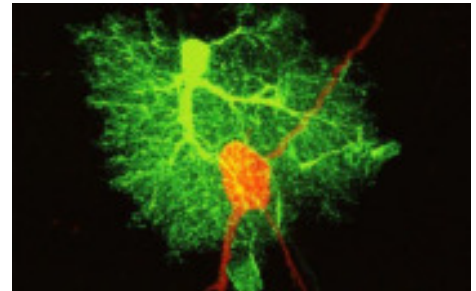


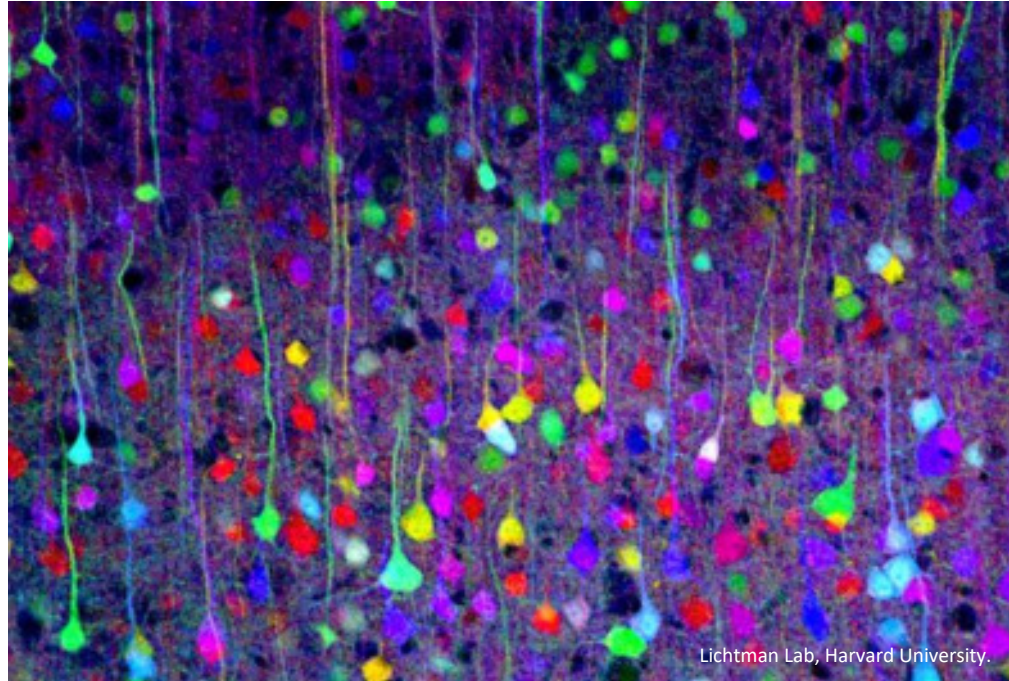
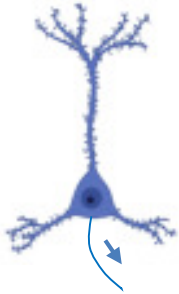
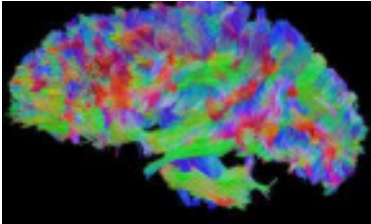
Calcium signals in glial cell astrocytes modulate synaptic transmission and plasticity in cerebral cortices.

Gabriele Losi

*Institute of Nanoscience-CNR,
c/o Dept. Biomed. Science, Metabolic and Neuroscience,
University of Modena and Reggio Emilia*



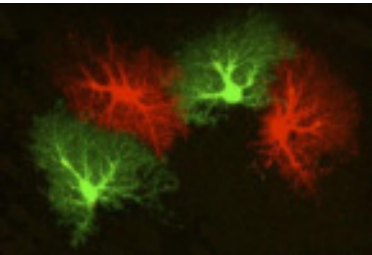
THE BRAIN OF NEURONS...



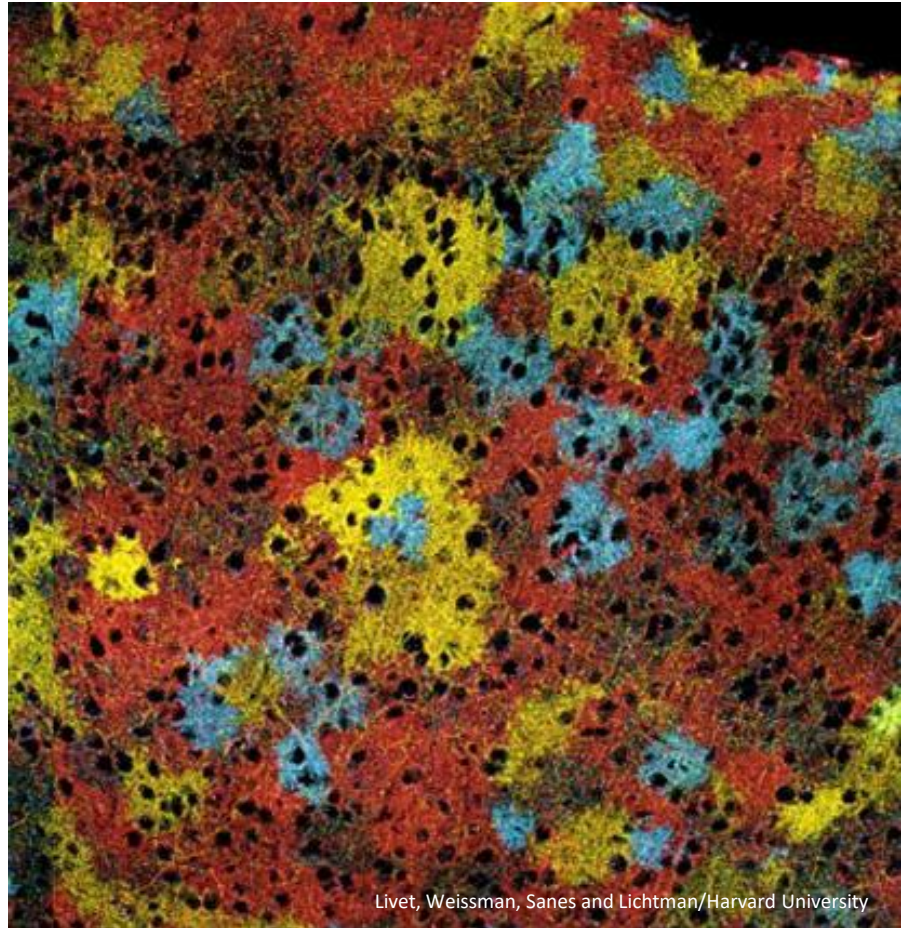
1 Brain = 80÷100 10^9 Neurons

1 neuron = 1-10K synapses

IS ALSO THE BRAIN OF ASTROCYTES !

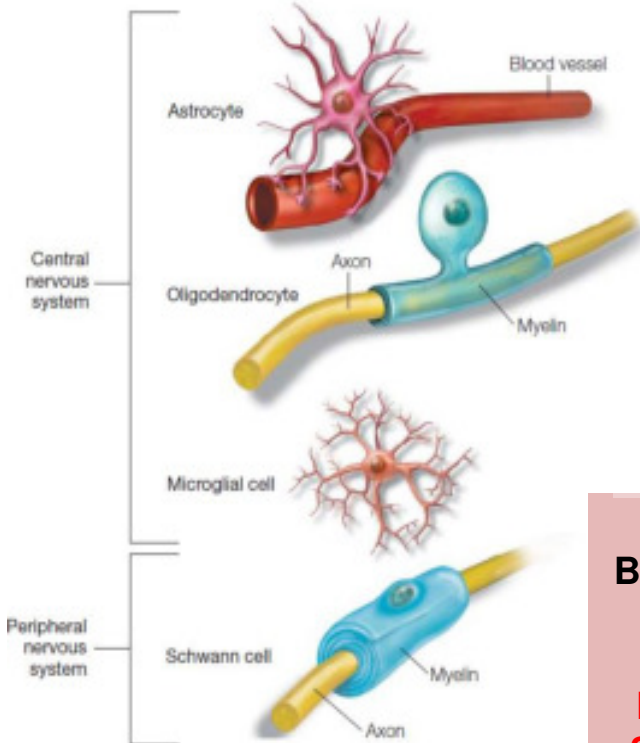


Astrocytes are as many as neurons in the human brain.



