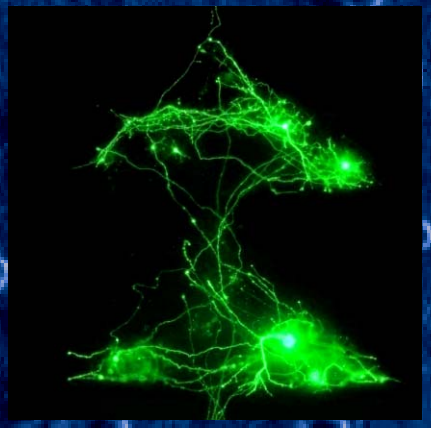
Architecture, Computation and Information Transport in Living Neuronal Networks



KITP October 2010

J. Neurophysiol 2005 2007 2010

J. Neurosci 2006

PRL 2006, Phys. Rep. 2007

PNAS 2008, Nat. Phys. 2008

Front. Neurosci. 2010

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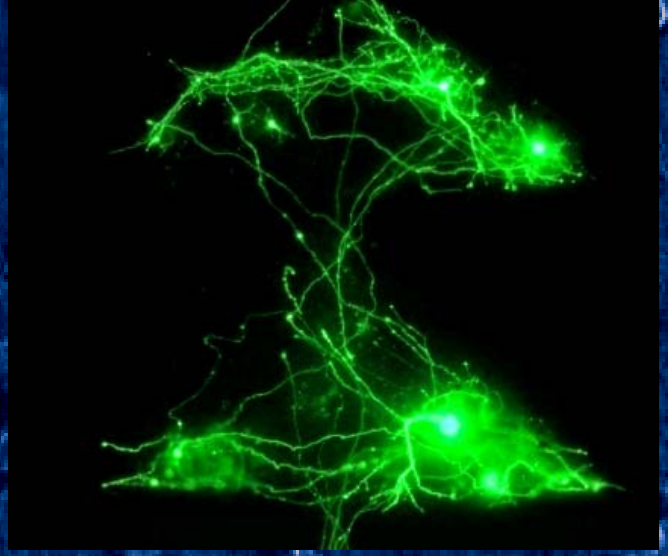
WEIZMANN INSTITUTE OF SCIENCE



The Team:

Ofer Feinerman
Assaf Rotem
Jordi Soriano
Shimshon Jacobi
Ilan Breskin
Shani Stern

Thanks to:

Theo Geisel
Fred Wolf
David Golomb
Amos Arieli

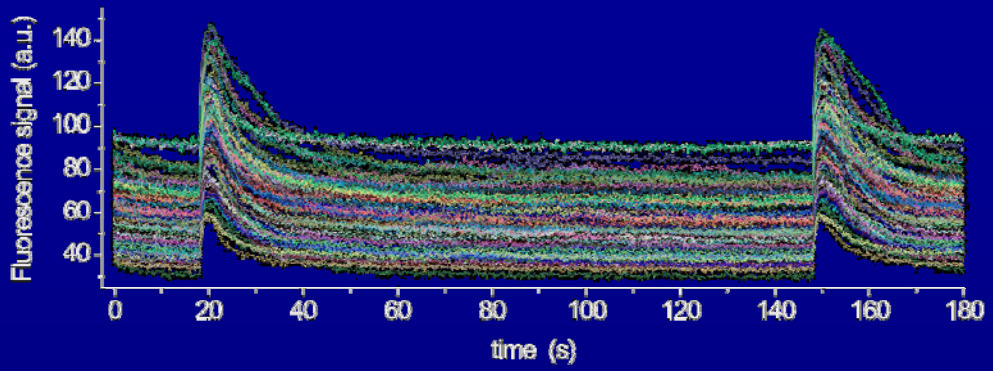
Credit Due:

David Kleinfeld

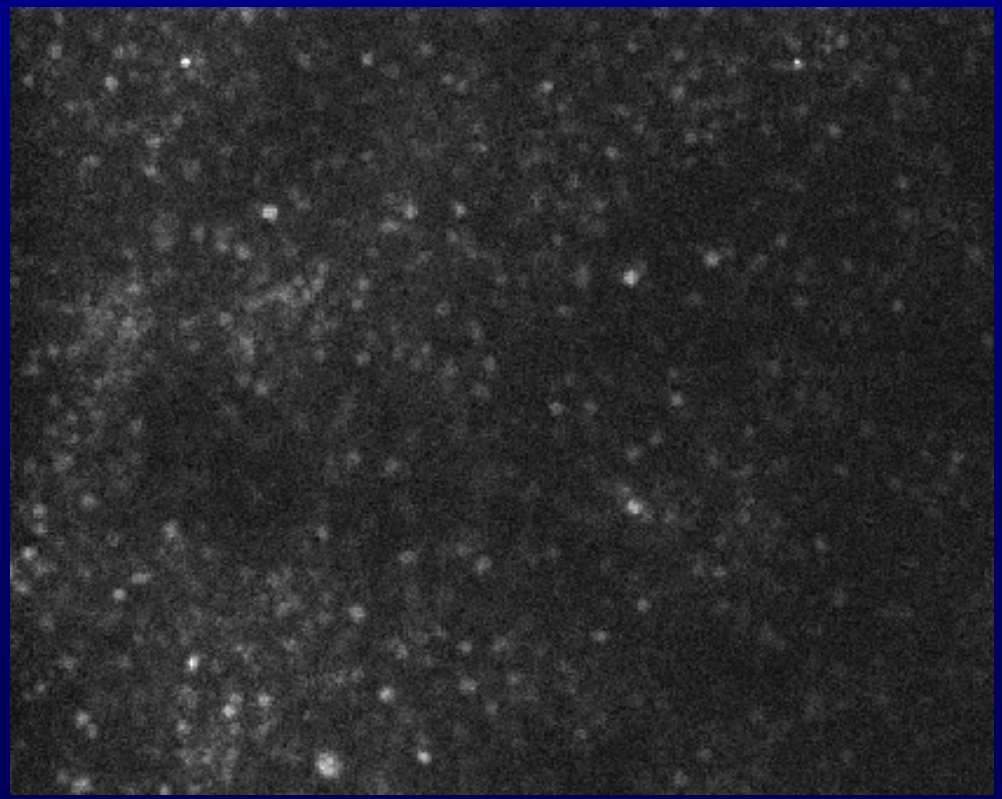
Collaborators:

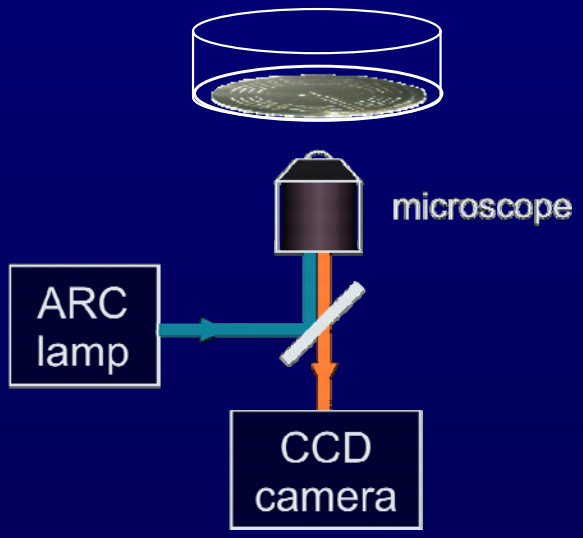
Menahem Segal
Tsvi Tlusty
Jean-Pierre Eckmann
Enric Alvarez-Lacalle
Cyrille Zbinden
Andreas Neef
Olav Stetter
Shimon Marom

Neuronal Cultures



Spontaneous bursts





Our way to study the Brain?

- Hit with small hammer (=magnetic stimulation) and observe linear response (=electroencephelogram, or EEG):

  look at schizophrenia, oscillations, synchrony

- New tools to probe it: WetSEM, “fountain pen”, Cloverleaf coil.

- Try to build one from scratch:

  using basic building blocks of neuron, self assemble into 1D and 2D living neuronal networks

2D Cultures:

The problem with cultures *in vitro*:

Limited repertoire in response to input

Almost all-or-none response.

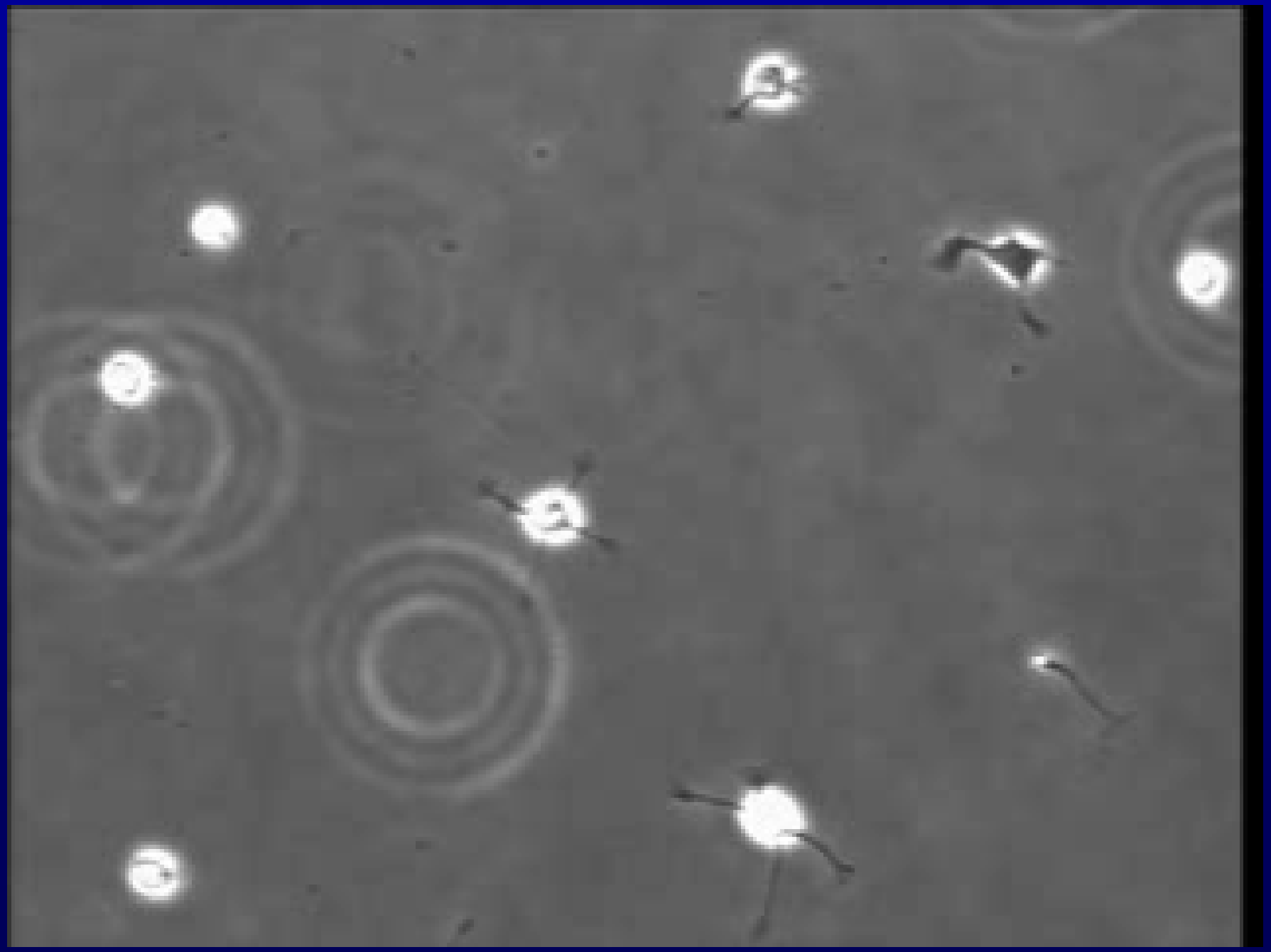
Is also an advantage:

