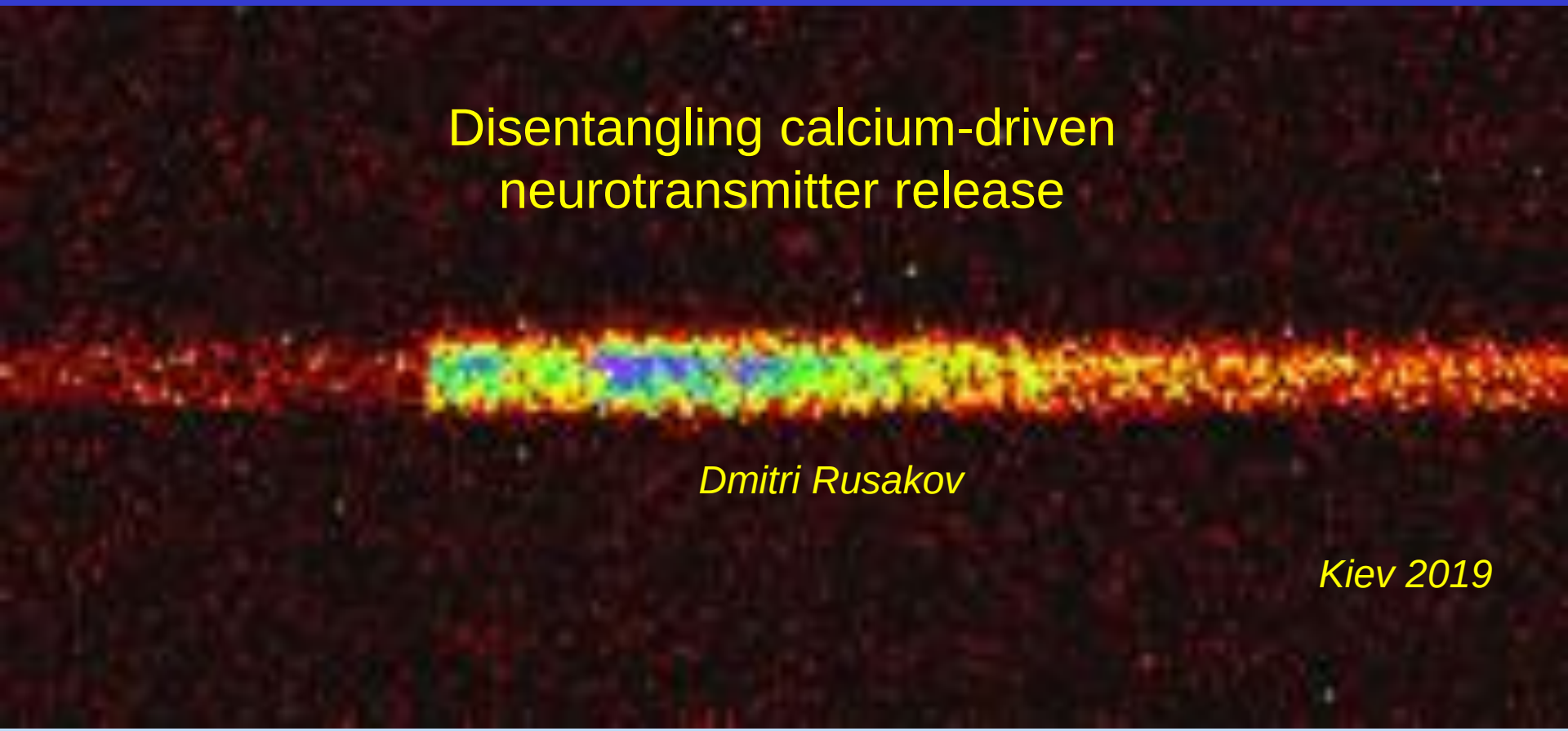
Disentangling calcium-driven neurotransmitter release



Dmitri Rusakov

Kiev 2019

Queen Square
Institute of Neurology

University College London

Neurotransmitter release is controlled by presynaptic Ca^{2+} entry

It is also regulated by nanomolar *resting* Ca^{2+}, which in turns depends on the history of cell firing

In the past, giant-synapse preparations provided fundamental insights into the machinery of release

What controls release probability and its dynamics at small central synapses remains poorly understood

Technological revolution – genetically encoded optical sensors for glutamate

nature methods

Article | Published: 13 January 2013

An optimized fluorescent probe for visualizing glutamate neurotransmission

Jonathan S Marvin, Bart G Borghuis, Lin Tian, Joseph Cichon, Mark T Harnett, Jasper Akerboom, Andrew Gordus, Sabine L Renninger, Tsai-Wen Chen, Cornelia I Bargmann, Michael B Orger, Eric R Schreiter, Jonathan B Demb, Wen-Biao Gan, S Andrew Hires & Loren L Looger

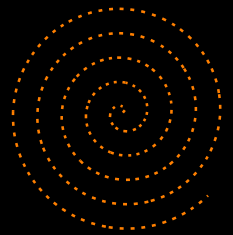
Monitoring quantal release: from EPSCs (glorious past) to glutamate imaging (challenging future)