GLIAL CELLS

KNOWN ASTROCYTIC FUNCTIONS:



- Ionic and water homeostasis
- Metabolic support
- Neurovascular coupling
- Neurotransmitter clearance
- Neuronal growth, synaptogenesis
- 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^{1,2}, Nicolas Toni² and Andrea Volterra^{3,4*}

Nature 2019

NE40CH03_Araque ARjats.cls December 25, 2020 11:33

Annual Review of Neuroscience

Astrocytes and Behavior

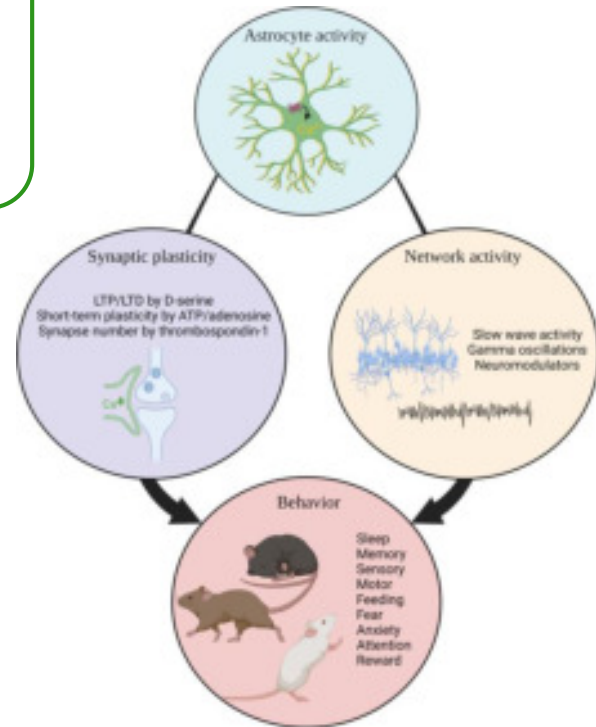
Paulo Kofuji and Alfonso Araque

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

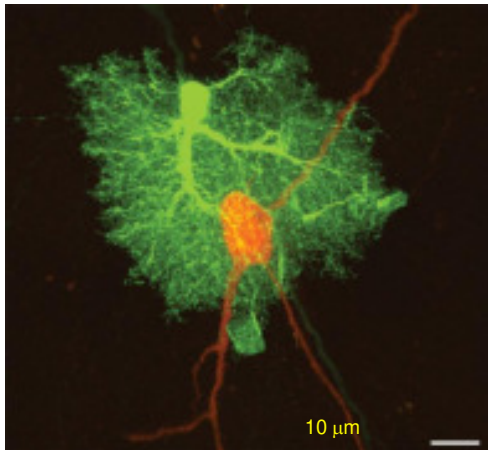
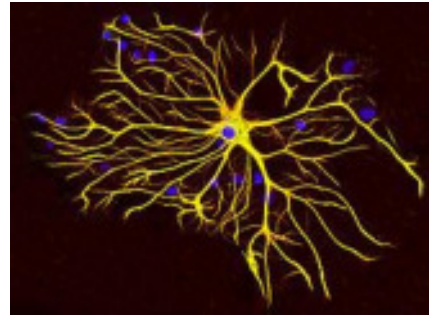
REVIEW
published: 04 January 2022
doi: 10.3389/fncel.2021.786293



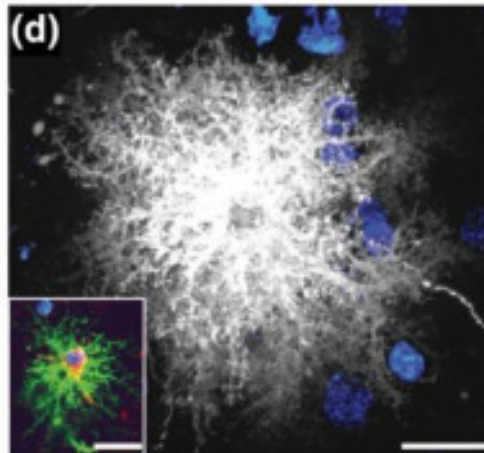
ASTROCYTE MORPHOLOGY

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 NANOSCOPIC.**



Allen and Barres 2009



Vasile and Rouach 2017

ASTROCYTE MORPHOLOGY

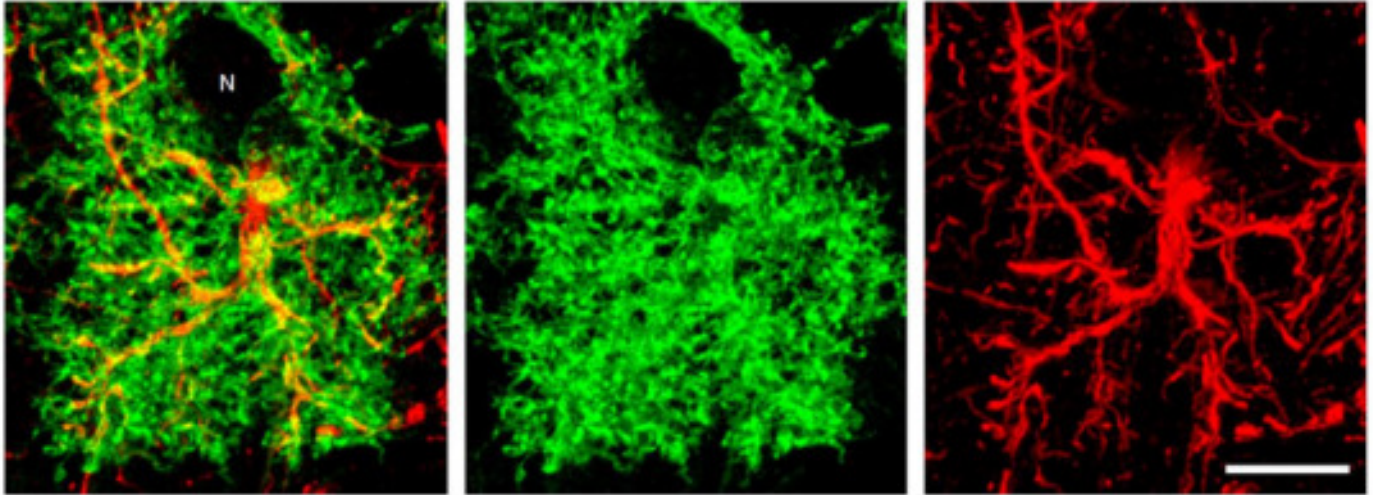


FIGURE 3.10 Astrocytes appear stellate when their intermediate filaments are stained (red, GFAP), but membrane labeling (green, membrane-associated EGFP) highlights the profusion of fine cellular processes that intercalate among other neuropil elements such as synapses and neurons (N). Scale bar = 10 μm . Image courtesy of Dr. M. C. Smith.

