Calcium signals in glial cell astrocytes modulate synaptic transmission and plasticity in cerebral cortices.

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THE BRAIN OF NEURONS...

1 Brain = 80÷100 \(10^9\) Neurons
1 neuron = 1-10K synapses

Lichtman Lab, Harvard University.
Astrocytes are as many as neurons in the human brain.
GLIAL CELLS

KNOWN ASTROCYTIC FUNCTIONS:

- Ionic and water homeostasis
- Metabolic support
- Neurovascular coupling
- Neurotransmitter clearance
- Neuronal growth, synaptogensis
- Inflammation, defensive response

RECENTLY DISCOVERED:

A DYNAMIC DIALOGUE BETWEEN NEURONS AND ASTROCYTES IS NECESSARY ALSO FOR NEURONAL INFORMATION PROCESSING, SYNAPTIC PLASTICITY, SPECIFIC COGNITIVE FUNCTIONS AND BEHAVIOR.
Astrocyte function from information processing to cognition and cognitive impairment
Mirko Santello, Nicolas Toni, and Andrea Volterra

Annual Review of Neuroscience
Astrocytes and Behavior
Paulo Kofuji and Alfonso Araque

From Synapses to Circuits, Astrocytes Regulate Behavior
Krissy A. Lyon and Nicola J. Allen
Molecular Neurobiology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA, United States

Nature 2019
CLASSICAL DESCRIPTION of STAR SHAPED CELLS IS **VERY LIMITED**

IT LACKS THE MOST IMPORTANT AND DYNAMIC SUBCELLULAR REGION, THE SPONGIFORM DOMAIN OF ULTRA-SMALL PROCESSES THAT ARE NANOSCOPYC.
FIGURE 3.10  Astrocytes appear stellate when their intermediate filaments are stained (red, GFAP), but membrane labeling (green, membrane-associated EGFP) highlights the profusion of fine cellular processes that intercalate among other neuropil elements such as synapses and neurons (N). Scale bar = 10 μm. Image courtesy of Dr. M. C. Smith.
THIN ASTROCYTIC PROCESSES CONTACT SYNAPSES

EACH ASTROCYTE CONTACTS THOUSANDS/MILION OF SYNAPSES IN ITS OWN DOMAIN (TERRITORY)

TRIPARTITE SYNAPSE:
SYNAPSES ARE COMPOSED BY PRE- and POST-SYNAPTIC ELEMENTS (NEURONAL) AND BY PERYSINAPTIC ASTROCYTIC PROCESSES (PAPs; ASTROCYTE)

Halassa et al. 2007
ASTROCYTE EXCITABILITY IS MEDIATED BY INTRACELLULAR CALCIUM TRANSIENTS

Astrocytes respond to synaptic activity with intracellular $Ca^{2+}$ transients and modulate synaptic transmission by releasing Gliotransmitters.

**METABOTROPIC RECEPTORS**
- mGluR, P2Rs, DARs, NERs, AChRs, GABA$_B$R

**GLIO-TRANSMITTERS**
- Glutamate
- D-serine
- ATP $\rightarrow$ Adenosine
- GABA
- other modulators (cytokines, neurotrophins)

Haydon PG. & Carmignoto G., Physiological Rev., 2006

Araque et al 2014

Bazargani and Attwell 2016
General Calcium functions:

- Protein kinases/phosphatases
- Gene expression (cell growth, differentiation, death)
- Membrane excitability
- Mitochondria
- Cell motility/muscle contraction

Shigetomi et al. 2016
STUDY OF Ca\(^{2+}\) OSCILLATIONS IN THIN PROCESSES with Genetically Encoded Calcium Indicators (GECI)

GCaMP6f

*Kd 88 nM @ 37°C*

AAV5.GfaABC1D.GCaMP6f

GFAP
GCaMP6f

NeuN
GCaMP6f
Disentangling calcium-driven astrocyte physiology

Dmitri A. Rusakov

D. Rusakov 2015
GABAergic signaling in somatosensory and visual cortex

WHAT...

HOW...

• 2P-LSM
• electrophysiology (patch-clamp; Lfp)
  • optogenetics, chemogenetics
  • Transgenic mice
• transgene delivery by viral vector (AAVs) intracranial injections
  • ANALYSIS with different algorithms
    (lab G. Carmignoto; G. Ratto)
The Inhibitory Neurotransmitter GABA Evokes Long-Lasting Ca^{2+} Oscillations in Cortical Astrocytes

Letizia Mariotti, Gabriele Losi, Michele Sessolo, Iacopo Marcon, and Giorgio Carmignoto
The Inhibitory Neurotransmitter GABA Evokes Long-Lasting Ca\textsuperscript{2+} Oscillations in Cortical Astrocytes

Letizia Mariotti, Gabriele Losi, Michele Sessolo, Iacopo Marcon, and Giorgio Carmignoto
GABA_B RECEPTOR ACTIVATION EVOKEES CALCIUM TRANSIENTS AND GLUTAMATE RELEASE FROM ASTROCYTE