Detecting quantal releases at CA3-CA1 synapses: optical glutamate sensor

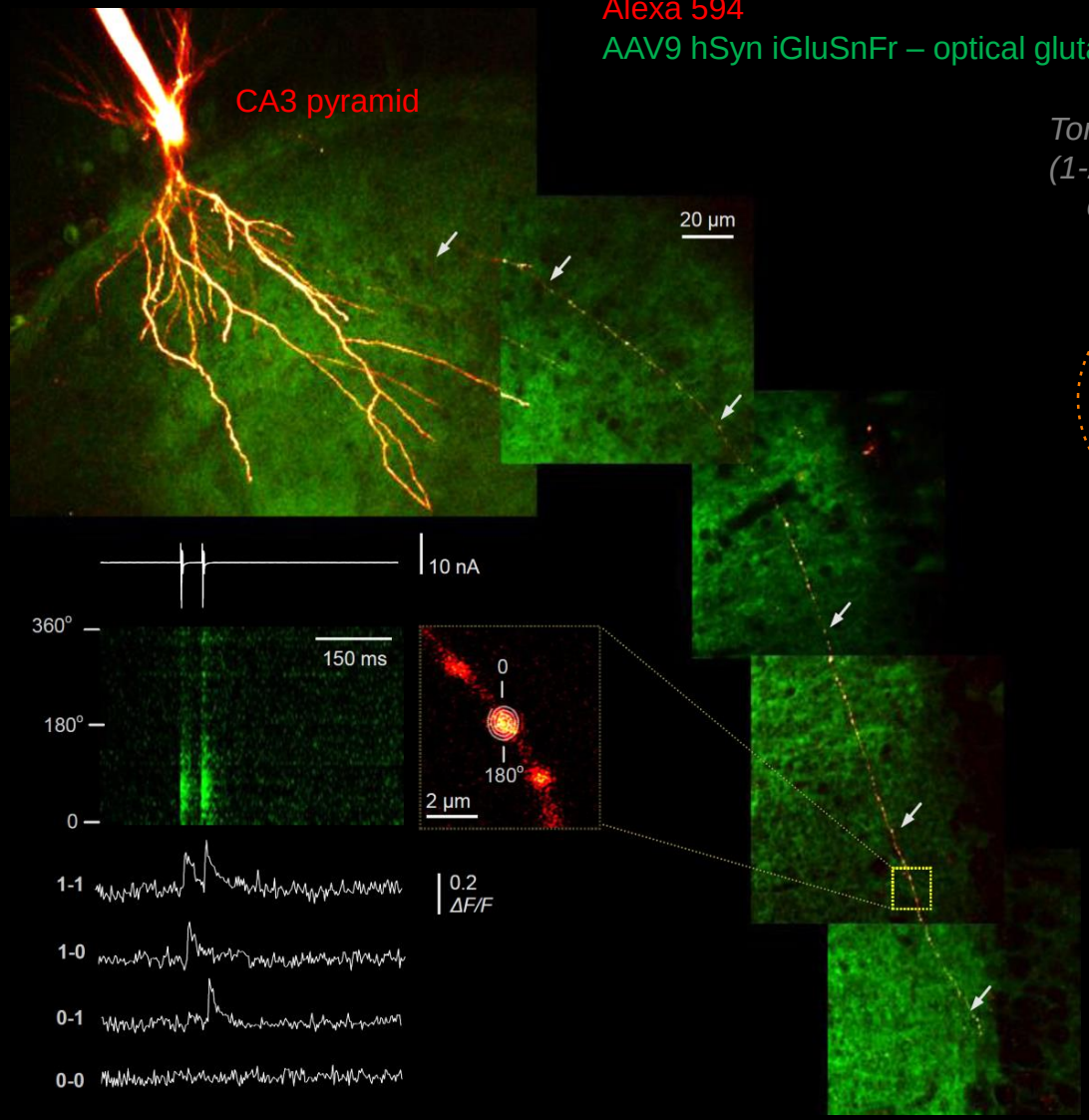
Thomas Jensen
James Reynolds

Alexa 594
AAV9 hSyn iGluSnFr – optical glutamate sensor

Tornado linescan (1-2 ms to cover circular area)

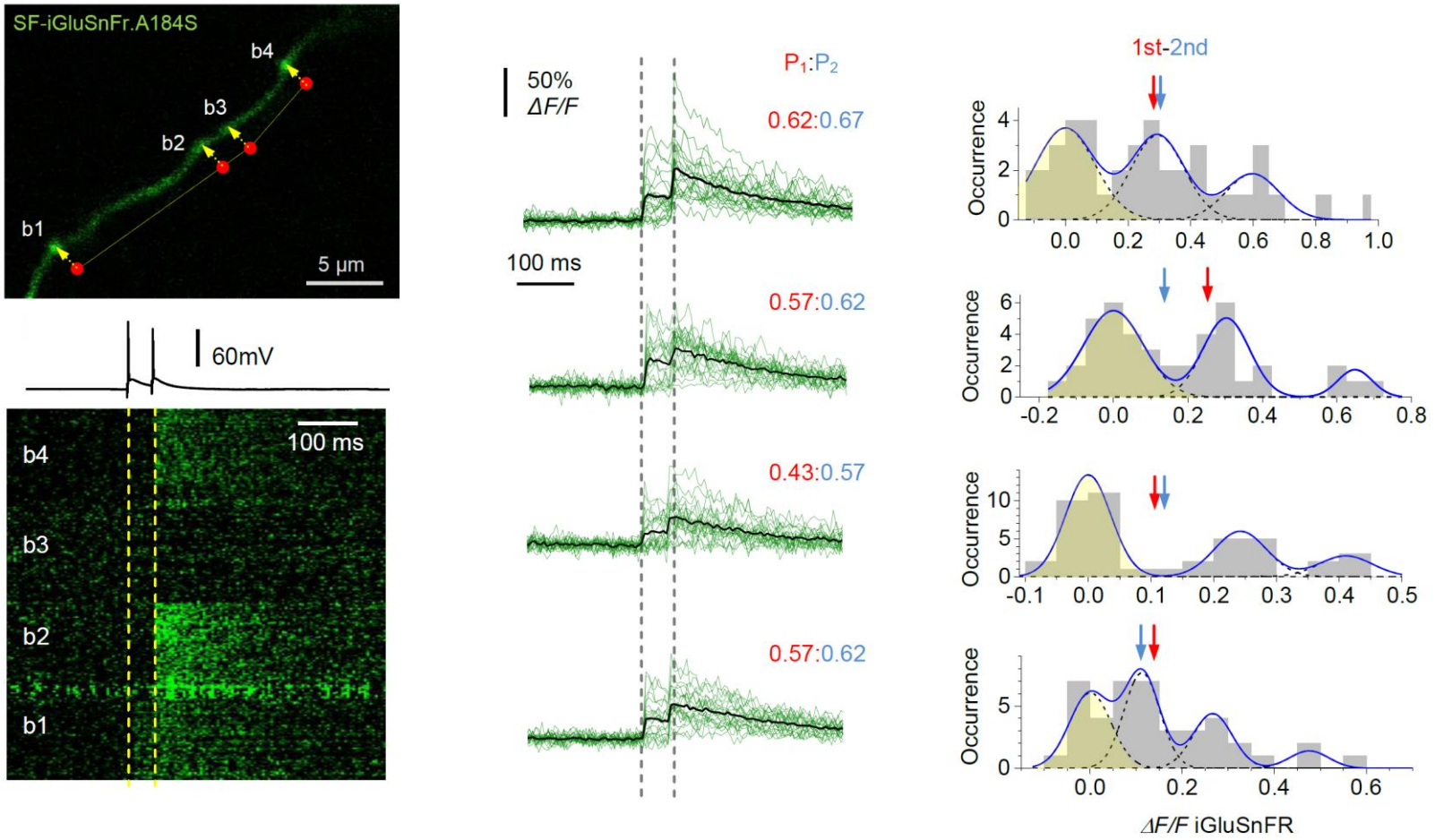


Tornado linescan



Monitoring glutamate release at multiple synapses

Organotypic hippocampal slices - Biolistic transduction with SF-iGluSnFr.A184S



The approach enables quantal analyses at multiple synapses

What about calcium?

Ratiometric fluorescence dyes pioneered in the 1970s enabled $[Ca^{2+}]$ monitoring in cultured cells over the physiological range (30-1000 nM)

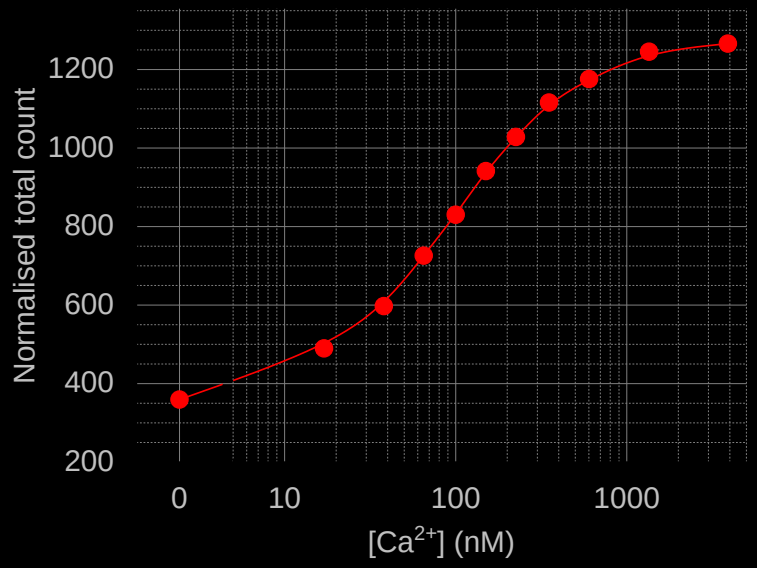
However, this method has basic limitations when applied in scattering tissue and during rapid $[Ca^{2+}]$ changes