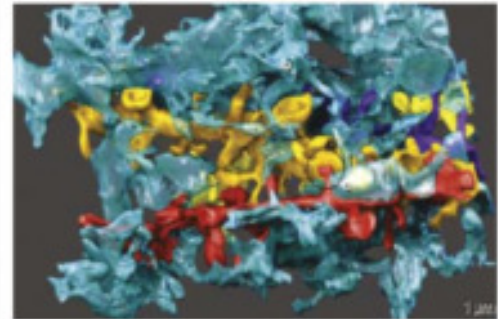
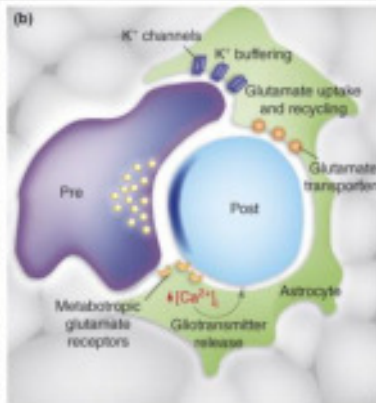
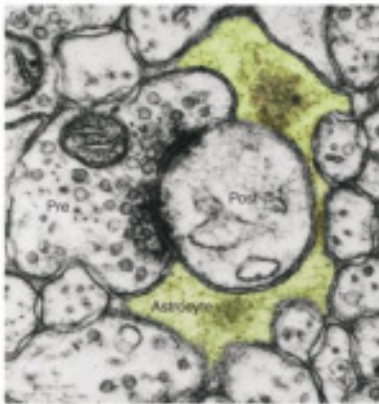
Squire et al; Fundamental
Neuroscience

THIN ASTROCYTIC PROCESSES CONTACT SYNAPSES

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

TRIPARTITE SYNAPSE:

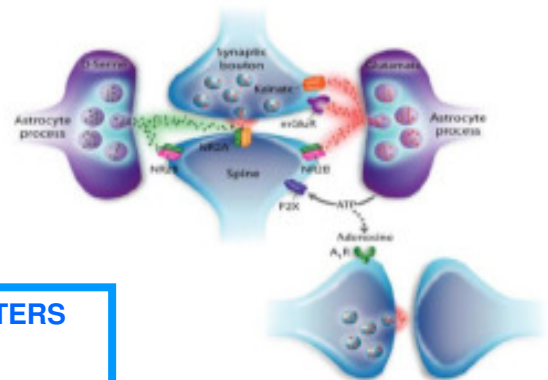
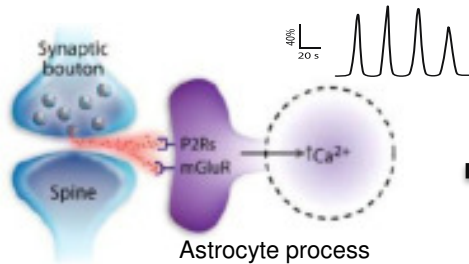
SYNAPSES ARE COMPOSED BY PRE- and POST-SYNAPTIC ELEMENTS (NEURONAL) AND BY **PERYSYNAPTIC ASTROCYTIC PROCESSES (PAPs; ASTROCYTE)**



SHENKIN in Molecular Medicine

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_BR

GLIO-TRANSMITTERS

- Glutamate
- D-serine
- ATP → Adenosine
- GABA
- other modulators
(cytokines, neurotrophins)

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

Araque et al 2014

Bazargani and Attwell 2016

CALCIUM IN ASTROCYTES

General Calcium functions:

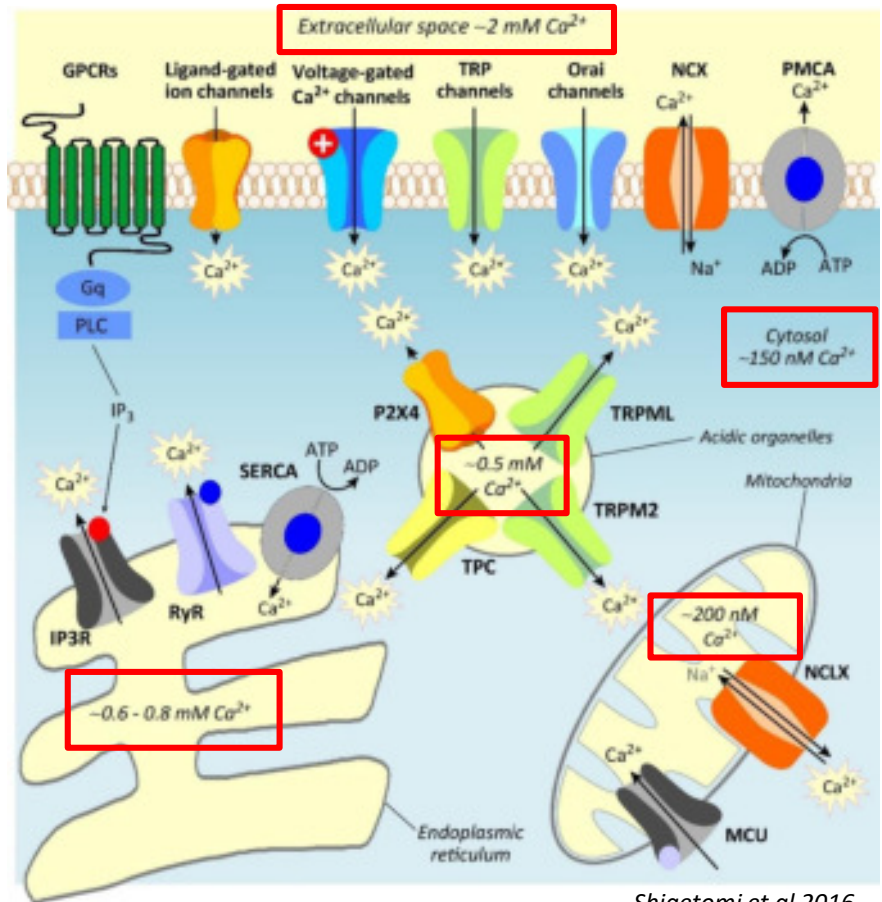
Protein kinases/phosphatases

Gene expression (cell growth, differentiation, death)

Membrane excitability

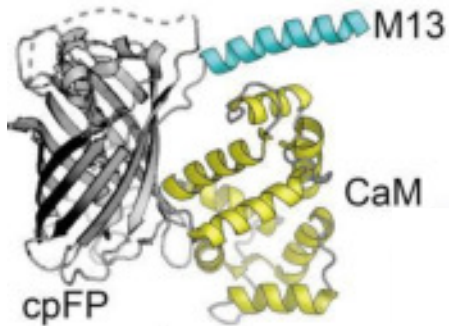
Mitochondria

Cell motility/muscle contraction



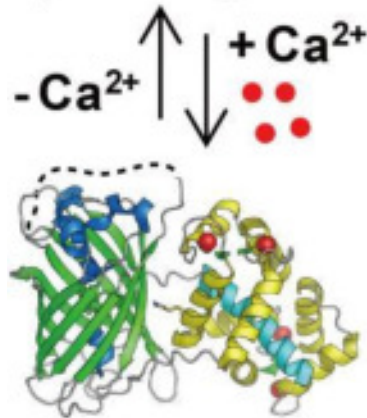
Shigetomi et al 2016

STUDY OF Ca^{2+} OSCILLATIONS IN THIN PROCESSES with Genetically Encoded Calcium Indicators (GECI)

