Characterizing astrocytic networks: Filling the “gaps”

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Introduction

Astrocyte functions

- Feed neurons with nutrients
- Participate in tissue repair
- Control extracellular ion levels
- Maintain the blood-brain barrier
- Facilitate synaptic plasticity
- Regulate synaptic transmission

Tripartite synapse

Astrocytes respond to neurotransmitters with intracellular calcium increases and release gliotransmitters that activate receptors either in the pre- or the post-synaptic terminal. Thus, synapses are tripartite, meaning they have three major components:

1. Pre-synaptic axon terminal
2. Post-synaptic neuron
3. An astrocyte which wraps around the synapse

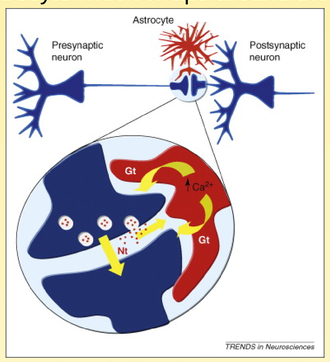

Figure 1. Illustration of tripartite synapse (Adapted from Perea et. al, 2009). Graphical illustration depicting information flow between astrocyte and neuronal elements.

Astrocytic networks

Connexins (gap junction proteins) are expressed in astrocytes and allow for the formation of glial networks. This enables diffusion of signaling molecules and the propagation of signals.

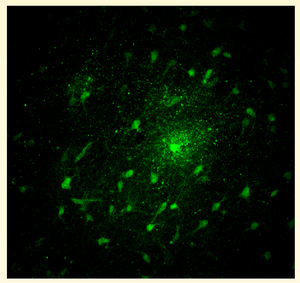

Figure 2. Astrocytic network. Astrocytic network labeled by dye diffusion from a single astrocyte in the hippocampus.

Objectives

1. Identify astrocytic networks by delivering dye to individual astrocytes and allowing it to diffuse
2. Characterize spatial properties of astrocytic networks in the hippocampus and nucleus accumbens

Methods

Identifying astrocytes

Before performing dye-filling experiments, we confirmed we were recording from astrocytes.

Astrocytes were identified by:

- Low membrane resistance
- Absence of active currents in response to hyperpolarizing and depolarizing voltage pulses
- Linear current-voltage relationship of steady state currents

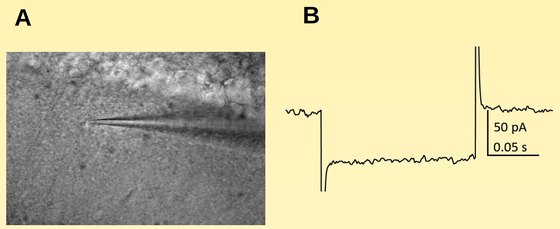

Figure 3. Astrocyte identity confirmed by low membrane resistance. A.) DIC image of a cell being recorded (Top: pyramidal layer. Center: recording pipette in stratum radiatum). B.) Whole cell current in response to 5 mV voltage pulse, used to deduce membrane resistance.

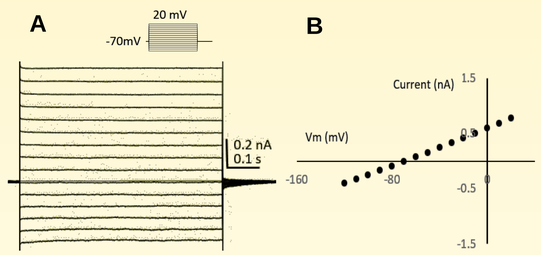

Figure 4. Astrocyte identify confirmed by absence of active currents and linear IV curve. A.) Whole cell currents in response to hyperpolarizing and depolarizing voltage pulses. B.) Steady state current plotted against pulse voltage

Identifying non-astrocytes

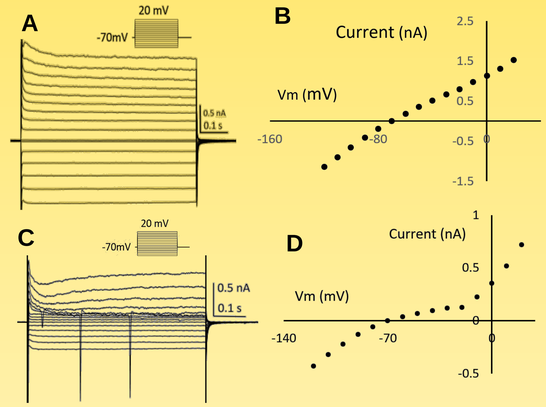

Figure 5. Non-astrocytes identified by active currents and non-linear IV curve A.) Inward currents in response to depolarization confirms identity of ng2^{+} glia. C.) Inward and outward currents indicative of action potentials confirm identity of neuron. B, D.) Non-linear steady-state IV curve confirms non-astrocyte identity.

Astrocyte filling

- A seal was formed between the pipette and an astrocyte membrane
- Biocytin dye was delivered to the cell for a desired length of time
- Slice was fixed overnight, fluorescently stained, then imaged

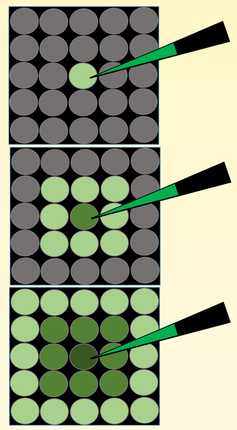

Figure 6. Graphical depiction of dye diffusion over time. A single plane of an astrocytic network is simulated, as it receives dye from neighboring cells. Each circle represents a cell. Triangle represents a pipette delivering dye.

Results

Qualitative comparison

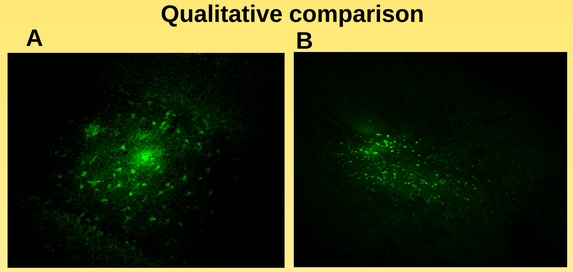

Figure 7. Astrocytic networks visualized. Images were obtained from a 2-photon excited fluorescence microscope. A.) Network in the stratum radiatum (CA1) of the hippocampus, constructed from a stack of 22 consecutive images (20.8 µm deep). B.) Network in the nucleus acumbens, constructed from a stack of 27 consecutive images (25.7 µm deep).

Quantitative comparison

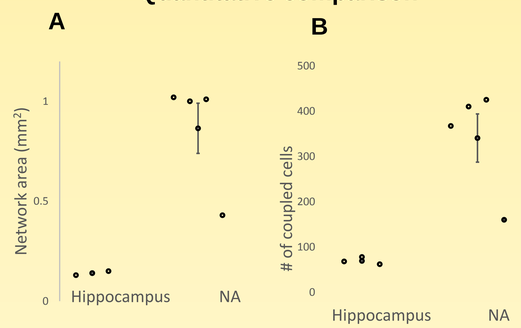

Figure 8. Quantification of network connectivity A.) Cells emitting fluorescence were counted manually. B.) Area of region containing fluorescent cells was measured. CA1: n=3. NA: n=4.

Future directions

1. Characterize networks after treatment amphetamines and 4 aminopyridine
2. Characterize networks of other brain regions, such as the striatum and amygdala

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