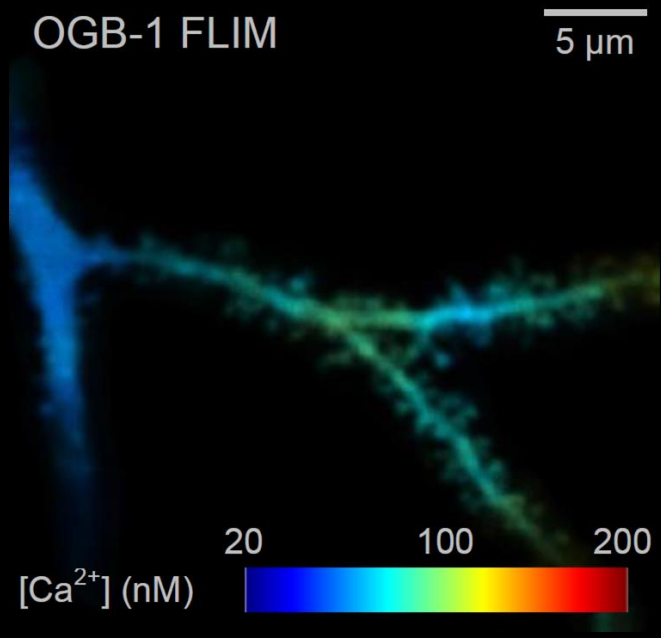
Thus, most imaging studies *in situ* have opted for intensity measurements ($\Delta F/R$, etc.)

which are, however, difficult to translate into $[Ca^{2+}]$, especially below ~100 nM

Reporting nanomolar Ca^{2+} in neuronal processes using FLIM of OGB-1

OGB-1 Ratiometric photon count readout vs $[Ca^{2+}]$





CA1 pyramidal cell , acute hippocampal slice

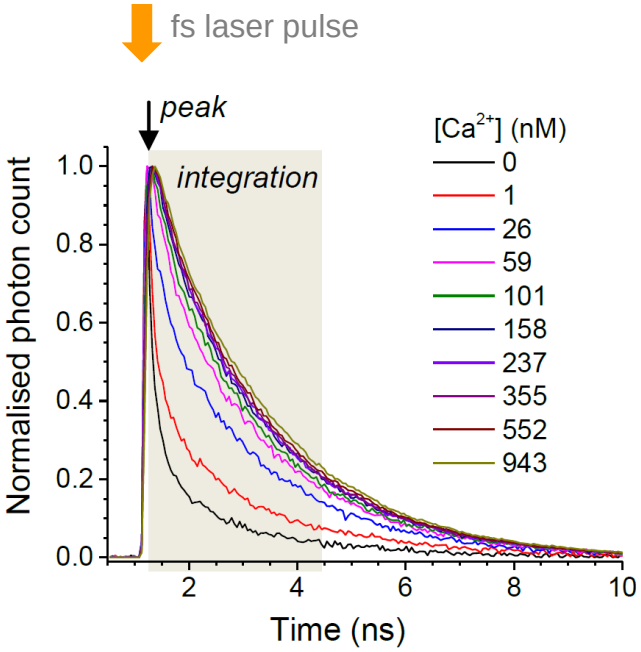
Zheng et al, *Neuron* 2015, *Nat Prot* 2018

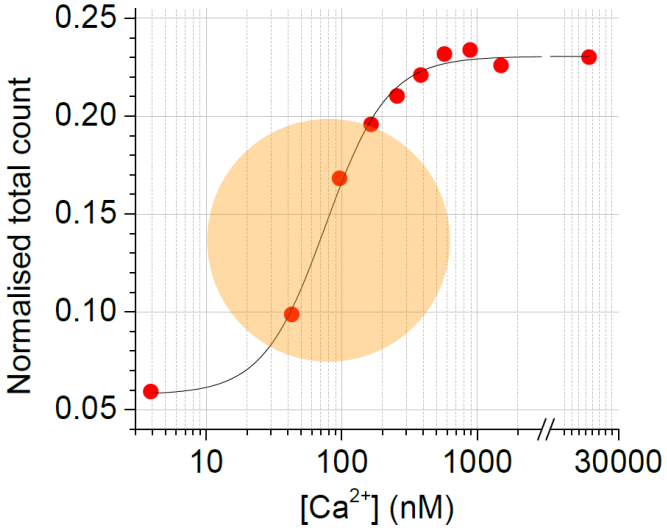
Challenge

Emission bands of OGB-1 and iGluSnFr (green dyes) are chromatically inseparable, precluding simultaneous monitoring of Ca^{2+} and glutamate

Is there a red-shifted Ca^{2+} indicator with Ca^{2+}-sensitive FLIM readout?

Cal-590





Cal-590 FLIM readout shows strong Ca^{2+} sensitivity in the 20-200 nM range (*in vitro* and *in situ*)