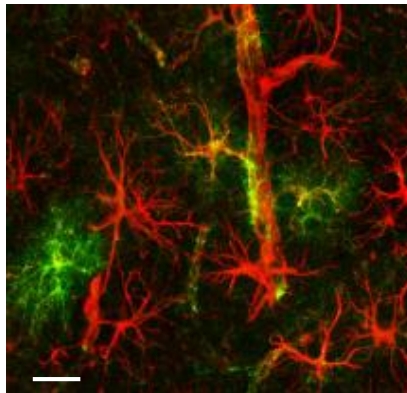


GCaMP6f

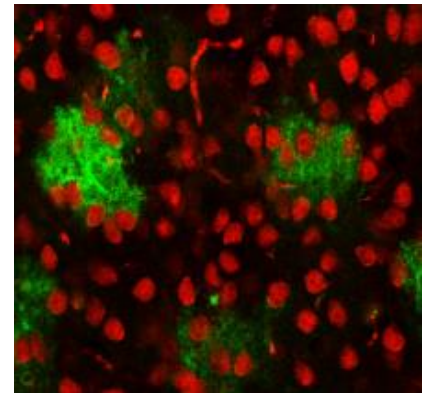
Kd 88 nM @ 37°C



AAV5.GfaABC1D.GCaMP6f



GFAP
GCaMP6f



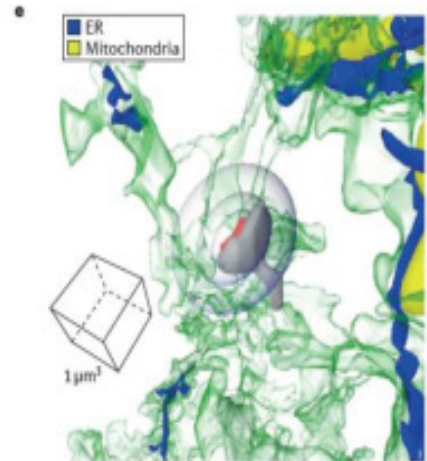
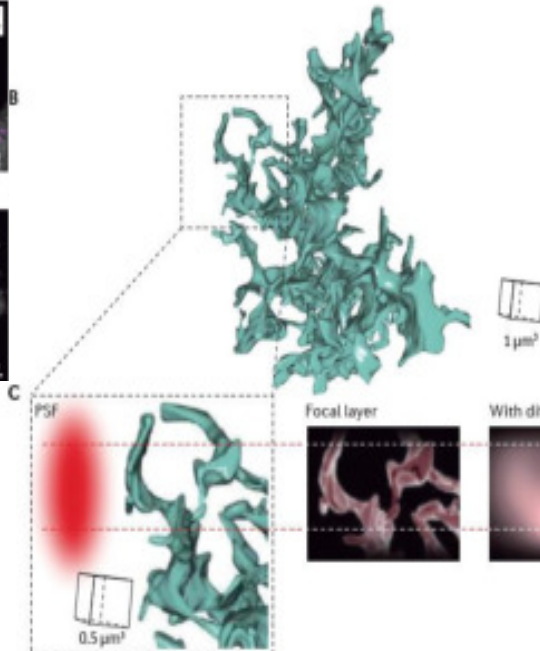
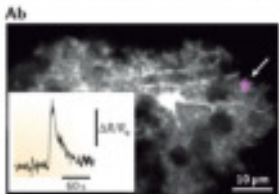
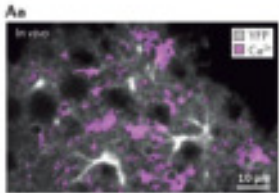
NeuN
GCaMP6f

THIN PROCESSES ARE NANOSCOPIC

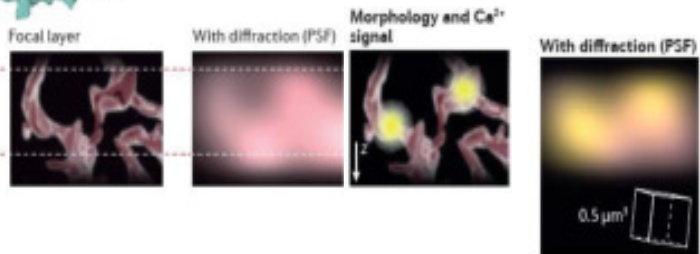
OPINION

Disentangling calcium-driven astrocyte physiology

Dmitri A. Rusakov



D. Rusakov 2015



Ca²⁺ ACTIVITY IN ASTROCYTES

WHAT...

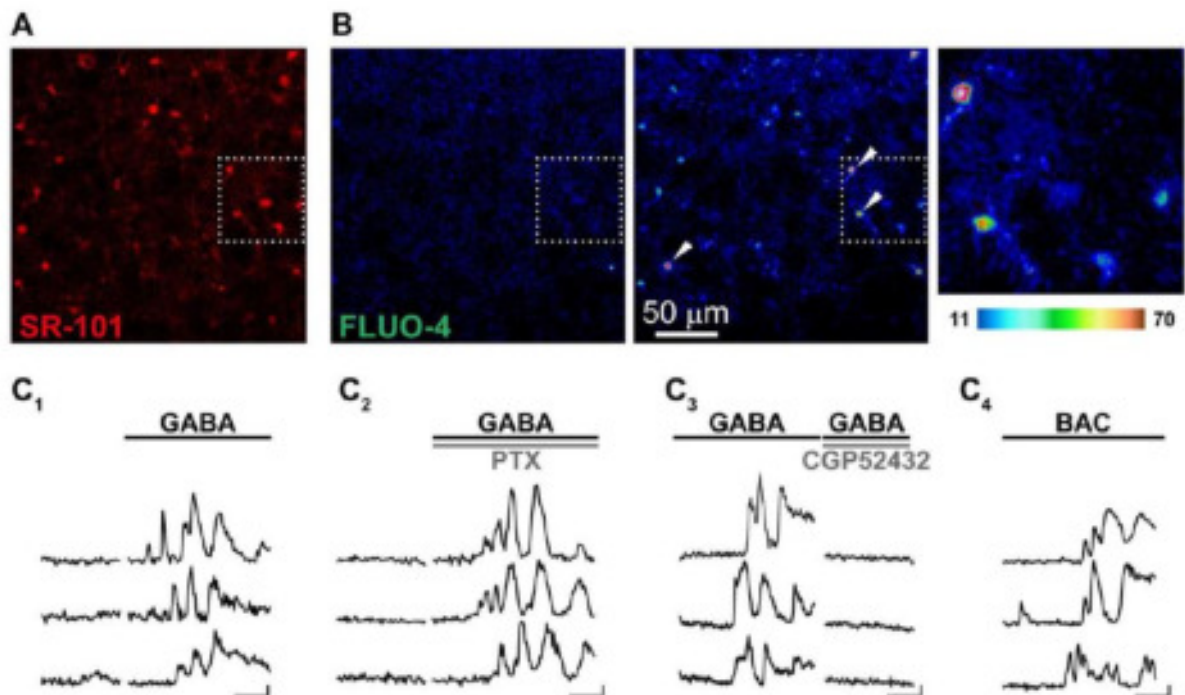
GABAergic signaling in
somatosensory and
visual cortex

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)

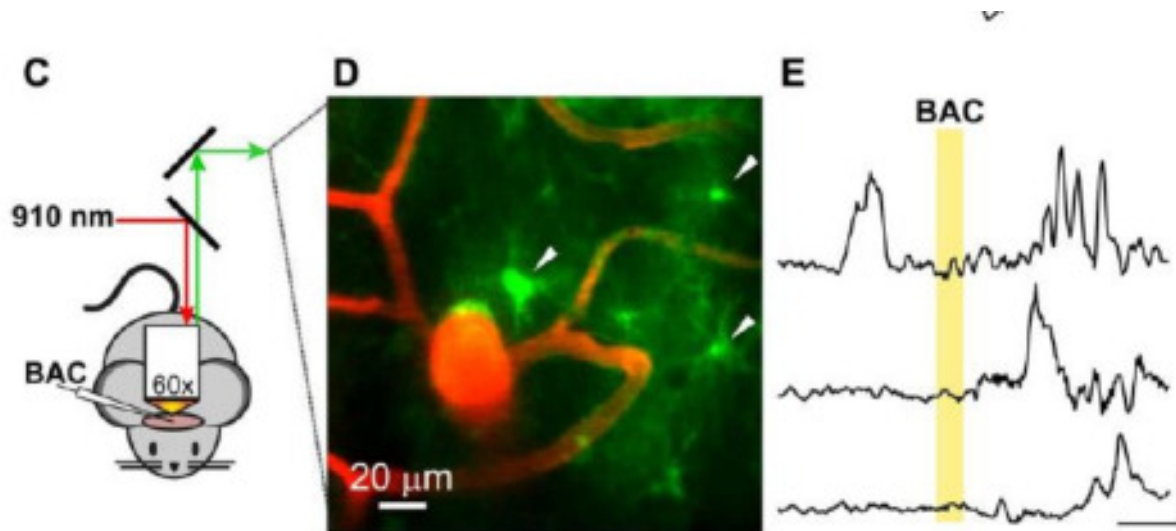
The Inhibitory Neurotransmitter GABA Evokes Long-Lasting Ca^{2+} Oscillations in Cortical Astrocytes

Letizia Mariotti, Gabriele Losi, Michele Sessolo, Iacopo Marcon,
and Giorgio Carmignoto