Patch-clamp from pyramidal neurons
ASTROCYTE RESPONSE TO SPECIFIC GABAERGIC INTERNEURONS

Adapted from Kawaguchi et al, 2001
OPTOGENETICS: LIGHT GATED ION CHANNELS

ChR2: channerhodopsin-2
Light gated cation channel (blue light)

ChR2 expressed on one cell type only
OPTOGENETIC STIMULATION OF SELECTIVE NEURONAL POPULATIONS.
SOMATOSTATIN or PARVALBUMIN INTERNEURONS

AAV5.GfaABC1D.GCaMP6f
AV2/1.EF1.dflox.hChR2-mCherry

OPTOGENETIC STIMULATION OF SELECTIVE NEURONAL POPULATIONS.
SOMATOSTATIN or PARVALBUMIN INTERNEURONS

2P-LSM
920 nm
470 nm

ChR2: channelrhodopsin-2
Light gated cation channel (blue light)

Ca²⁺

Analysis: GECI Quant+AstroResp
L. Mariotti
A. Lia
M. Zonta

150 ms@1Hz

GCaMP6f expressing astrocytes

AAV5.GfaABC1D.GCaMP6f
AV2/1.EF1.dflox.hChR2-mCherry

2-3 wks
OPTOGENETIC PV- or SST-INTERNEURON STIMULATION in vivo


Response is mediated by \( \text{GABA}_B \) receptors
OPTOGENETIC PV- or SST-INTERNEURON STIMULATION EVOKES DISTINCT RESPONSE IN ASTROCYTES

ASTROCYTE RESPONSE TO SST-INs IS POTENTIATING

Evoked firing is constant
Evoked currents are constant

WHICH MECHANISM FOR POTENTIATION?
SST-INTERNEURONS ALSO RELEASE SOMATOSTATIN
Also known as SRIF (somatotropin release inhibiting factor)

Large dense-core vescicles in axon, soma, dendrites

Periphery and hypothalamus: reduces hormone release
Brain: reduces NTs release, favors hyperpolarization,
Protective and favors cognitive functions
REDUCED IN AGEING AND ALZHEIMER’S DISEASE
SOMATOSTATIN EFFECTS ARE COMPLEX AND POORLY UNDERSTOOD IN BRAIN CORTEX

Large dense-core vesicles in axon, soma, dendrites

SST-Receptors 1-5

SOMATOSTATIN INCREASES CALCIUM EVENTS IN CORTICAL ASTROCYTES IN SITU
ASTROCYTIC POTENTIATING RESPONSE TO SST-INTERNEURONs DEPENDS ON SST-RECEPTOR ACTIVATION


150 ms@1Hz

10s

30s

CYN 154806

Soma

Proximal processes

Microdomains
In somatosensory cortex:
Astrocytes show weak and depressing Ca\(^{2+}\) oscillations in response to PV interneuron activity, strong and potentiating oscillations in response to SST interneurons.

Astrocytes discriminate specific interneuron activity through somatostatin receptors activation.

Future studies may unveil similar specific responses to other neuropetides.
Astrocytes Modulate Somatostatin Interneuron Signaling in the Visual Cortex

Vanessa Jorge Henriques, Angela Chiavegato, Giorgio Carmignoto and Marta Gómez-Gonzalo

B

Basal  S1  S2  Merge

C

1Hz 30 s  1Hz 30 s

D

IPSC (%)  mean IPSC (%)

Time (min)  S1  S2

May 2022
DEVELOPMENT OF NEW ANALYTICAL TOOLS:

- AstroRespNew (G. Carmignoto’s Lab, CNR-IN, Padova)
- Image Gateway (G. Ratto’s Lab, CNR-NANO, Pisa)

ASTROCYTE CALCIUM SIGNAL IN PHYSIOLOGY (MEMORY) AND PATHOLOGY (ALZHEIMER’S DISEASE; NEUROINFLAMMATION)

2-Photon Laser Scanning Microscopy on brain tissue
HUMAN ASTROCYTES

MOST COMPLEX MORPHOLOGY IN HUMAN ASTROCYTES

IN HUMANS ASTROCYTES MAIN PROCESSES ARE 10 TIMES MORE THAN IN RODENTS

ALSO THE TOTAL NUMBER OF ASTROCYTES IN HUMANS IS THE HIGHEST AMONG SPECIES

Mohan et al 2015
Astrocyte functions in brain physiology: and pathology:
• LTP and memory
• Alzheimer’s Disease
• Neuroinflammation

Mechanisms of Seizure generation
Dravet Syndrome
Giorgio Carmignoto
Letizia Mariotti
Marta Gomez-Gonzalo
Micaela Zonta
Angela Chiavegato
Annamaria Lia
Rosa Chiara Goisis
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