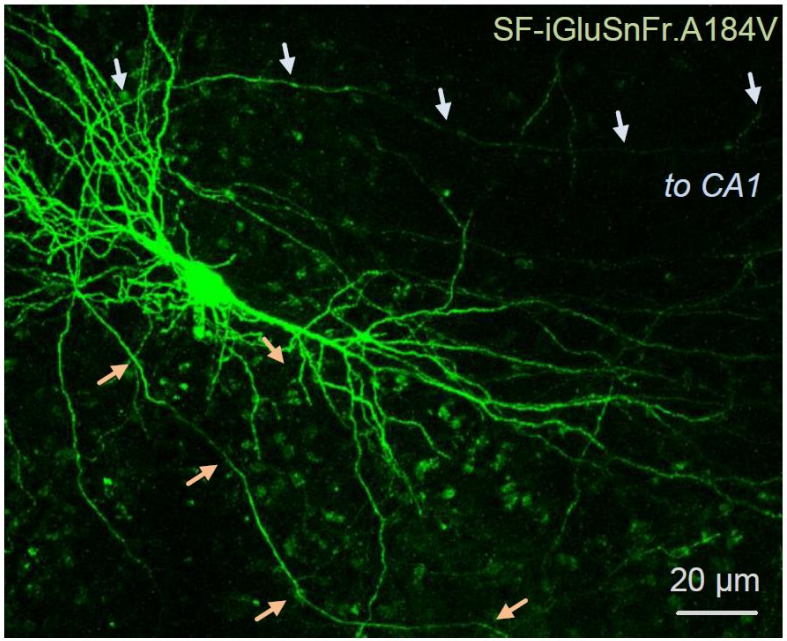
Jensen et al, *Nat Commun* 2019

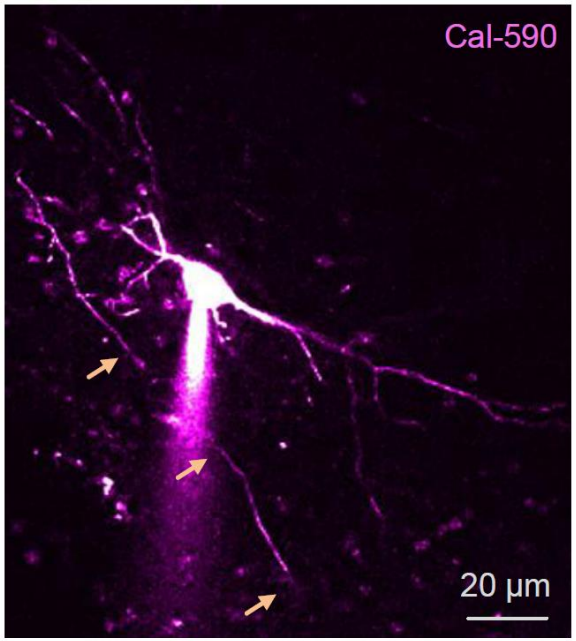
Multiplex SF-iGluSnFr.A184S/V – Cal-560 imaging

(CA3-CA1 connections in organotypic hippocampal slices)

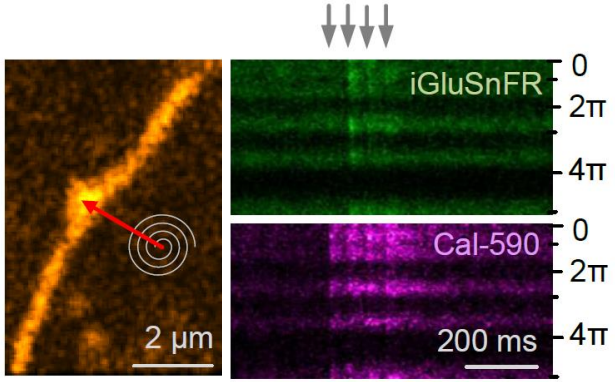
Biolistic transduction with SF-iGluSnFr.A184V

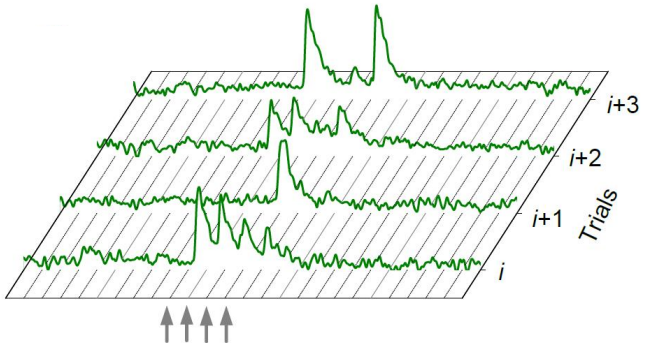
Whole-cell Cal-590 dialysis and axon tracing into CA1



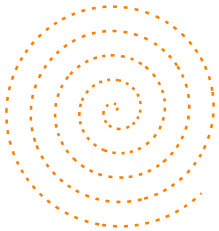


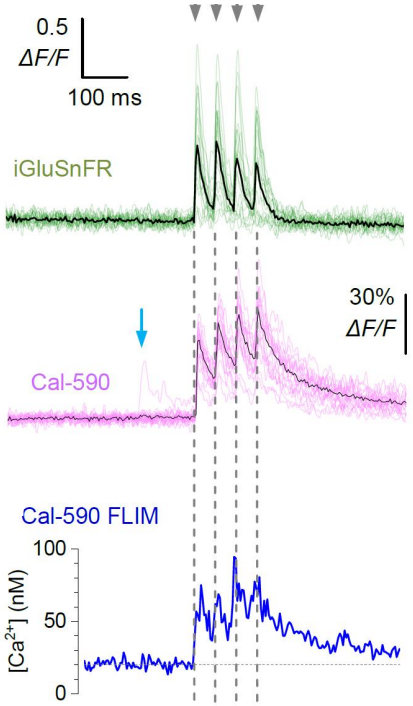
Multipex glutamate- presynaptic Ca^{2+} imaging

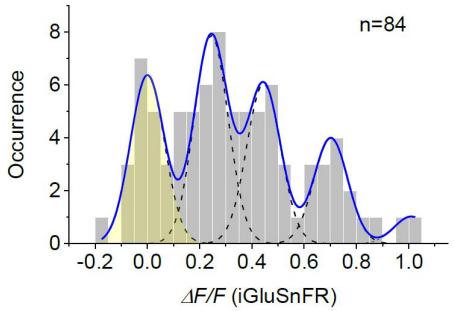


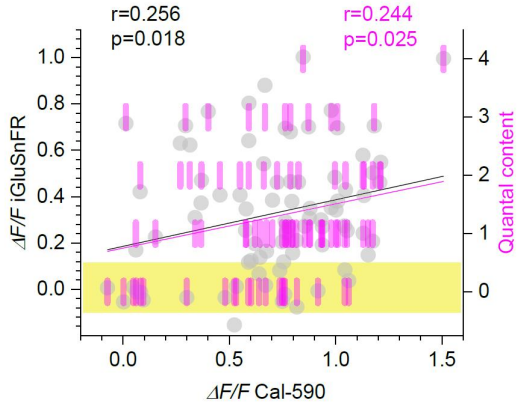


Tornado linescan (1-2 ms to cover circular area)



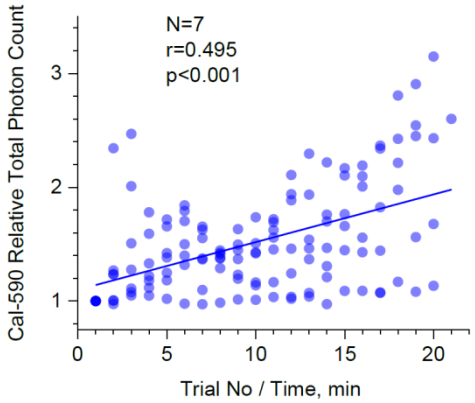




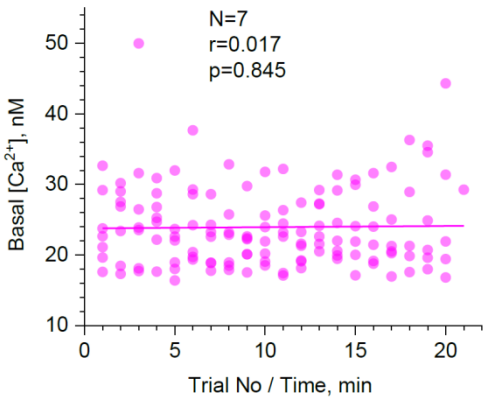


Testing the Ca^{2+} buffering effect of Cal-590 on release probability P_r

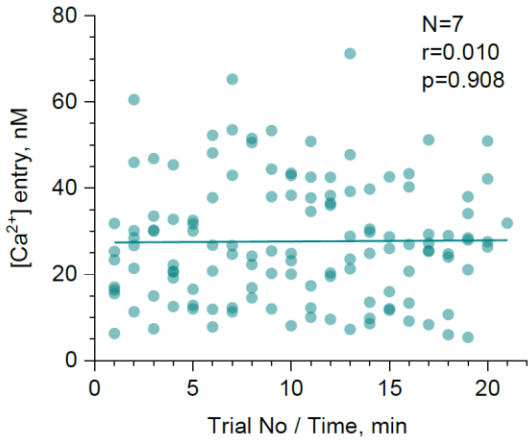
Shortly after whole-cell break-in, axonal [Cal-590] steadily increases