Can investigate necessary conditions for computation: Connectivity Dimensionality, Inputs

Proof of Concept:

Engineer structures that produce a “minimal computation”

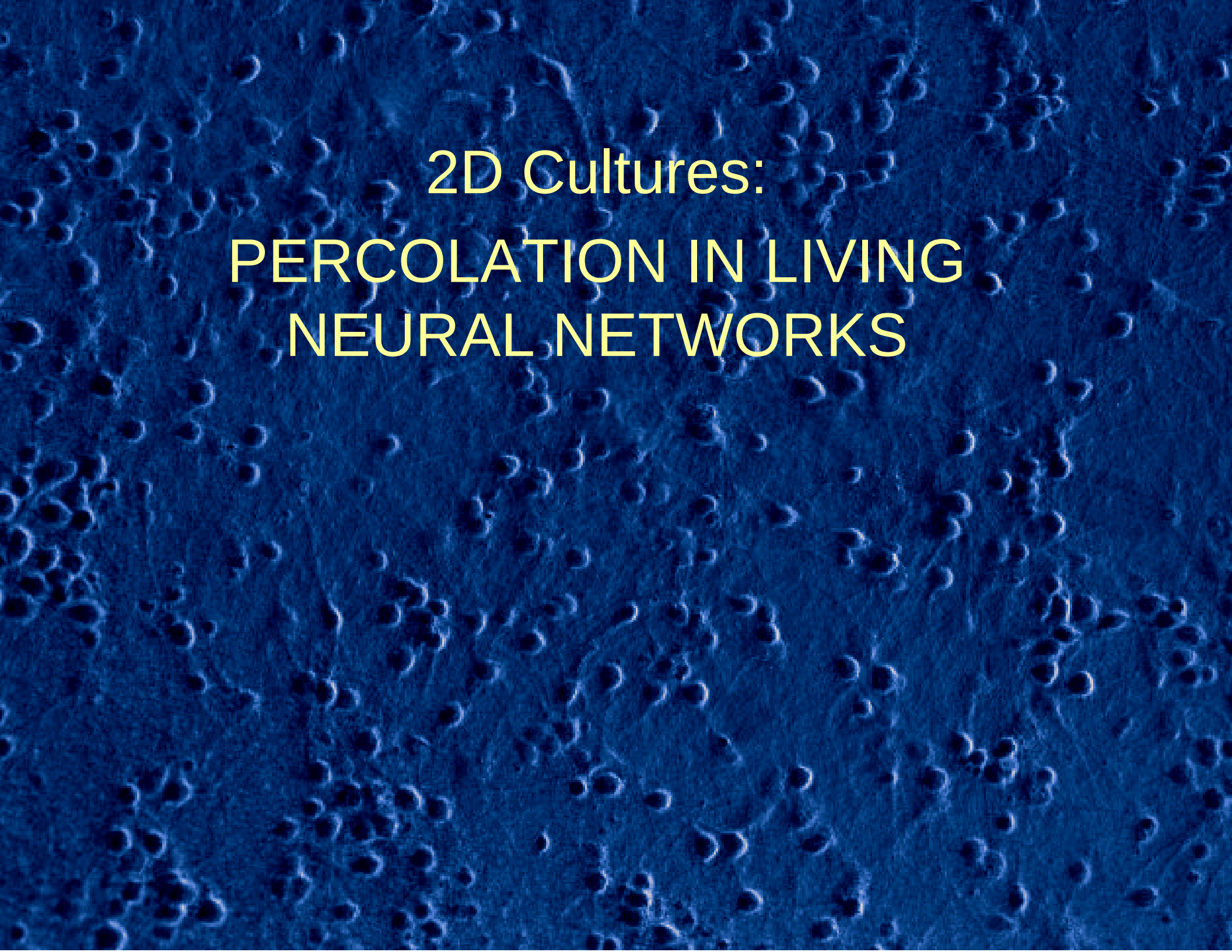
Connections of a single neuron



The Problem:

Why do neurons which are so ‘intelligent’ in the brain become so ‘stupid’ in the dish

- Loss of one dimension 3D → 10,000 neighbors, 2D → 100 neighbors
- No ‘blueprint’ for connections – random connectivity
- No input during development of connections

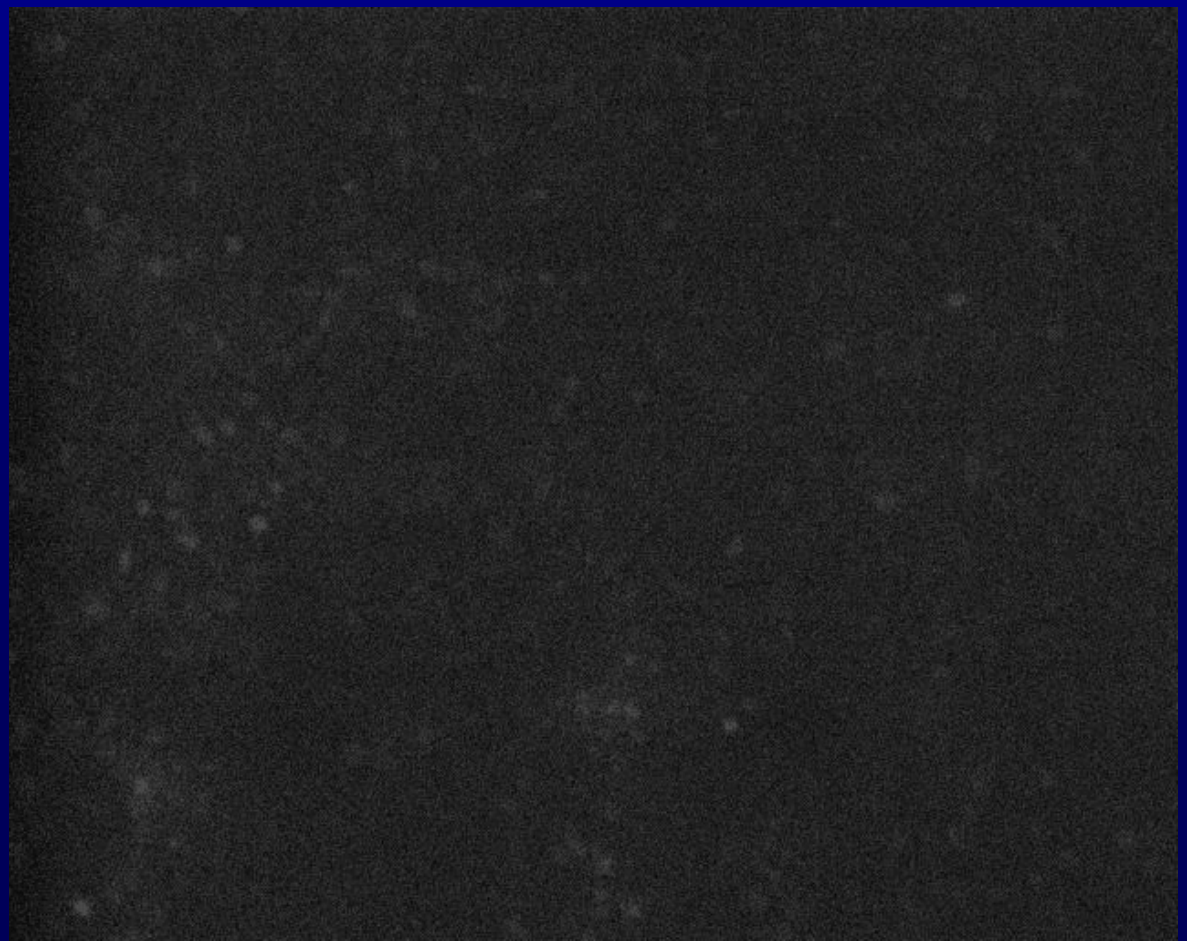

2D Cultures:
PERCOLATION IN LIVING
NEURAL NETWORKS

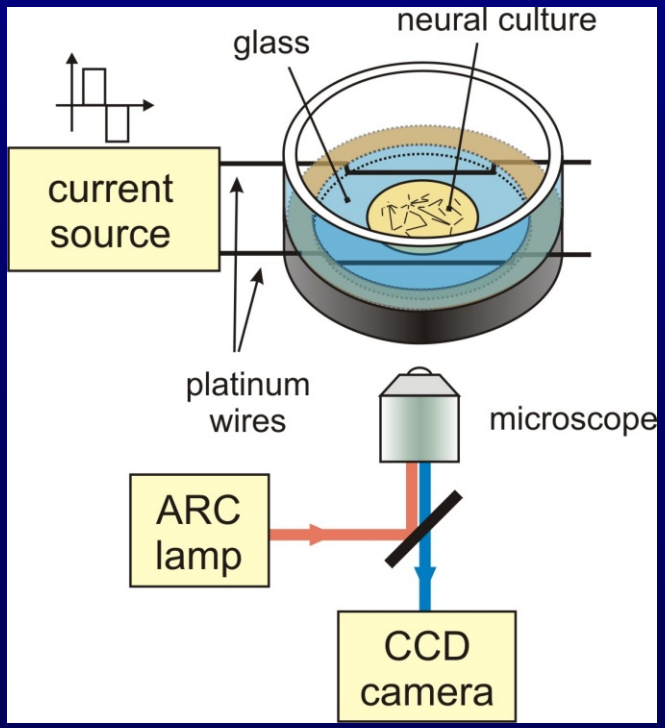
Network of rat-embryo hippocampal neurons, containing about 10^5 neurons.

