks

06

Conclusion

Conclusion

- Using the Olympus microscope, our results for the microglia cells were inconclusive. However, using the Zeiss microscope and Z-Stacks we were able to observe activated microglia.
- In support of our hypothesis, we found an increase of PSD-95 protein in the Lupus mouse indicating an increase in postsynaptic connections.

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Citations

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THANK YOU!!!

Any Questions?