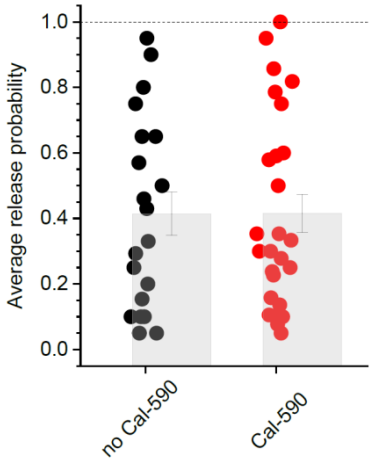
Basal presynaptic $[Ca^{2+}]$



Evoked presynaptic Ca^{2+} entry

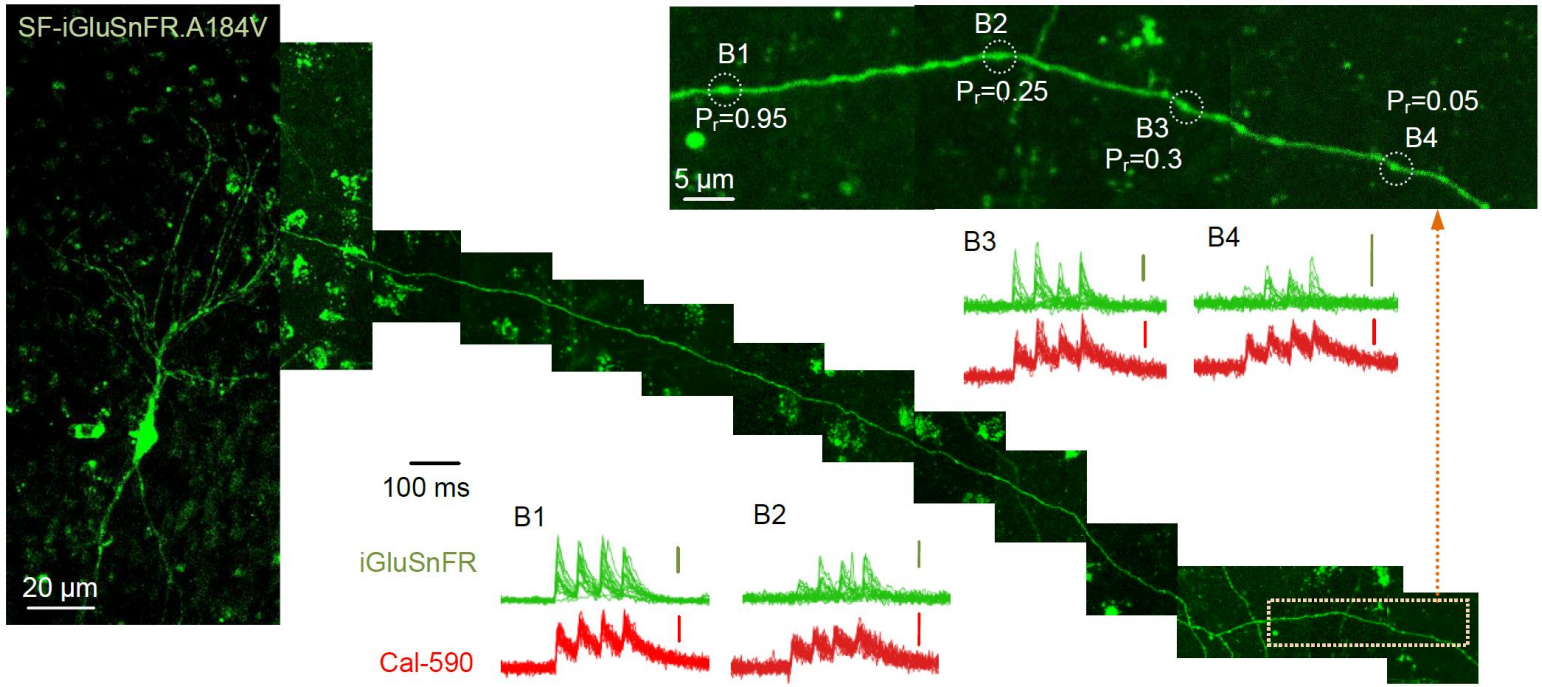


Average P_r



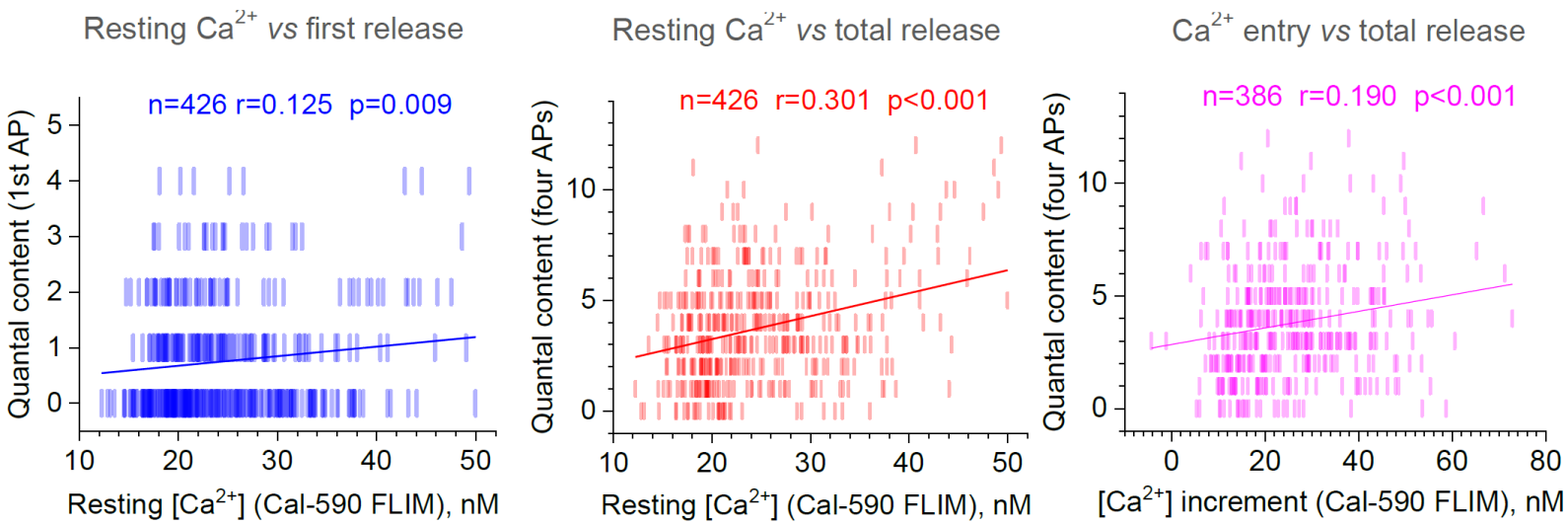
No detectable effect of Cal-590 dialysis on P_r