A region of the culture is selected (~ 1 mm^2), and the response of ~900 neurons monitored.

Main idea:

Study the collective response of neurons to an electric stimulation, for different connection strength between neurons.

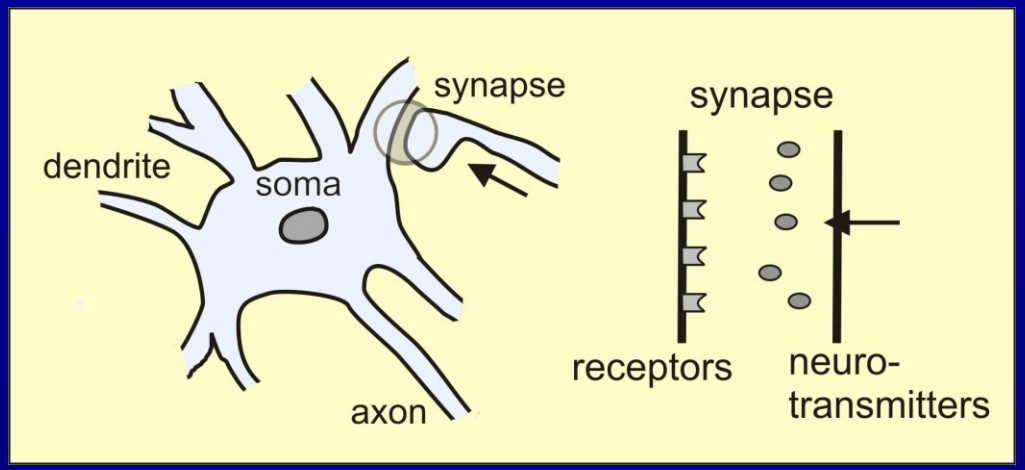




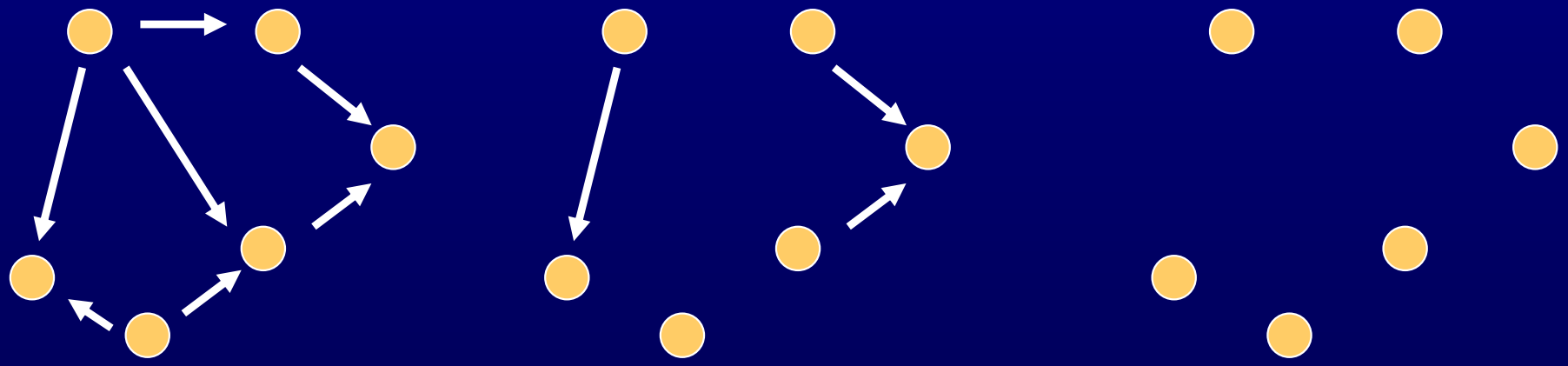
WEAKENING THE NETWORK

Signals between neurons are transmitted with neurotransmitters.

Connections can be weakened and broken by blocking the corresponding neuroreceptors.



Receptor	Receptor antagonist
AMPA	CNQX

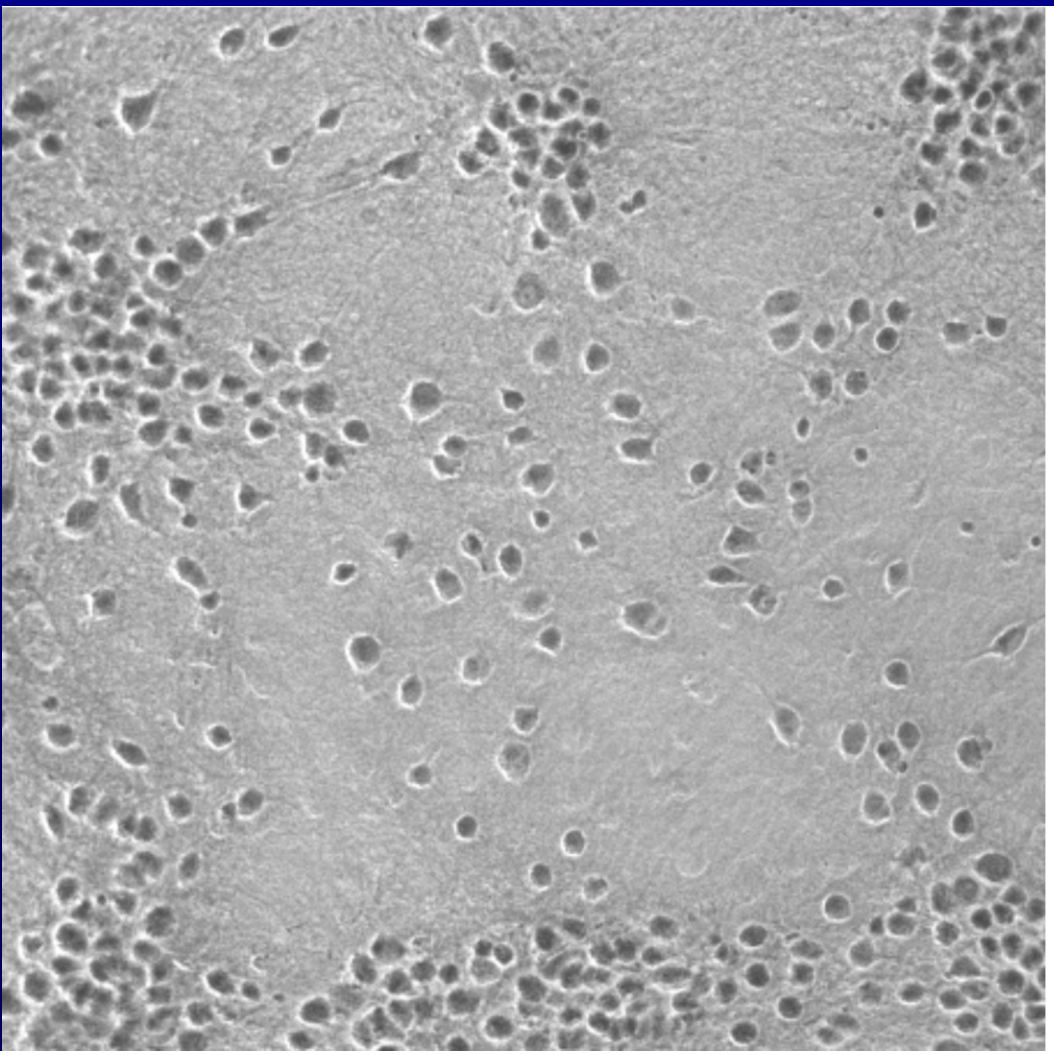


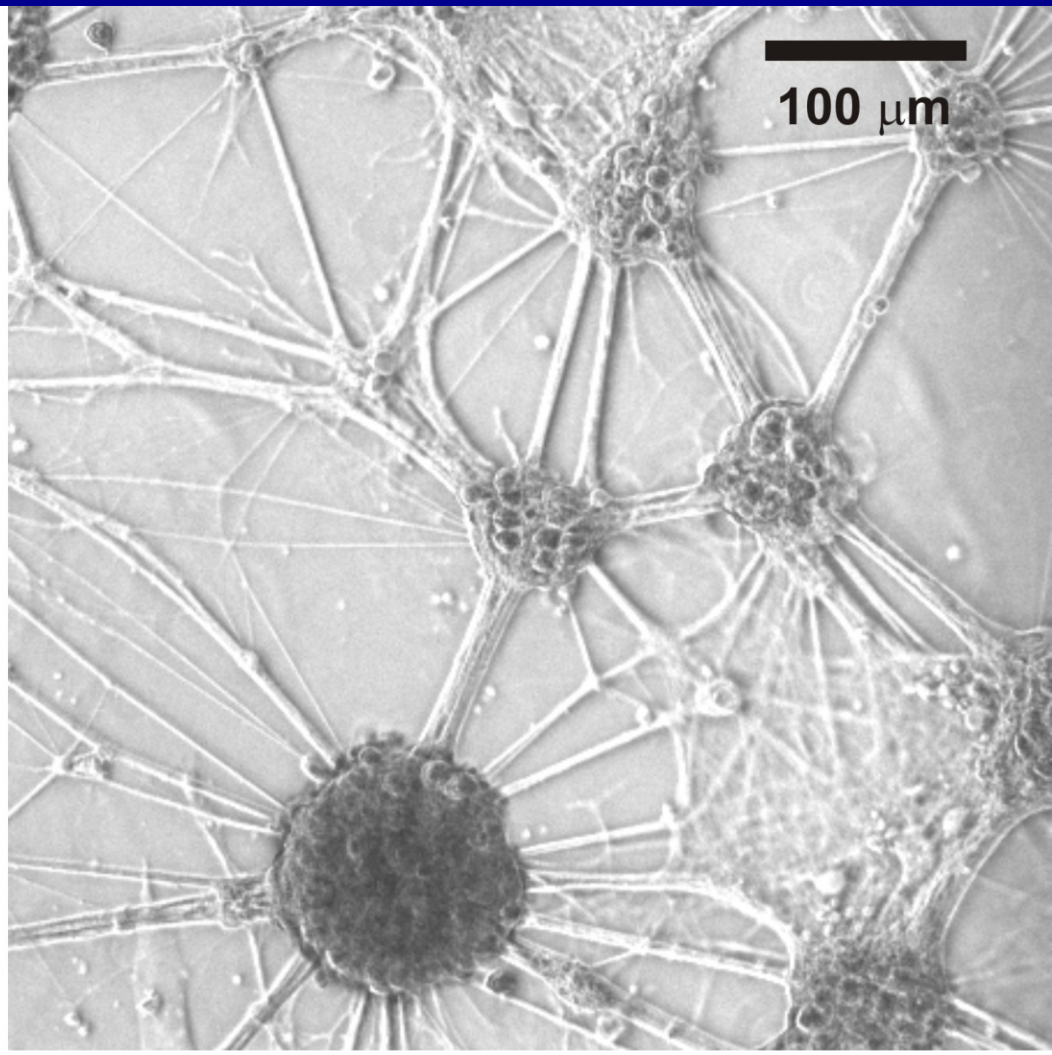
[CNQX]=0 [CNQX]=high [CNQX]=saturating

The cultures:

