Effects of Glia Cell Density in the Plasticity of Neuronal Astrocytic Co-culture

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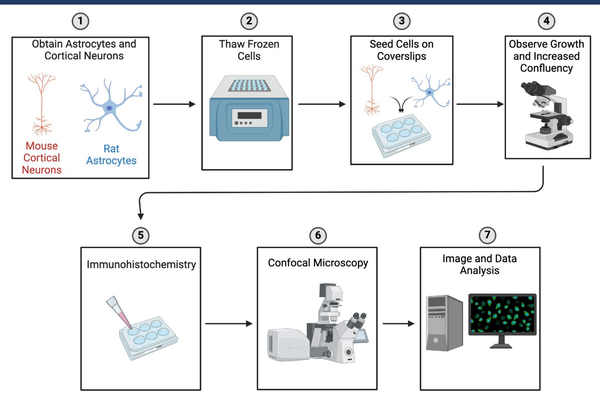
Summary

Glial cells have an important function in the nervous system supporting neural development, signaling, and neuronal structure. Astrocytes are a type of glial cell found in the central nervous system (CNS) that carry out several functions including neurotransmitter regulation, release of signaling molecules called gliotransmitters, and energy regulation in neurons. While there is ongoing research investigating the role of glial cells in the development and signaling of neurons, little is known about the significance and mechanisms that glia have in the development of synaptic connections between neurons. Synaptic plasticity is the process by which neurons alter the strength of their connections, and is believed to be an important component in development and learning. Therefore, we aim to investigate the importance of astrocytes in synaptic plasticity by assessing the optimal density in which to culture cortical neurons and astrocytes *in vitro*. We are using immunohistochemistry to visualize the connections between neurons and recording neuronal activity with high-density microarray electrodes. Determining the optimal astrocytic density to co-culture with cortical neurons is crucial for future electrophysiology experiments to elucidate the specific mechanisms of synaptic plasticity using electrical and light stimulation. This can give critical insight to how synapses enhance or prune their connections, and can lead to better understanding of learning and the pathophysiology observed in neurodegenerative diseases.

Methods



Cell Plating:

Mouse cortical neurons and rat astrocytes were kept frozen in liquid nitrogen until thawing. Once thawed, cells were seeded on coverslips at a ratio of 1 astrocyte to 8 neurons. The number of neurons varied between 100,000, 200,000, and 300,000 per dish. These cultures were maintained in incubators at 37°C and 5% CO_2 between 2-3 weeks.

Immunohistochemistry:

Each coverslip was fixed with 4% paraformaldehyde for 15 minutes before replacing the media with PBS. 3 washes of PBS were conducted for 10 minutes each before blocking with 5% donkey serum, 0.3% triton buffer, and PBS for one hour. Primary antibodies were then incubated for 2 hours at room temperature. Primary antibodies included MAP2 (microtubule-associated protein 2), which is a cytoskeletal protein in neurons (anti-chicken, 1:500) and GFAP (glial fibrillary acid protein), which is an intermediate filamentous protein expressed in astrocytes (anti-rabbit, 1:500). After primary antibody incubation, 3 subsequent washes with PBS were conducted for 10 minutes. Secondary antibodies were incubated for 2 hours away from light. Secondary antibodies included Alexa 488 (donkey anti-mouse, 1:500) and Alexa 594 (donkey anti-rabbit, 1:500). Following 3 final washes for 10 minutes in PBS, each coverslip was mounted using Fluoromount-G mounting media. Cells were quantified using DAPI for nuclear staining.

Low Density Neuronal Cultures may Lack Sufficient Development or Undergo Apoptosis

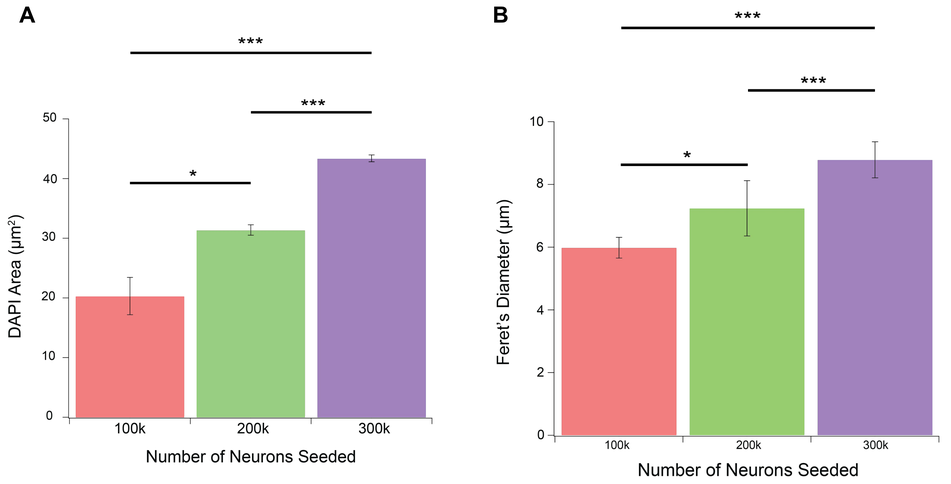
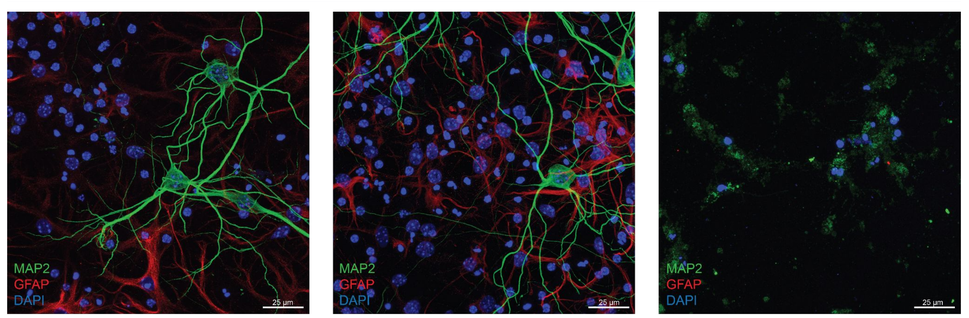