Collecting multiplex data from individual synapses



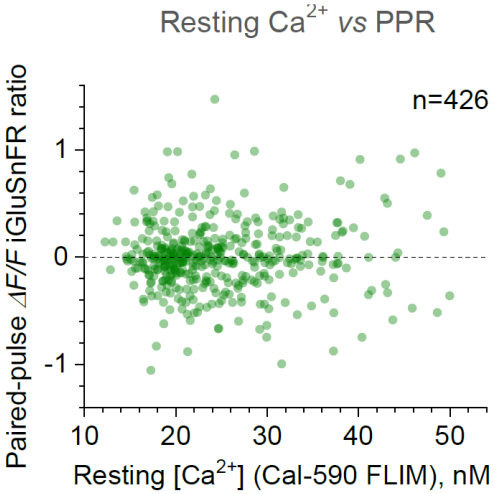
Jensen et al, *Nat Commun* 2019

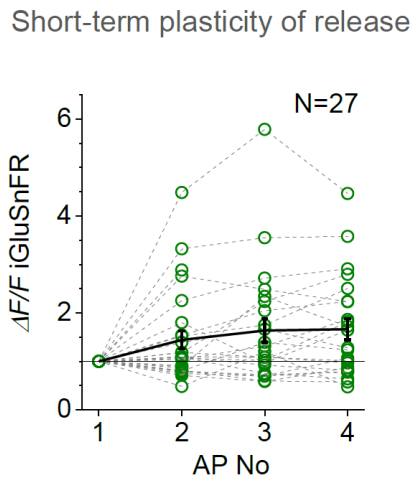
Fluctuations in presynaptic resting [Ca^{2+}] and evoked Ca^{2+} entry *versus* glutamate release

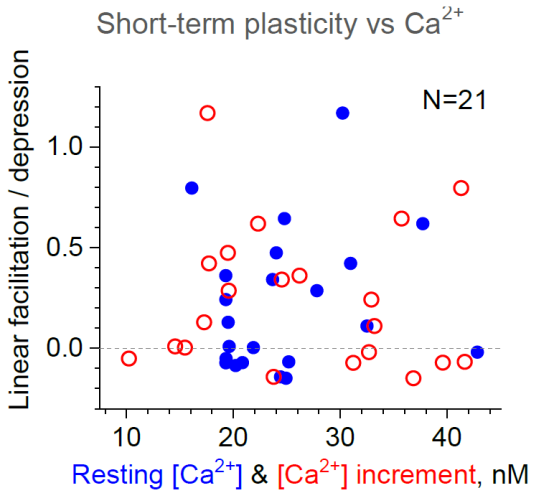


Vesicular release probability fluctuates with resting [Ca^{2+}] and evoked Ca^{2+} entry, from one event to the next

Does presynaptic resting [Ca^{2+}] affect short-term plasticity?







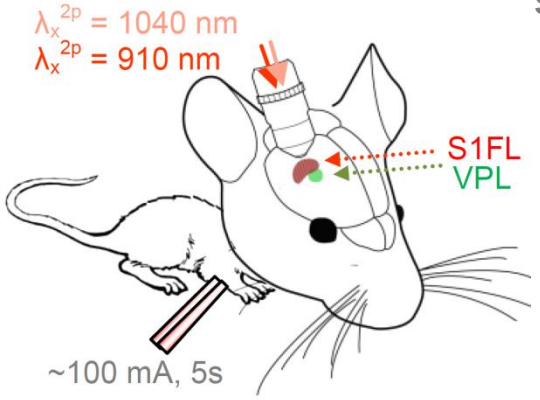
Fluctuations in resting [Ca^{2+}], from one event to the next, have no detectable effects on STP

Attempting to monitor synaptic efficacy *in vivo*

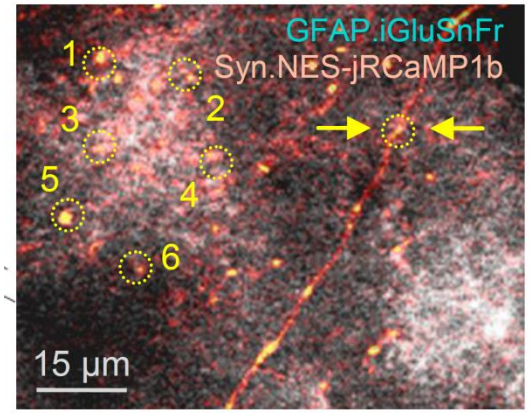
Viral transduction

Cortical astroglia
↓
GFAP.iGuSnF (green)

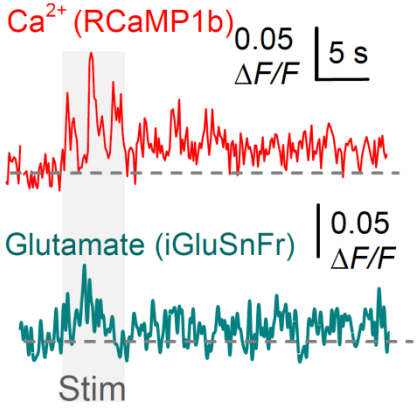
Somatosensoty cortex
↑
Syn.NES-jRCaMP1b (red)
Ventral posterolateral nucleus



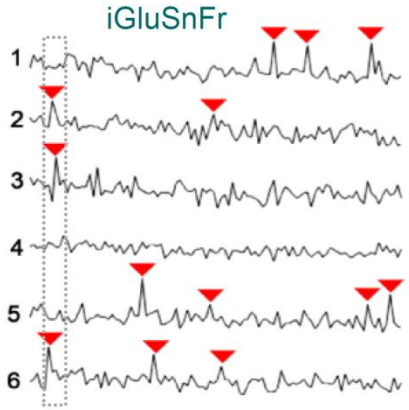
Somatosensory cortex, ~150 µm deep



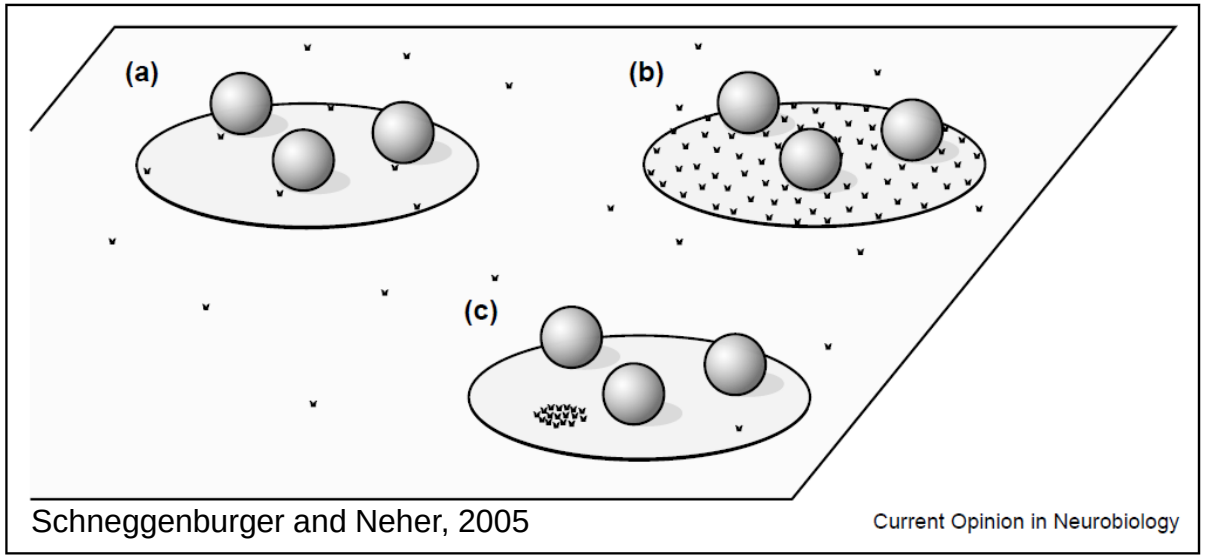
One-bouton evoked response



Spontaneous bouton responses



Are Ca^{2+} entry and glutamate release sites co-localised?