Hippocampus

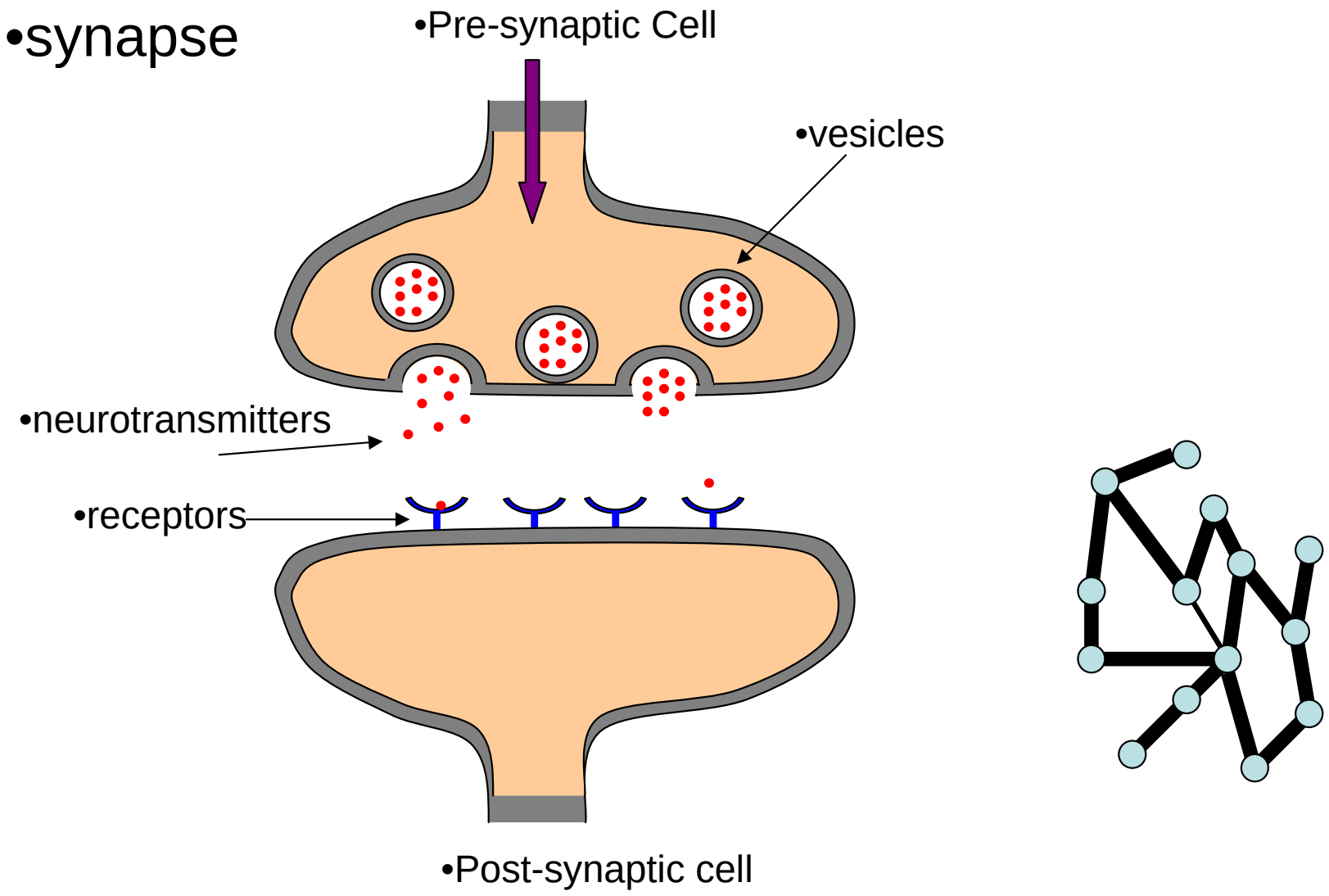
Cortex





•REDUCING NEURAL CONNECTIVITY

- Signals between neurons are transmitted with neurotransmitters.
- Connections weakened by blocking neuroreceptors.



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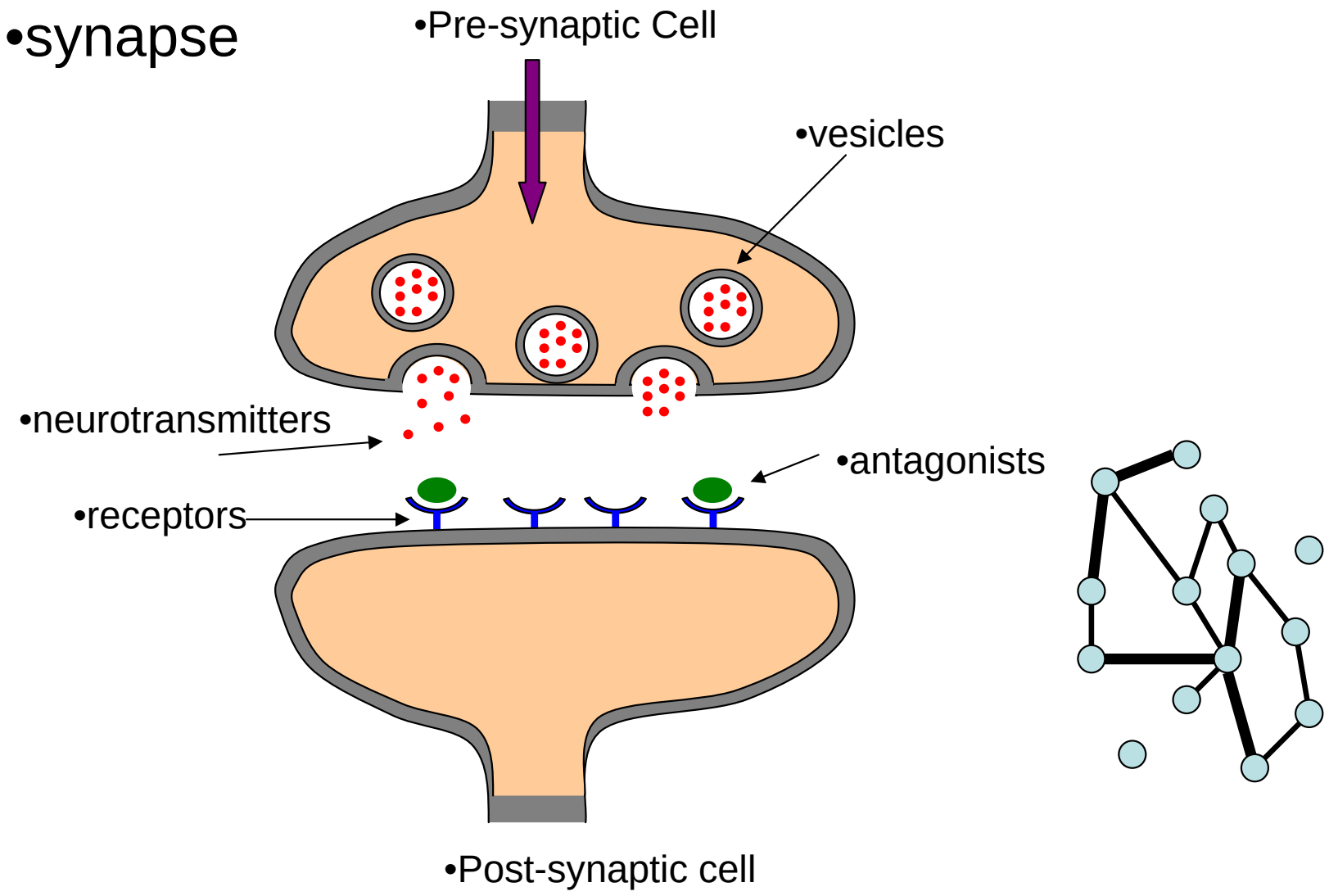