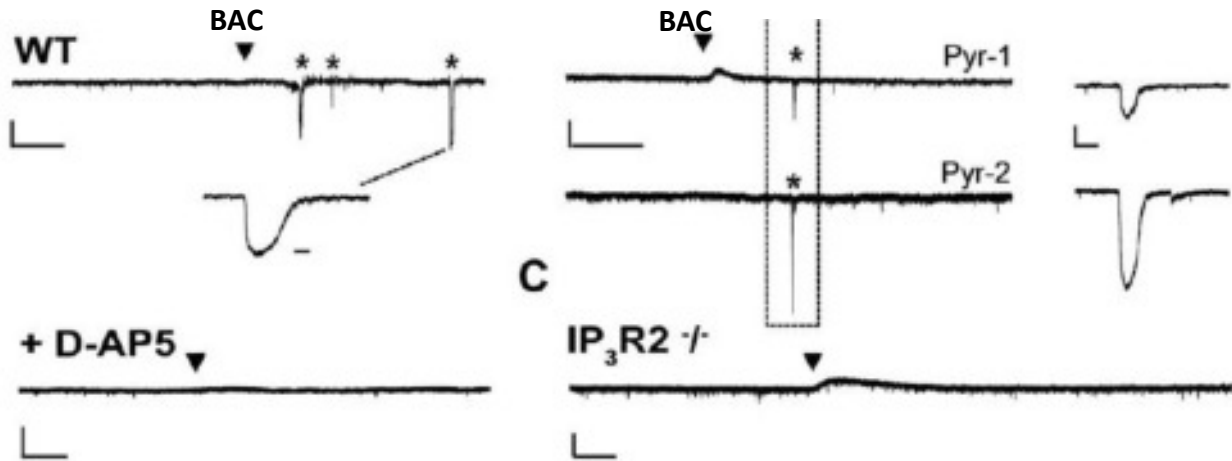
The Inhibitory Neurotransmitter GABA Evokes Long-Lasting Ca^{2+} Oscillations in Cortical Astrocytes

Letizia Mariotti, Gabriele Losi, Michele Sessolo, Iacopo Marcon,
and Giorgio Carmignoto

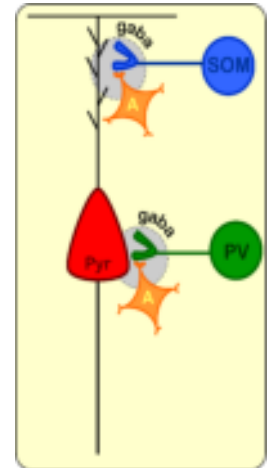
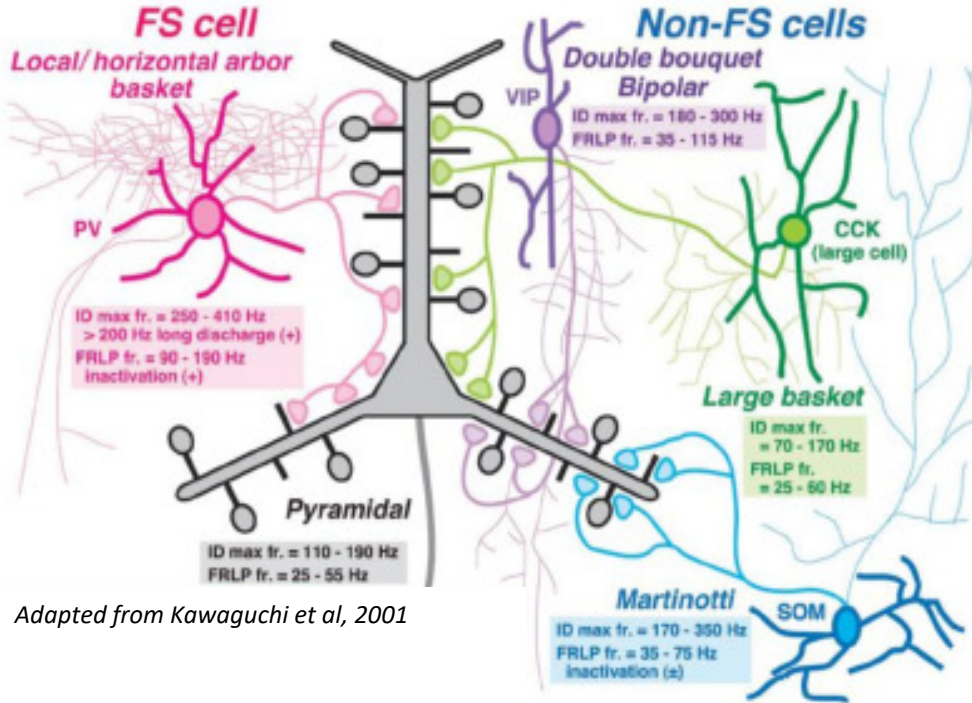


GABA_B RECEPTOR ACTIVATION EVOKES CALCIUM TRANSIENTS AND GLUTAMATE RELEASE FROM ASTROCYTE

Patch-clamp from pyramidal neurons



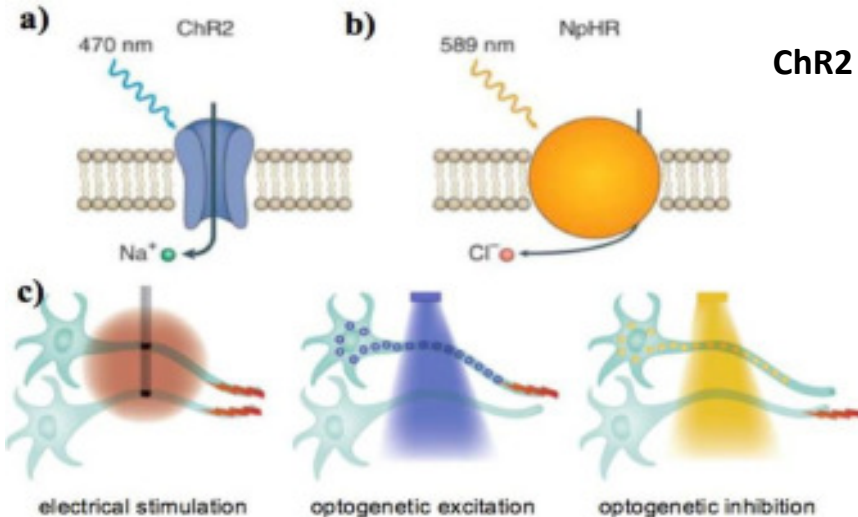
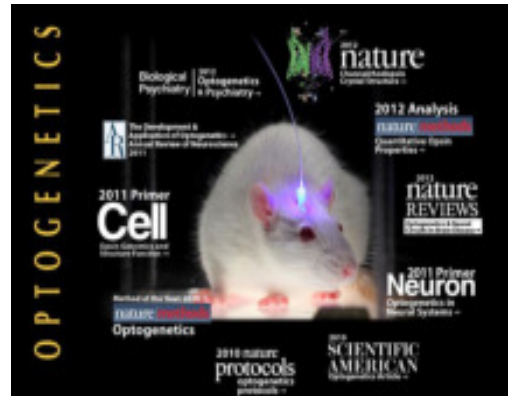
ASTROCYTE RESPONSE TO SPECIFIC GABAERGIC INTERNEURONS