Synaptic vesicle release could be controlled by either

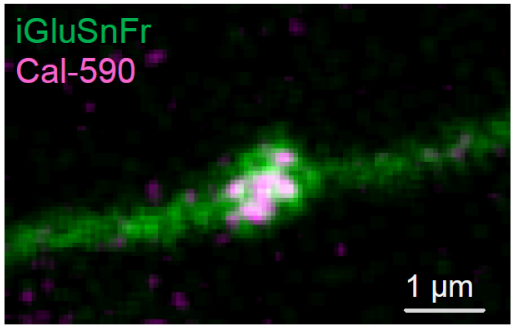
(a) a close association between individual Ca^{2+} channels and docking sites,

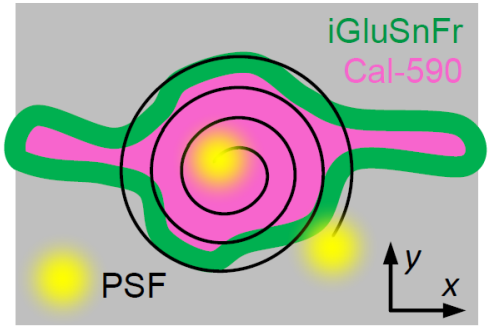
(b) overlapping Ca^{2+} domains affecting the vesicular pool, or

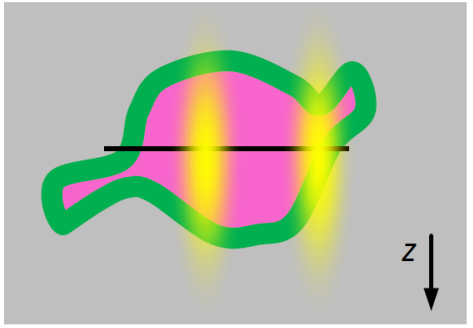
(c) a Ca^{2+}-channel cluster with a distance-dependent effect on release

Critical quest: to understand whether presynaptic Ca^{2+} entry and glutamate release sites are co-localised

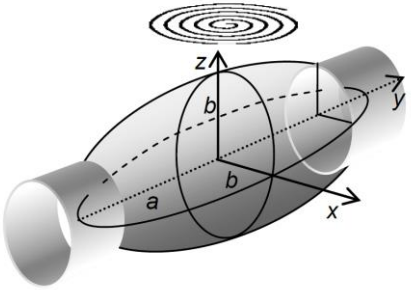
Experimental arrangement on the nanoscale