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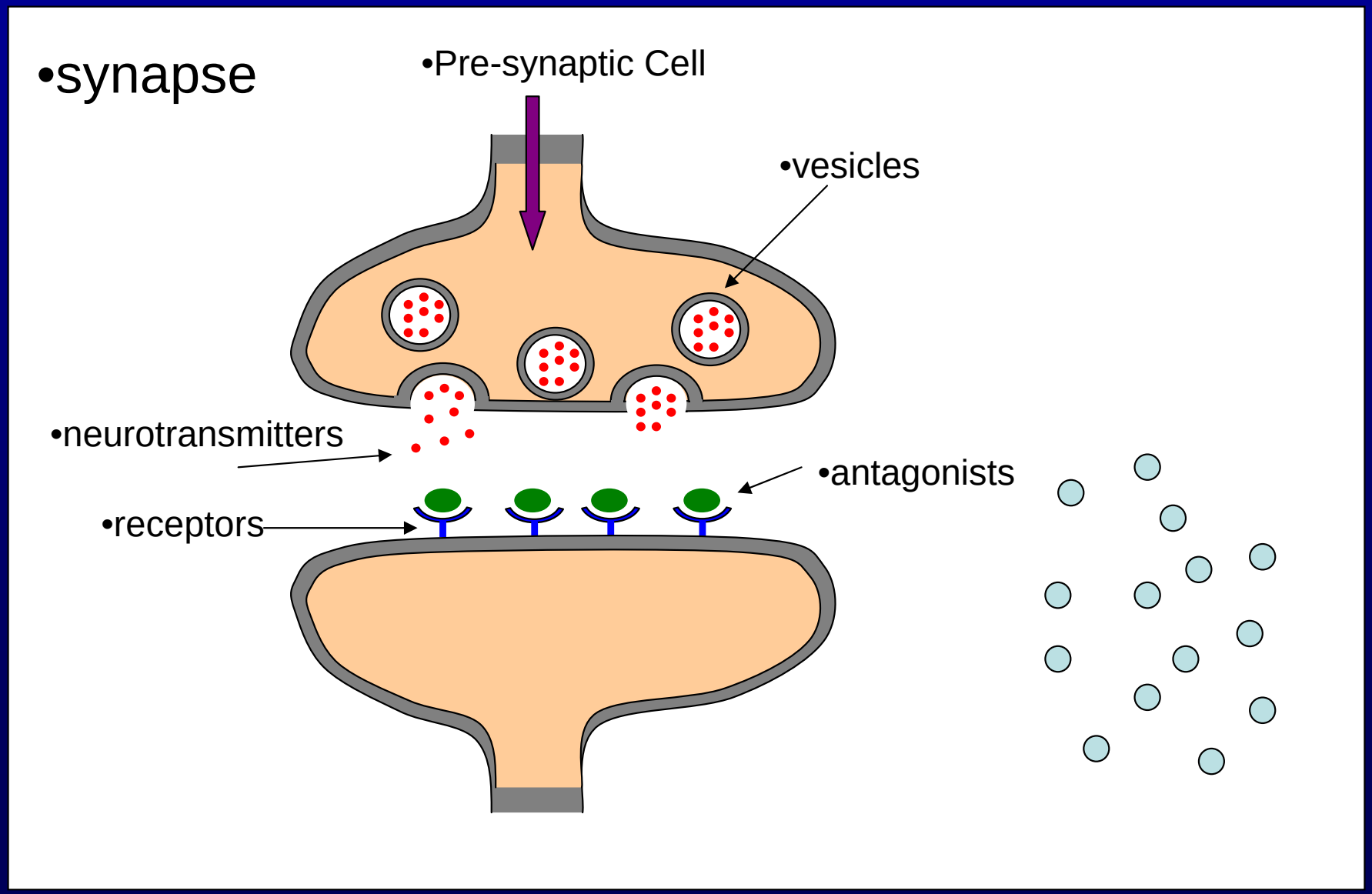
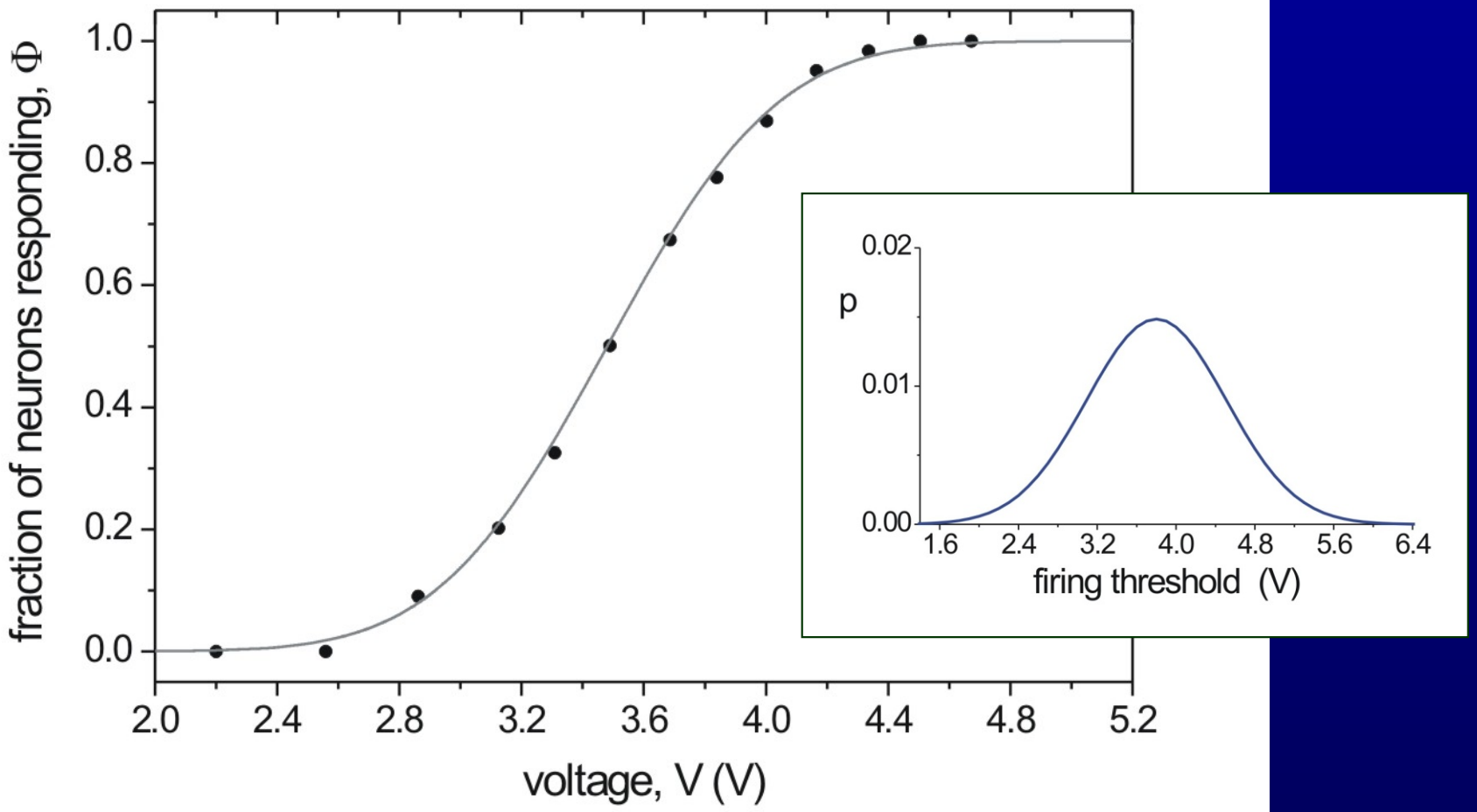