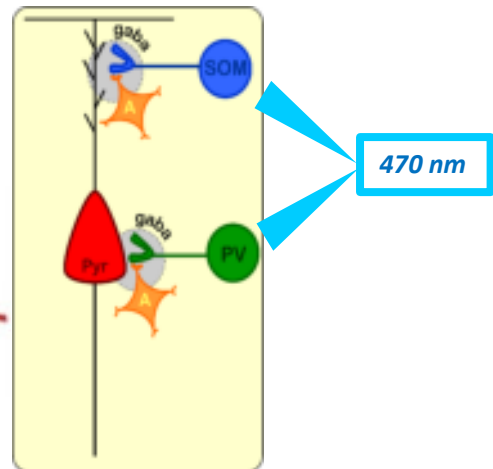
Adapted from Kawaguchi et al, 2001

OPTOGENETICS: LIGHT GATED ION CHANNELS

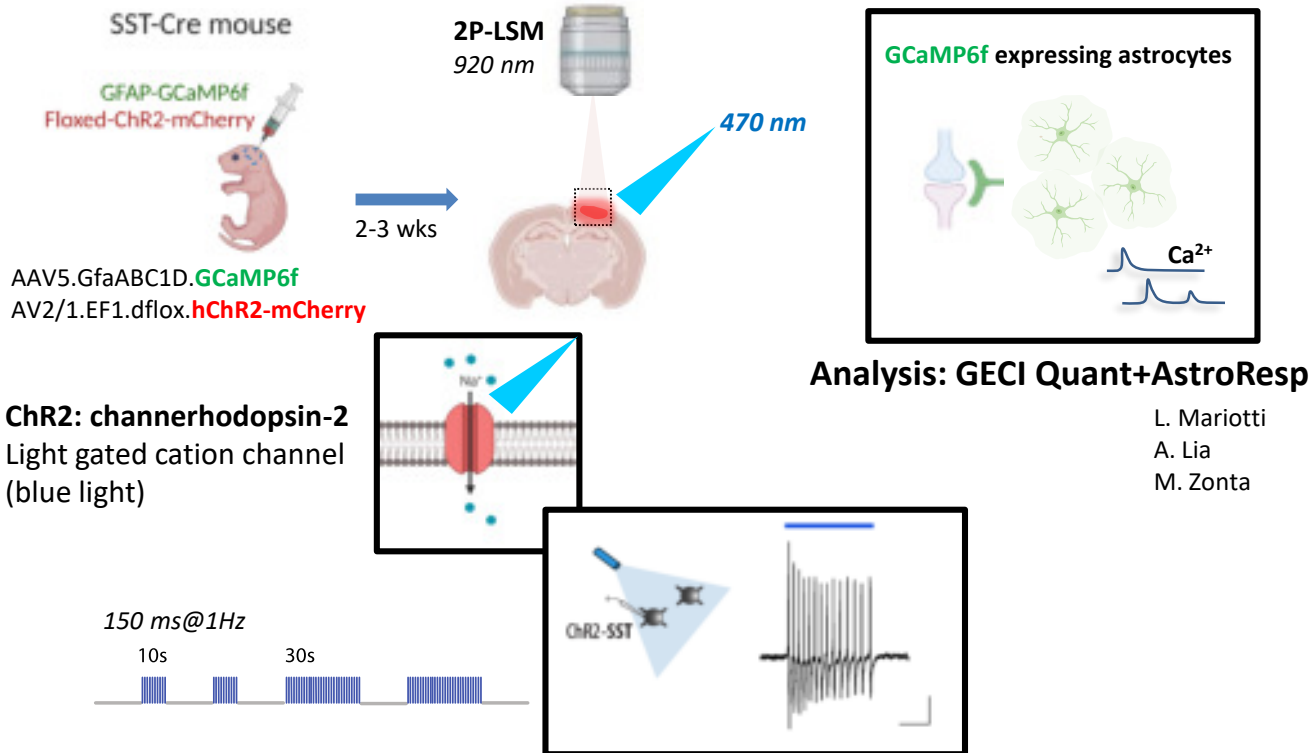
ChR2: channelrhodopsin-2
Light gated cation channel (blue light)



ChR2 expressed on one cell type only



OPTOGENETIC STIMULATION OF SELECTIVE NEURONAL POPULATIONS. SOMATOSTATIN or PARVALBUMIN INTERNEURONS



OPTOGENETIC PV- or SST-INTERNEURON STIMULATION *in vivo*

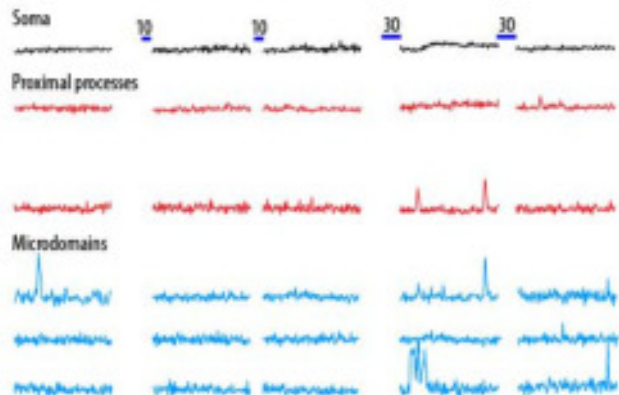
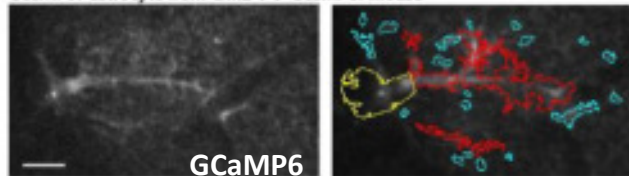
Mariotti, Losi et al 2018, Nat. Comm.

ChR2-PV

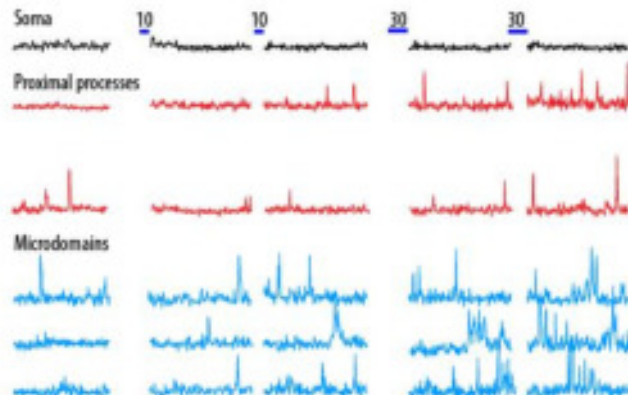
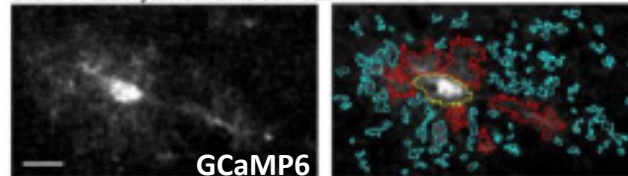
Response is mediated by $GABA_B$ receptors

ChR2-SST

GCaMP6f-astrocyte from ChR2-PV-GCaMP6f mouse



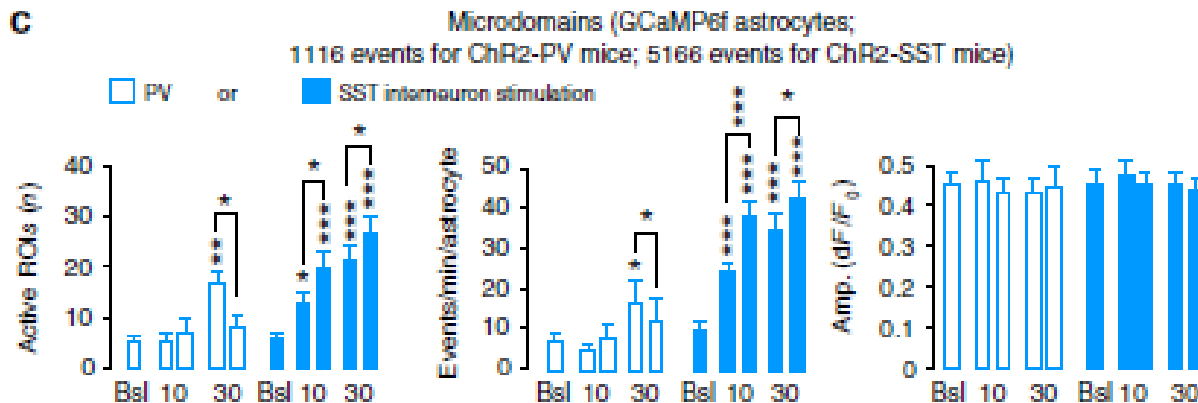
GCaMP6f-astrocyte from ChR2-SST-GCaMP6f mouse



20% DF/F0
5 s

OPTOGENETIC PV- or SST-INTERNEURON STIMULATION EVOKES DISTICT RESPONSE IN ASTROCYTES

ASTROCYTE RESPONSE TO SST-INs IS POTENTIATING



Evoked firing is constant

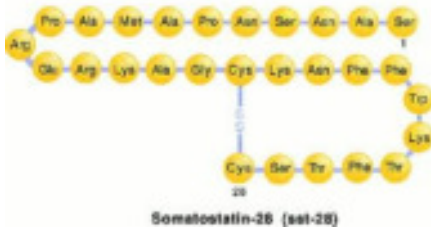
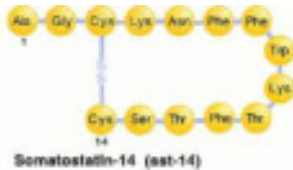
Evoked currents are constant

WHICH MECHANISM FOR POTENTIATION?