Figure 1. **(A)** Immunofluorescent images were acquired at 2-3 weeks *in vitro* for cultured neurons at different densities, seeded at a ratio of 1 astrocyte to 8 neurons. The different imaging channels for DAPI, MAP2 and GFAP staining were processed using ImageJ. The area of DAPI staining for neurons and astrocytes at each density was measured in μm^2. High-density cultures (300,000 neurons) and medium density cultures (200,000 neurons) had a greater nuclear size than the low-density cultures, suggesting a lack of development or induction of apoptosis in low-density cultures (One-way ANOVA, Tukey's test, * $p<0.05$, *** $p<0.0001$). **(B)** Feret's diameter was measured for the same images as (A), which measures the distance across each DAPI staining. High and medium-density cultures contained a significantly larger Feret's diameter than the low-density culture (One-way ANOVA, Tukey's test, * $p<0.05$, *** $p<0.0001$).

MAP2 and GFAP Staining to Characterize Neuronal Cell Culture



300k Neurons 200k Neurons 100k Neurons

Figure 2. Representative immunofluorescent images of cell cultures at low, medium, and high-densities taken at 63X. MAP2 is depicted in green and GFAP is shown in red. DAPI stains the nucleus of all cells and is shown in blue. Images were processed using ImageJ. At medium and high-density cultures, neurons and glia were able to develop; however, the low-density culture shows no staining for neurons or glia, suggesting a lack of development or induction of apoptosis. Scale bar represents 25 µm.

Sholl Analysis Quantifies Neuronal Arborization

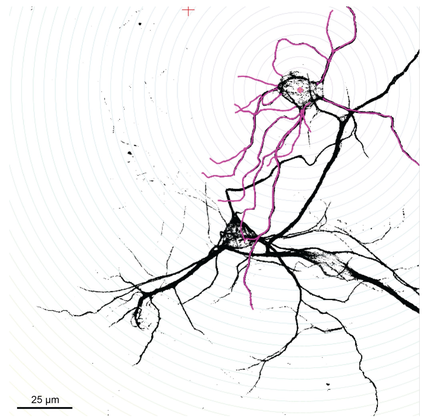


Figure 3. Representative sholl analysis of a neuron observed at 63X. The density of the neuronal culture is 300,000 neurons, representing the high-density neuronal culture. The sholl analysis can give relevant information regarding neurite development and branching, which are important characteristics to consider when assessing synaptic plasticity. Neuronal processes were semi-automatically reconstructed and are quantitatively analyzed using ImageJ. The upper neuron was traced and has an average arbor length of 59.01 µm ± 36.98 µm and a total of 13 branches with 4 branch points. Analysis of several neurons in the high-density and medium-density cell cultures can elucidate whether glia density plays a role in the development of neuronal processes. Additionally, sholl analysis can be used with astrocytes to determine how culture density impacts astrocytic branching. Scale bar represents 25 µm.

Conclusions and Current Work

- Low-density cell cultures seeded with 100,000 show little viability as suggested by the the DAPI staining with small and fragmented nuclei.
- At higher densities, the nuclear area and diameter grow larger for neurons and astrocytes.
- Analysis of the difference in the number of astrocytes between initial seeding and fixation can provide insights into how glia proliferate and develop in a density-dependent manner.
- Future experiments will characterize synaptic plasticity using electrophysiological assessment and correlate these results with immunohistochemistry data.

References

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2. Mong, J. A., Glaser, E., & McCarthy, M. M. (1999). Gonadal Steroids Promote Glial Differentiation and Alter Neuronal Morphology in the Developing Hypothalamus in a Regionally Specific Manner. *Journal of Neuroscience*, *19*(4), 1464–1472.