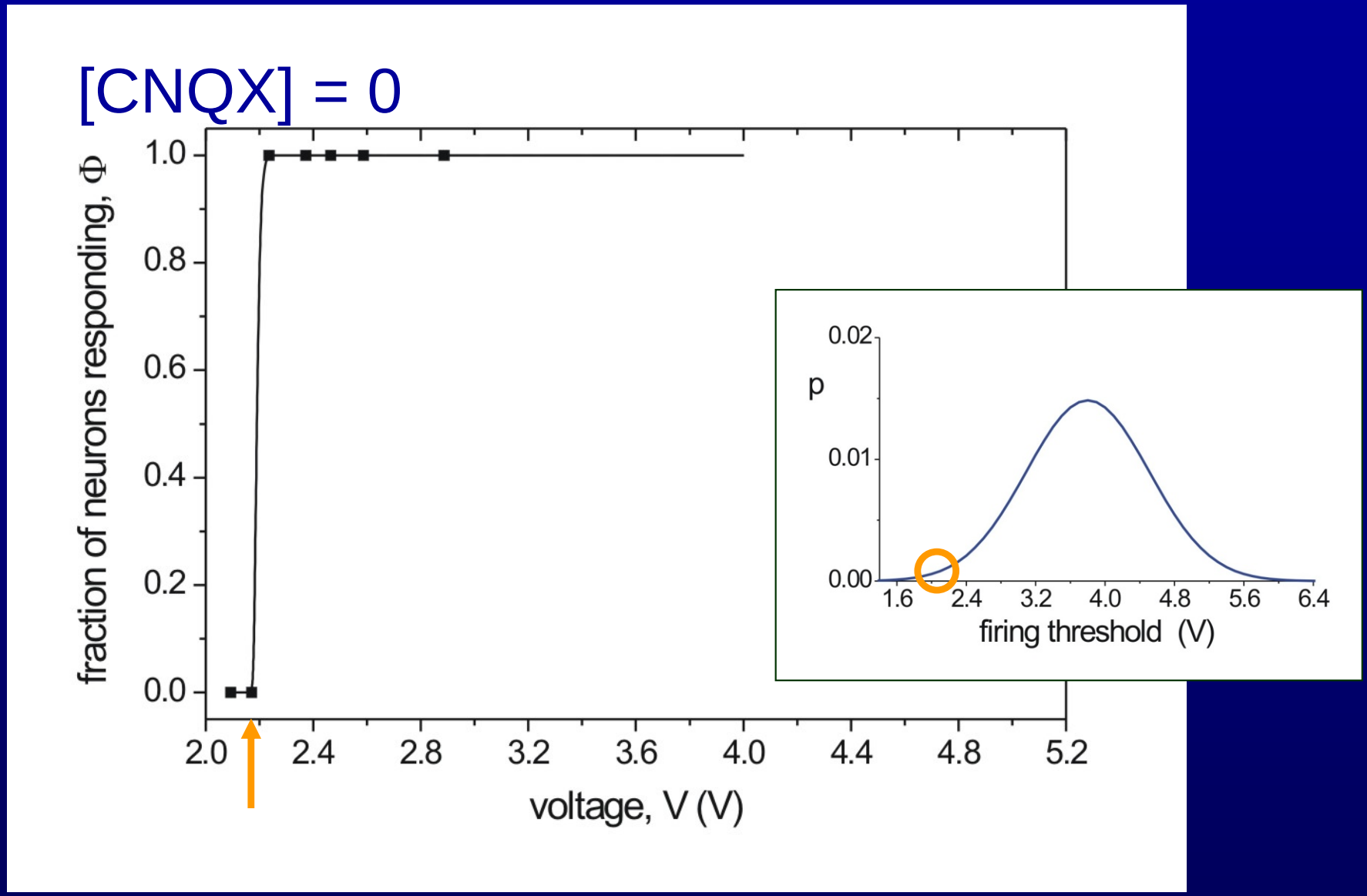
NETWORK RESPONSE

[CNQX] = saturating



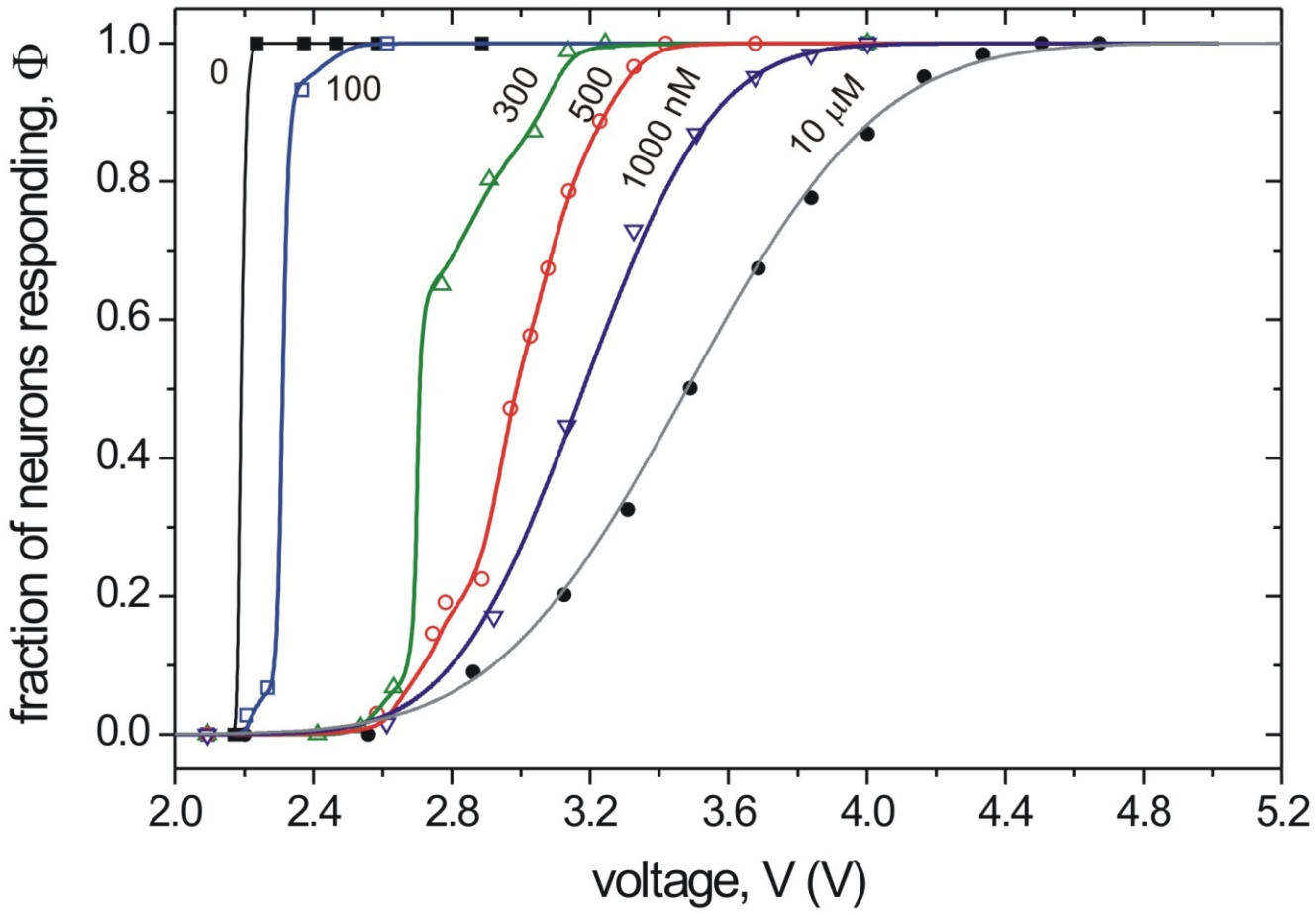
Disconnected network → probe response of individual neurons to voltage