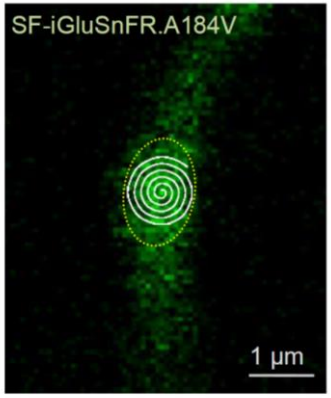


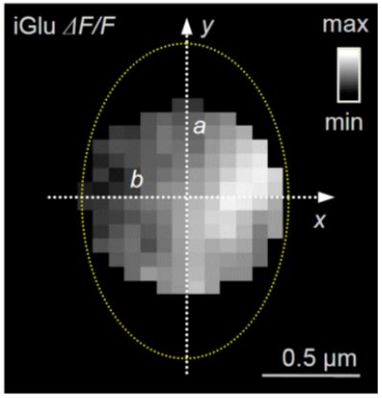


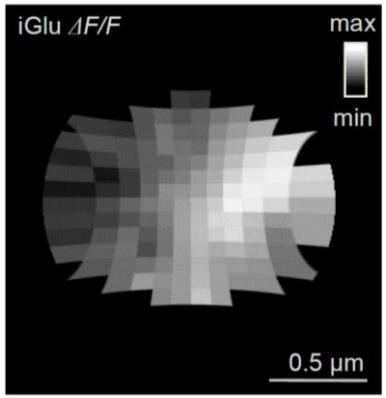


Glutamate signal - geodesic correction for lateral membrane distances

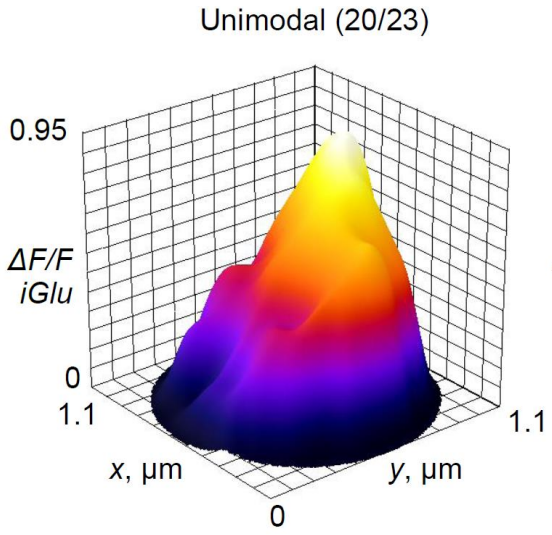


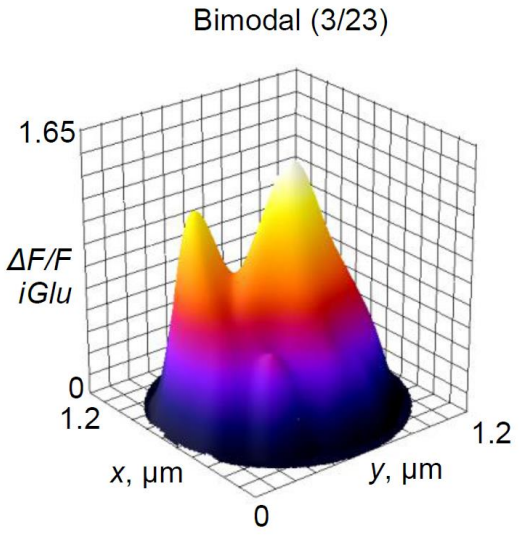


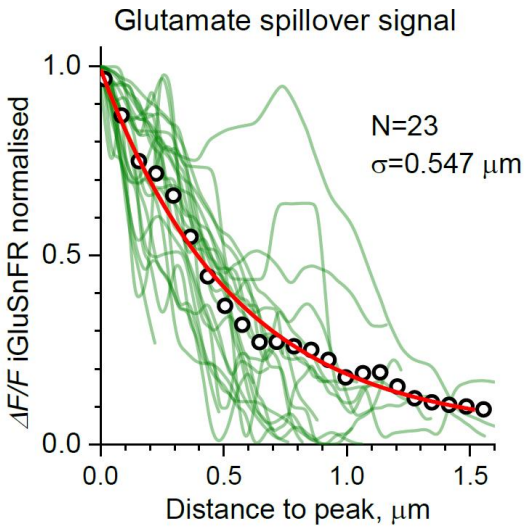




Extracellular signal profile of glutamate



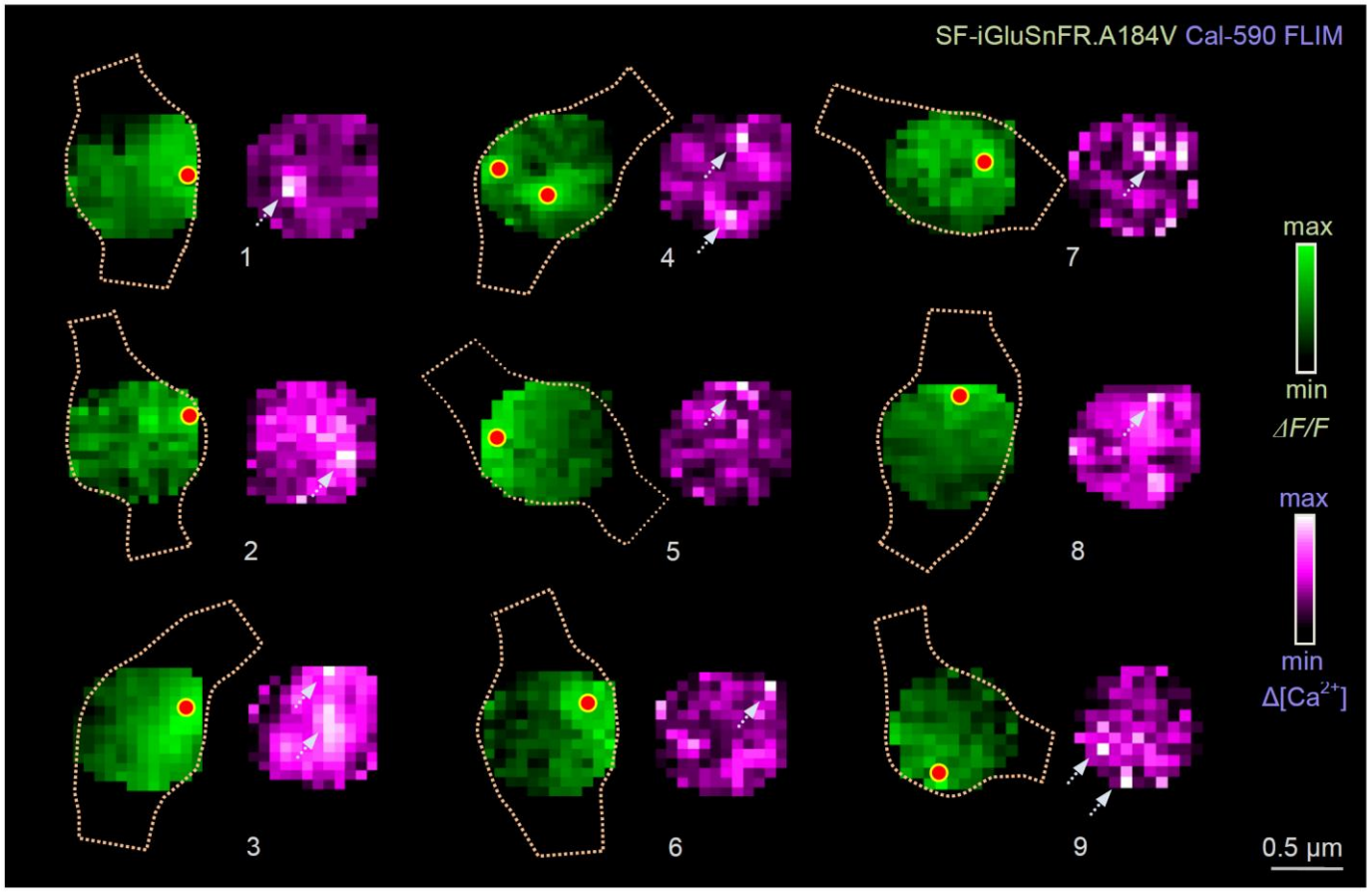




Glutamate signal is predominantly unimodal and fades away with a ~0.55 µm constant

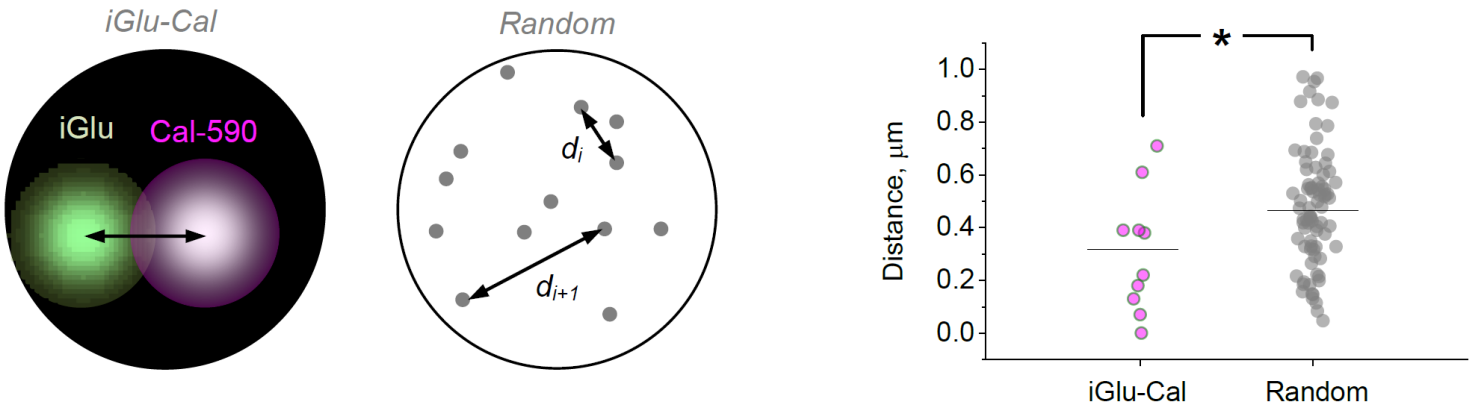
The likely presynaptic sites of glutamate release and Ca^{2+} entry

Only 9/23 boutons showed detectable Ca^{2+} entry hotspot (FLIM detection)



The data suggest loose-coupling between release machinery and Ca^{2+} entry

Is the juxtaposition of glutamate release and Ca^{2+} entry sites random?



Glutamate release and Ca^{2+} sites tend to co-occur but not on the nanoscale

Thomas Jensen	synaptic imaging
Kaiyu Zheng	FLIM
Sylvain Rama	Mossy fibres
James Reynolds	FLIM *in vivo*
Leonid Savtchenko	simulations
Olga Kopach	in vivo
Janosch Heller	molecular makeup
Piotr Michaluk	transporters

Glutamate sniffers: HHMI Janelia Campus
Jonathan Marvin, Loren Looger

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Fin