SST-INTERNEURONS ALSO RELEASE SOMATOSTATIN

Also known as **SRIF** (somatotropin release inhibiting factor)

Large dense-core vesicles
in axon, soma, dendrites



Periphery and hypothalamus: reduces hormone release

Brain: reduces NTs release, favors hyperpolarization,

Protective and favors cognitive functions

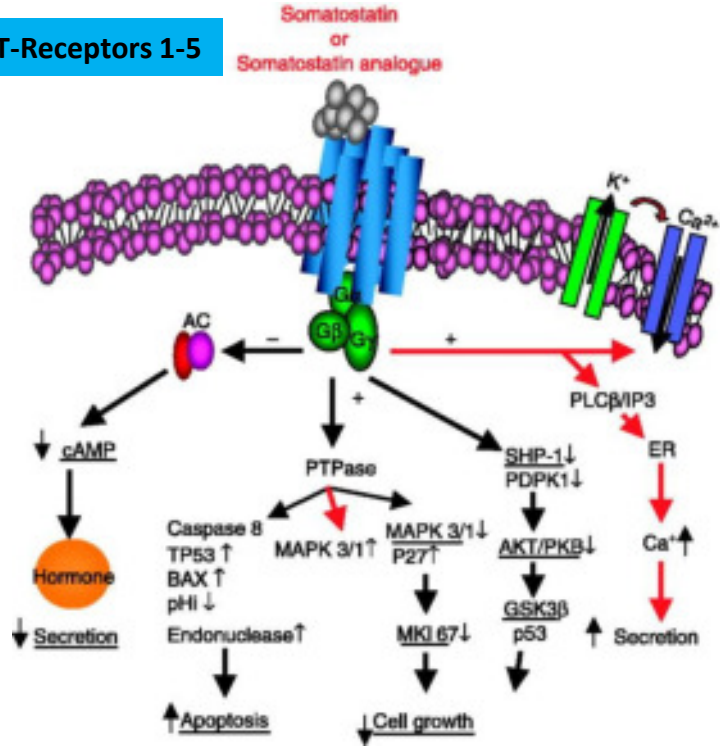
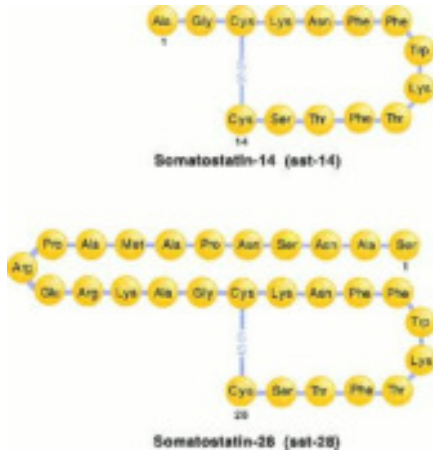
REDUCED IN AGEING AND ALZHEIMER'S DISEASE

SOMATOSTATIN EFFECTS ARE COMPLEX AND POORLY UNDERSTOOD IN BRAIN CORTEX

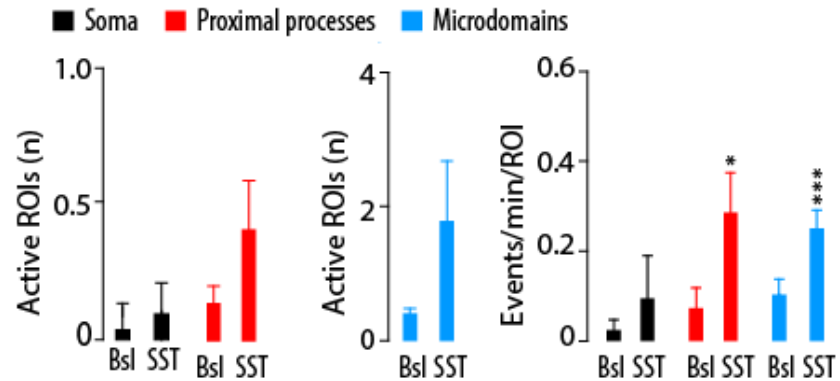
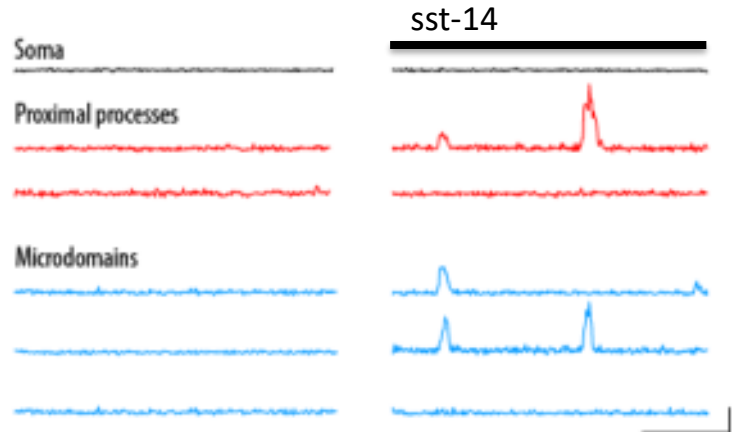
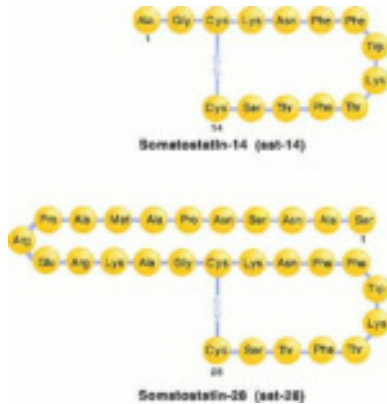
Large dense-core vesicles in axon, soma, dendrites

SST-Receptors 1-5

Somatostatin or Somatostatin analogue

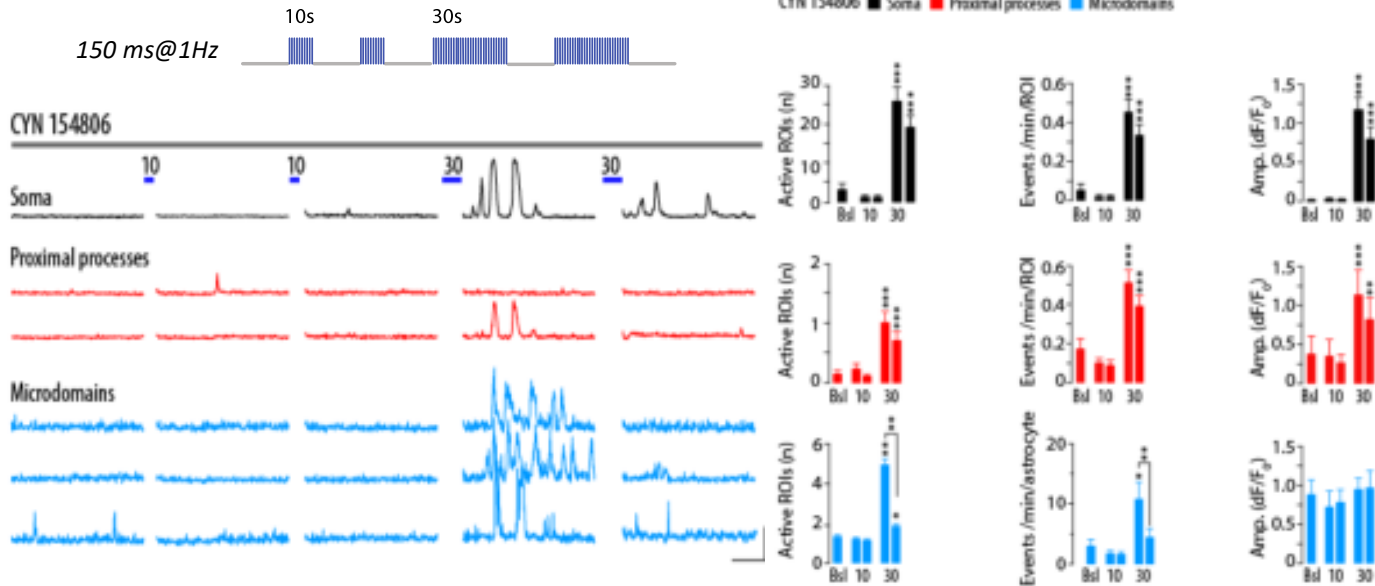


SOMATOSTATIN INCREASES CALCIUM EVENTS IN CORTICAL ASTROCYTES IN SITU



ASTROCYTIC POTENTIATING RESPONSE TO SST-INTERNEURONS DEPENDS ON SST-RECEPTOR ACTIVATION

Mariotti, Losi et al 2018, Nat. Comm.