NETWORK RESPONSE

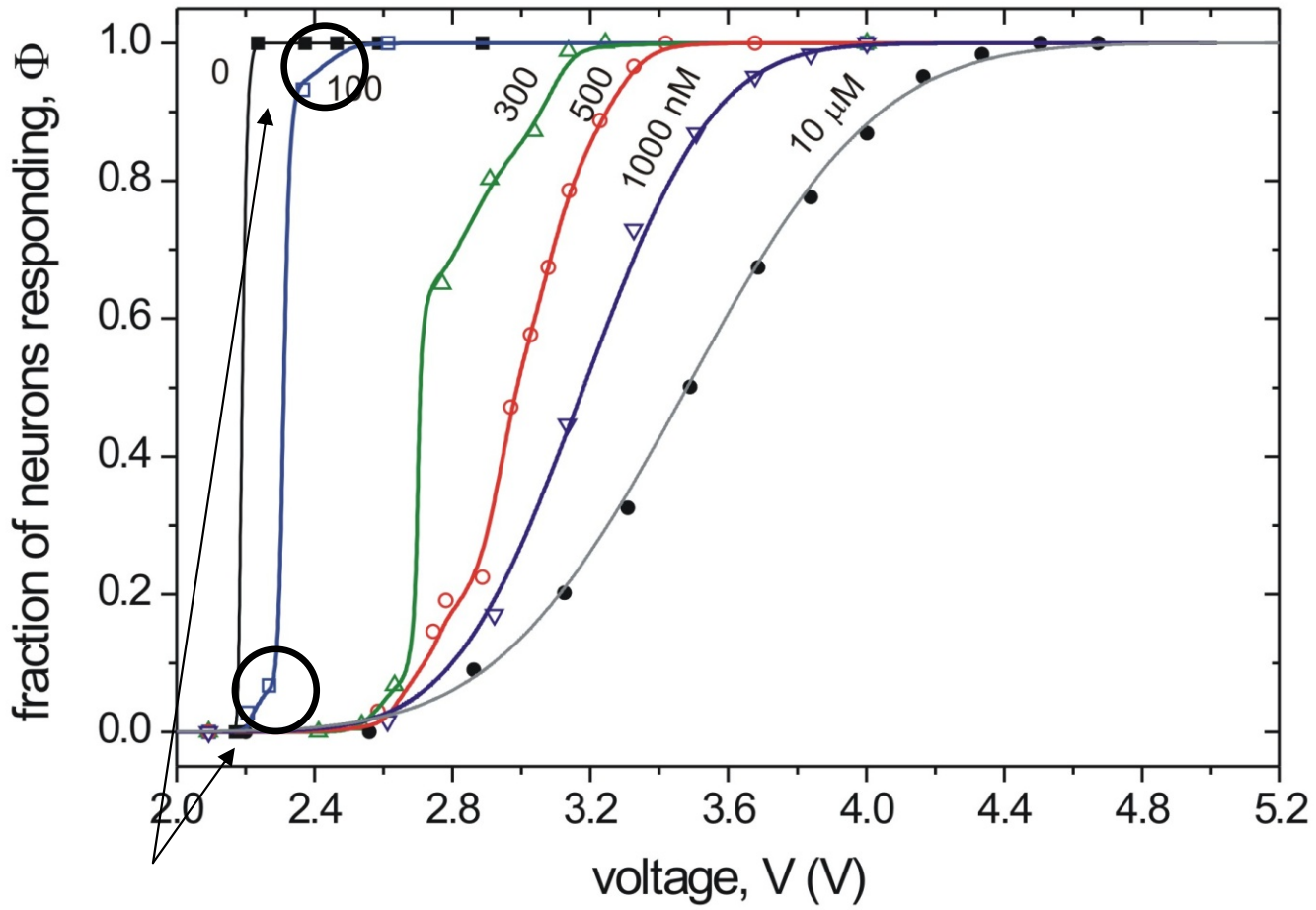


Fully connected network a few sensitive neurons ignite whole network

NETWORK RESPONSE

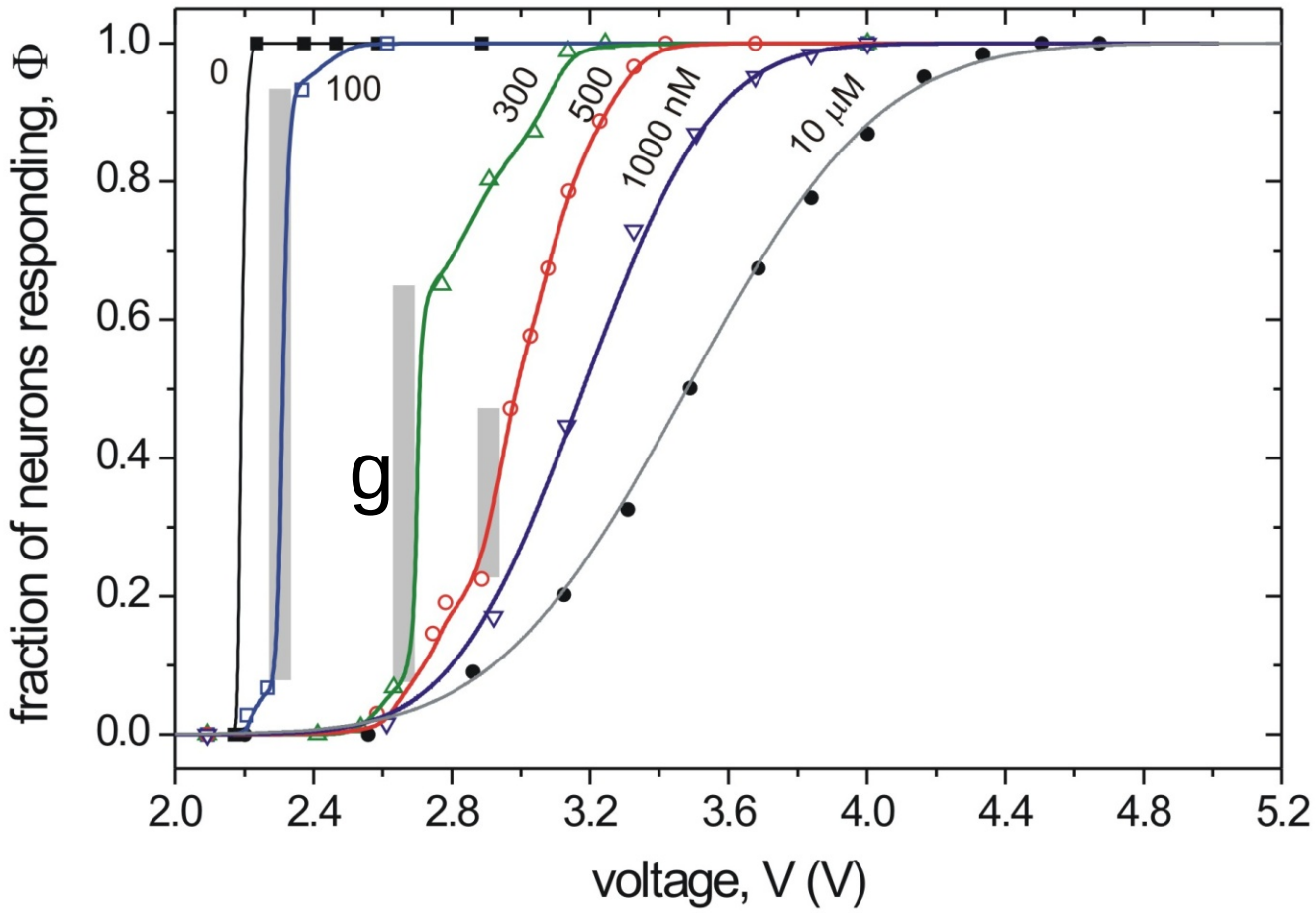


NETWORK RESPONSE

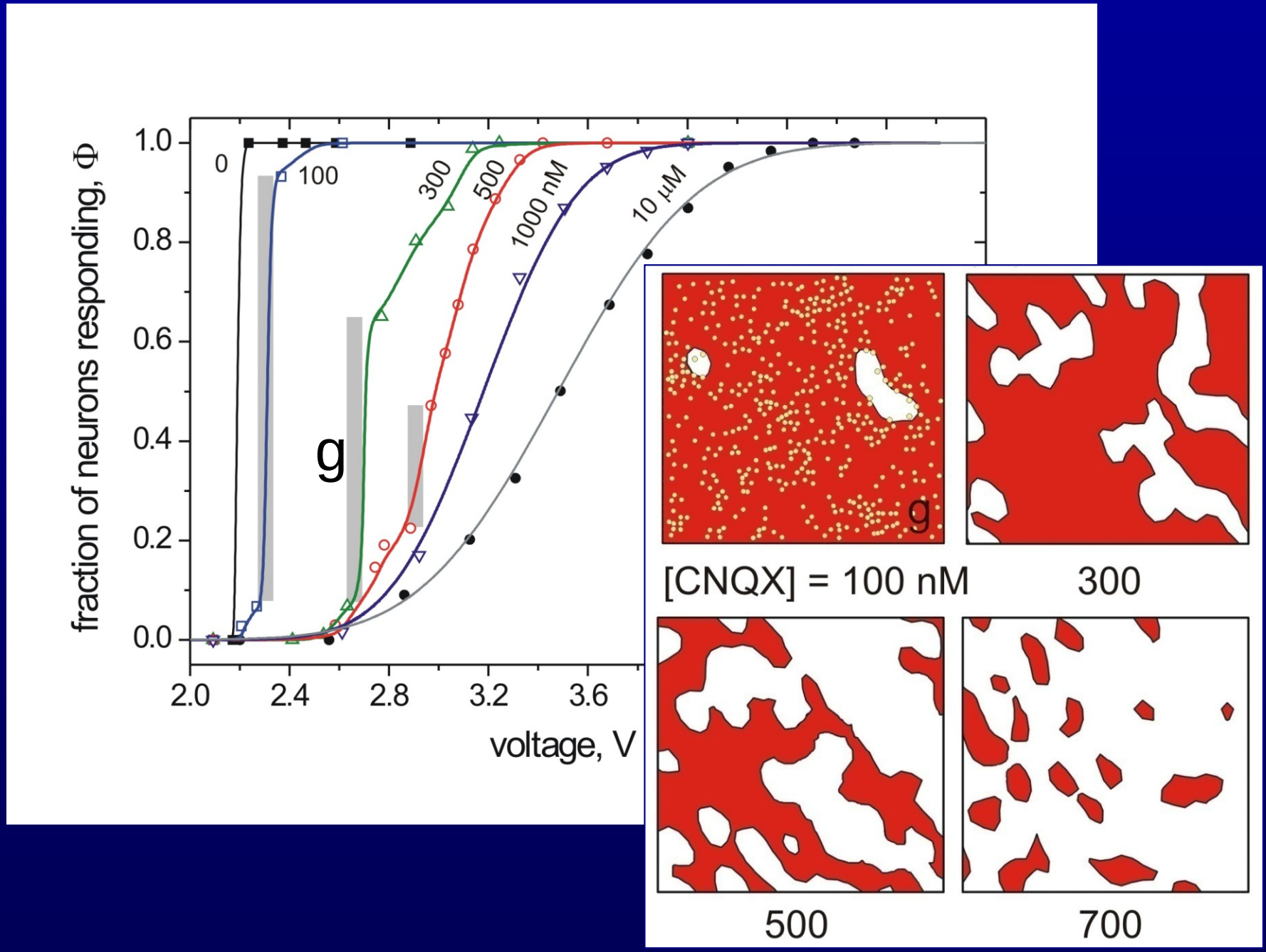


small clusters

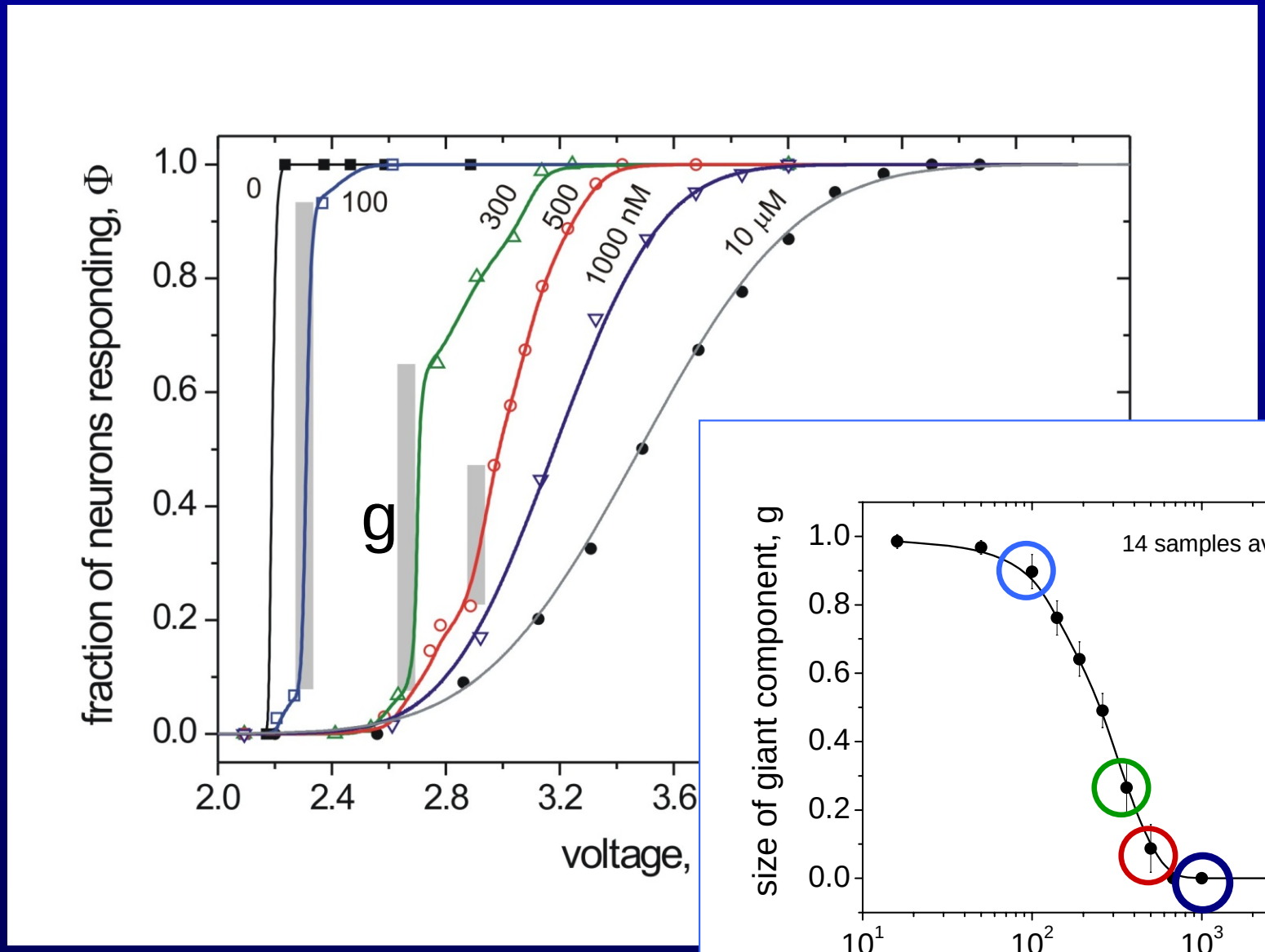
NETWORK RESPONSE. GIANT COMPONENT



NETWORK RESPONSE. GIANT COMPONENT



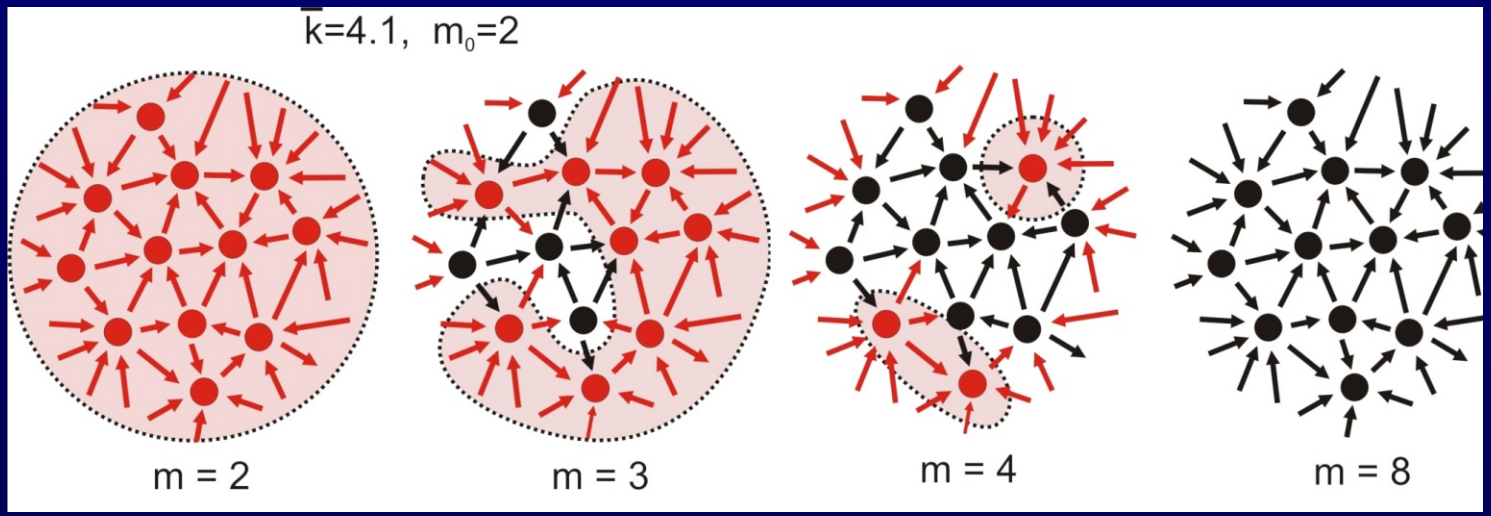
NETWORK RESPONSE. GIANT COMPONENT



This is Quorum- Percolation

Giant connected (or ‘spanning’) component.