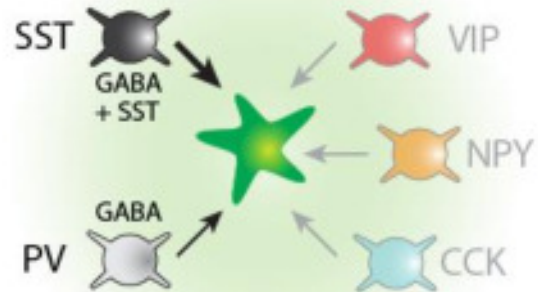
CONCLUSIONS-1

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 neuropeptides.



OPTOGENETIC SST-INTERNEURON STIMULATION IN VISUAL CORTEX



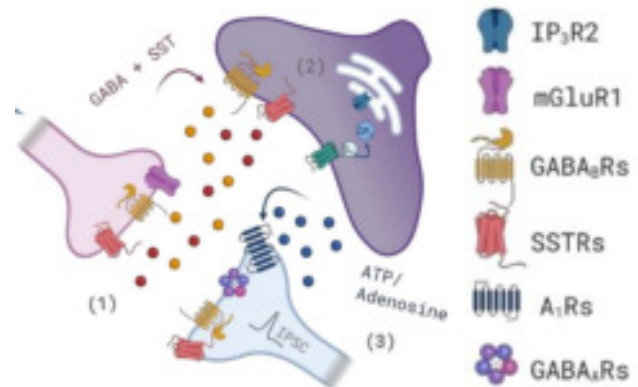
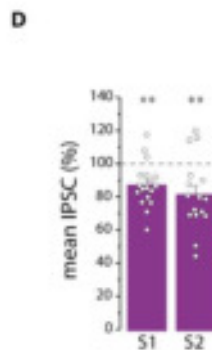
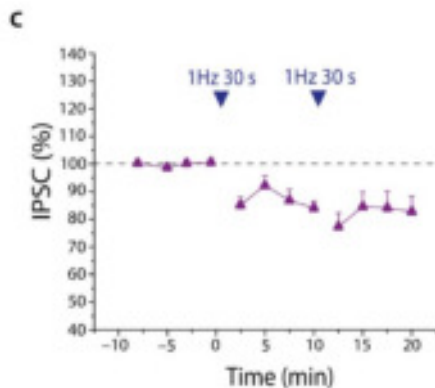
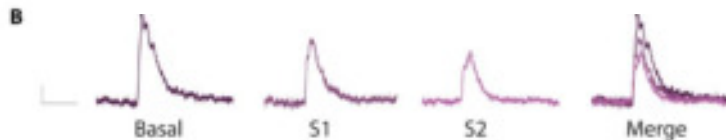
May 2022



Article

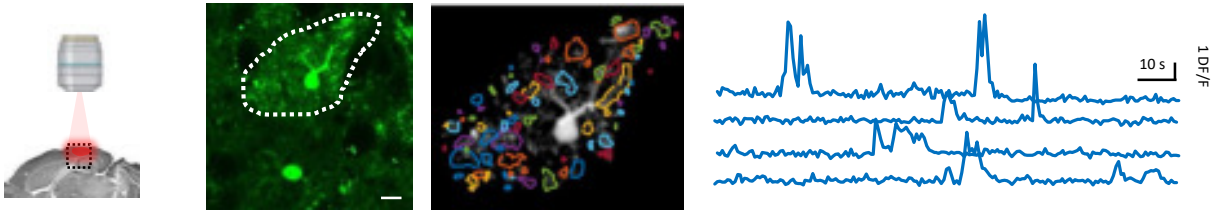
Astrocytes Modulate Somatostatin Interneuron Signaling in the Visual Cortex

Vanessa Jorge Henriques^{1,2}, Angela Chiavegato², Giorgio Carmignoto^{1,2,*} and Marta Gómez-Gonzalo^{1,2,*,†}



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

2-Photon Laser Scanning Microscopy on brain tissue



DEVELOPMENT OF NEW ANALYTICAL TOOLS:

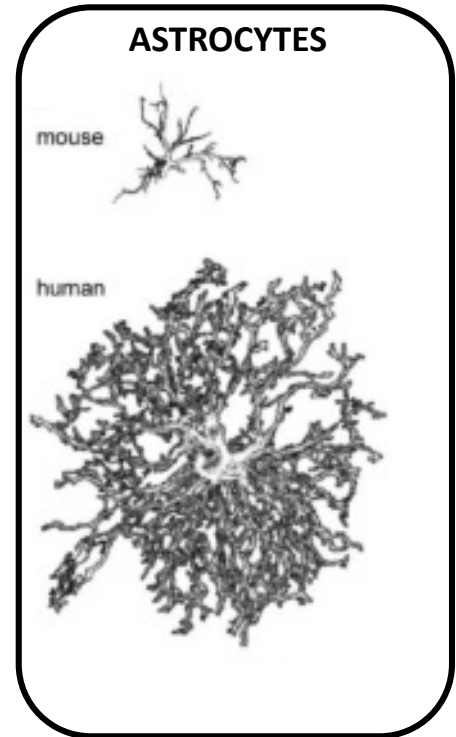
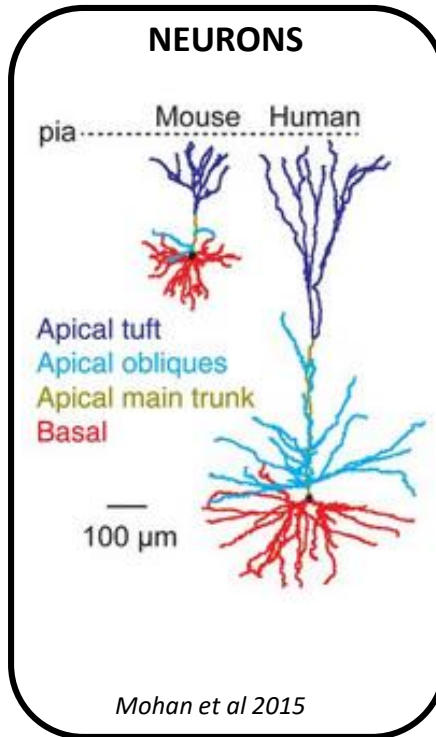
- AstroRespNew (G. Carmignoto's Lab, CNR-IN, Padova)
- Image Gateway (G. Ratto's Lab. , CNR-NANO, Pisa)

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**



ONGOING AND FUTURE STUDIES

Astrocyte functions in brain physiology: and pathology:

- **LTP and memory**
- **Alzheimer's Disease**
- **Neuroinflammation**

Mechanisms of Seizure generation

Dravet Syndrome

BIO @ NANO

<https://bio.nano.cnr.it/>

**Giorgio
Carmignoto**

Letizia Mariotti

**Marta Gomez-
Gonzalo**

Micaela Zonta

Angela Chiavegato

Annamaria Lia

Rosa Chiara Goisis

Vanessa Henriques

Michele Speggiorin

Michele Sessolo



DSBMN-UNIMORE: **DSV-UNIMORE:**

*Michele Zoli
Jonathan Mapelli
Rita Bardoni
Antonietta Vilella
Paolo Pozzi
Miriam Cavagnini*

*Giulia Puja
Silvia Alboni
Rossella Avallone*



**Marco Canossa,
Beatrice Vignoli**
Univ. Trento

**Gianmichele Ratto
Rocco Granata**
CNR-NANO, Pisa

Fiorenzo Conti
*Università Politecnica delle
Marche, Ancona*

Tommaso Fellin
IIT Genova

Daniela Puzzo
Univ. Catania

Claudio Grassi
*Univ. Cattolica Sacro Cuore,
Roma*

Marco DeCurtis
*Ist. Neurol. C.
Besta, Milano*
Stefano Vicini
*Georgetown University
Washington DC, USA*