A neuron fires either if it is excited by the external voltage or if it gets at least *m* inputs.



QUORUM PERCOLATION THEORY

Disintegration of the network:

Control parameter *m* : “effective” number of required inputs.

$$m = \frac{V_T}{g_{\mathrm{syn}}}$$

Voltage threshold (30 mV)
synaptic voltage (1-2 mV)
} → $m_0 = 15$

as the connectivity is reduced: $g_{syn} \longrightarrow c\, g_{syn}$

$$C = \frac{1}{1 + [\mathrm{CNQX}] / K_d}$$

$$\frac{m}{m_0} = 1 + \frac{[\mathrm{CNQX}]}{K_d}.$$

m is the control parameter

Theory- Tsvi Tlusty

p_k = degree probability distribution (links per node)

f = fraction fire in response to voltage V

Φ = total fraction of neurons that fire

$$\Phi(f) = f + (1-f)Prob.(\text{at least } m \text{ input neurons fire}) =$$

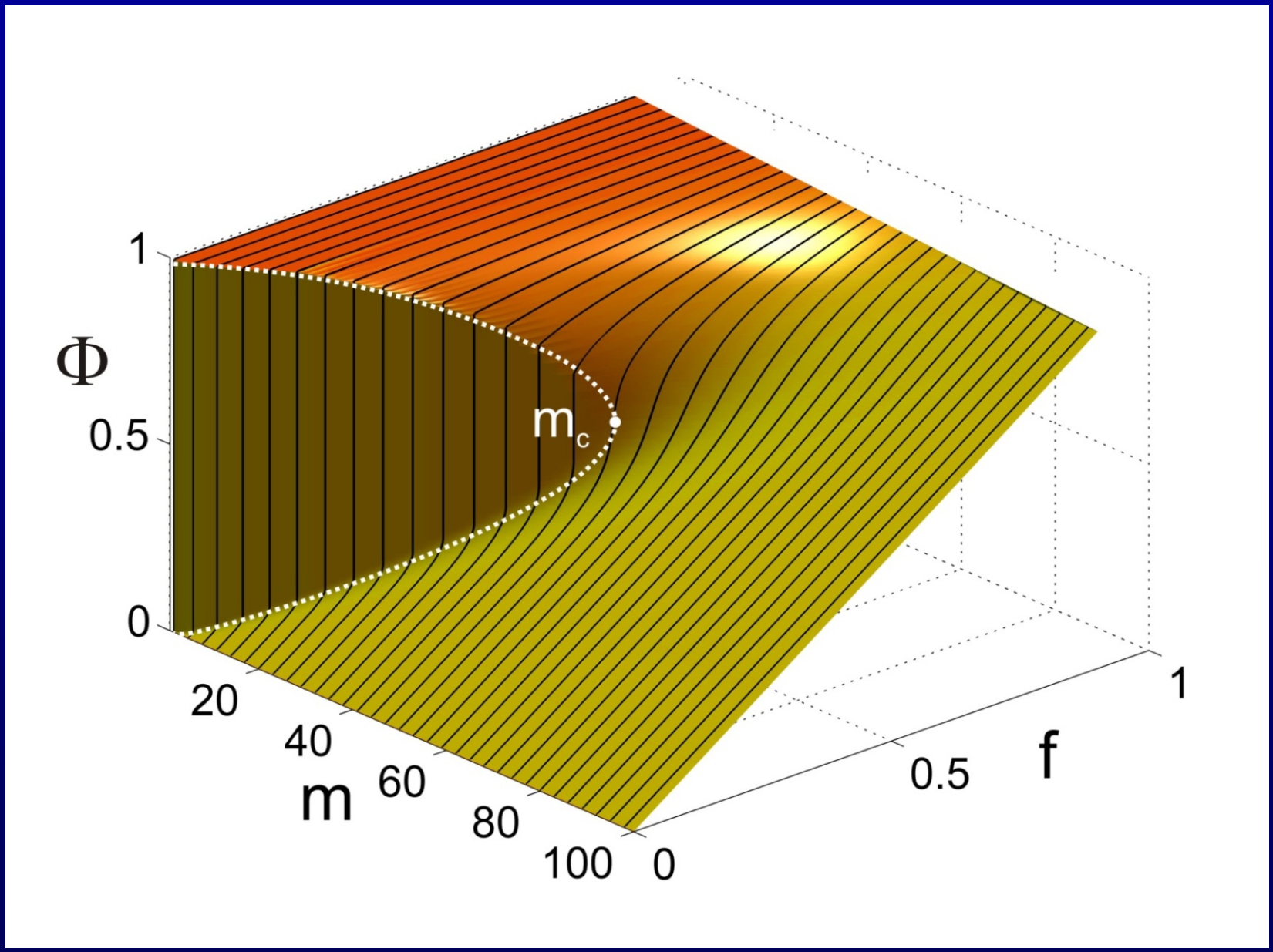
$$= f + (1-f)\sum_{k=m}^{\infty} p_k \sum_{l=m}^{k} \binom{k}{l} \Phi^l (1-\Phi)^{k-l}$$

Solved for a given p_k by considering the generating function (or z-transform) of the degree distribution and its derivatives.

$$\sum_{k=0}^{\infty} p_k z^k$$

with z = 1-Φ.

Φ as a function of (m, f) displays a line of transitions f^* ending at a critical threshold $m=m_c$



"Conventional" percolation is m=1
Continuous transition as function of m

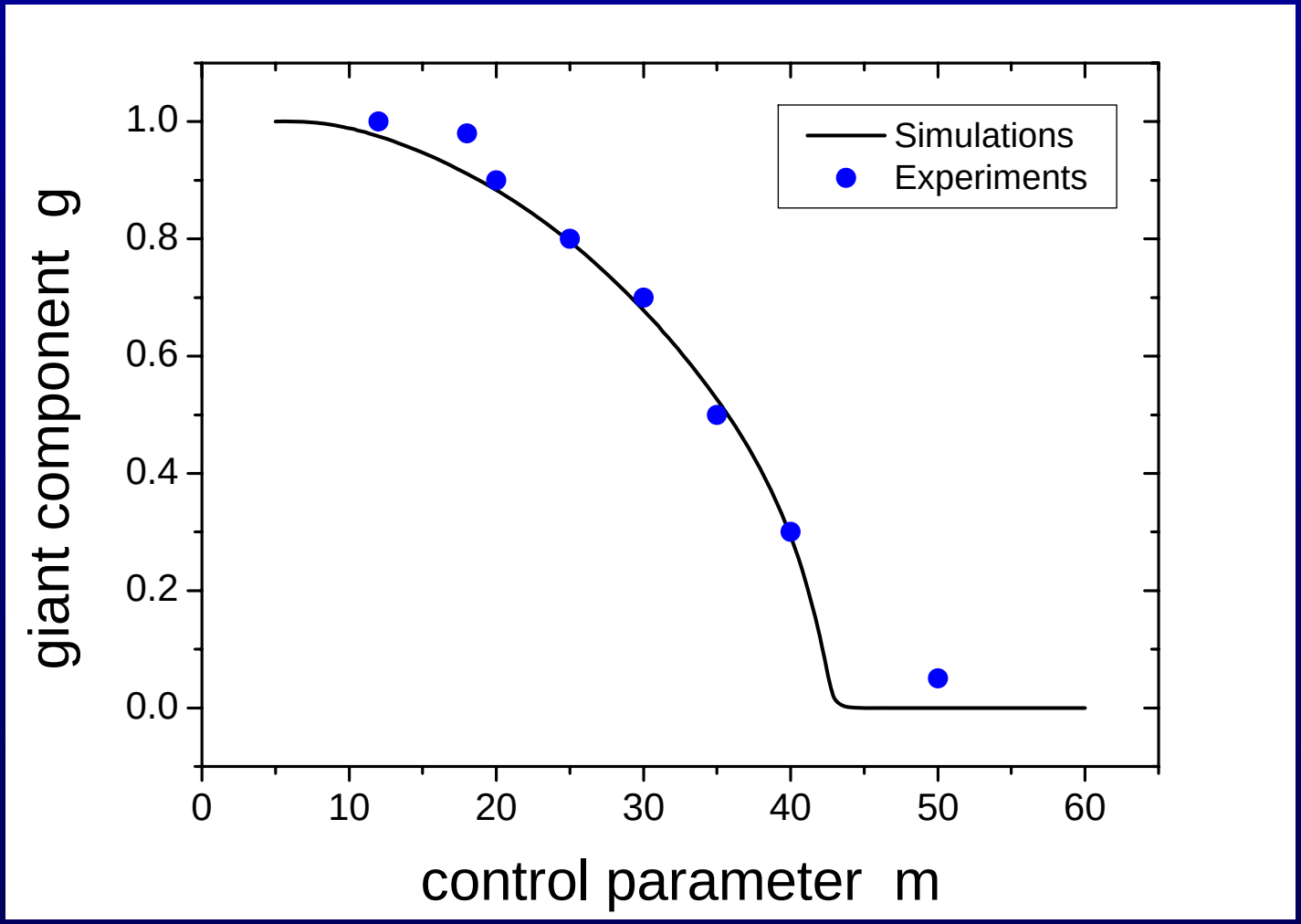
Gausiann distribution of inputs:

So what can we learn about the “Biology”???

EXTRACTING CONNECTIVITY

Gaussian degree distribution provides best fit to data

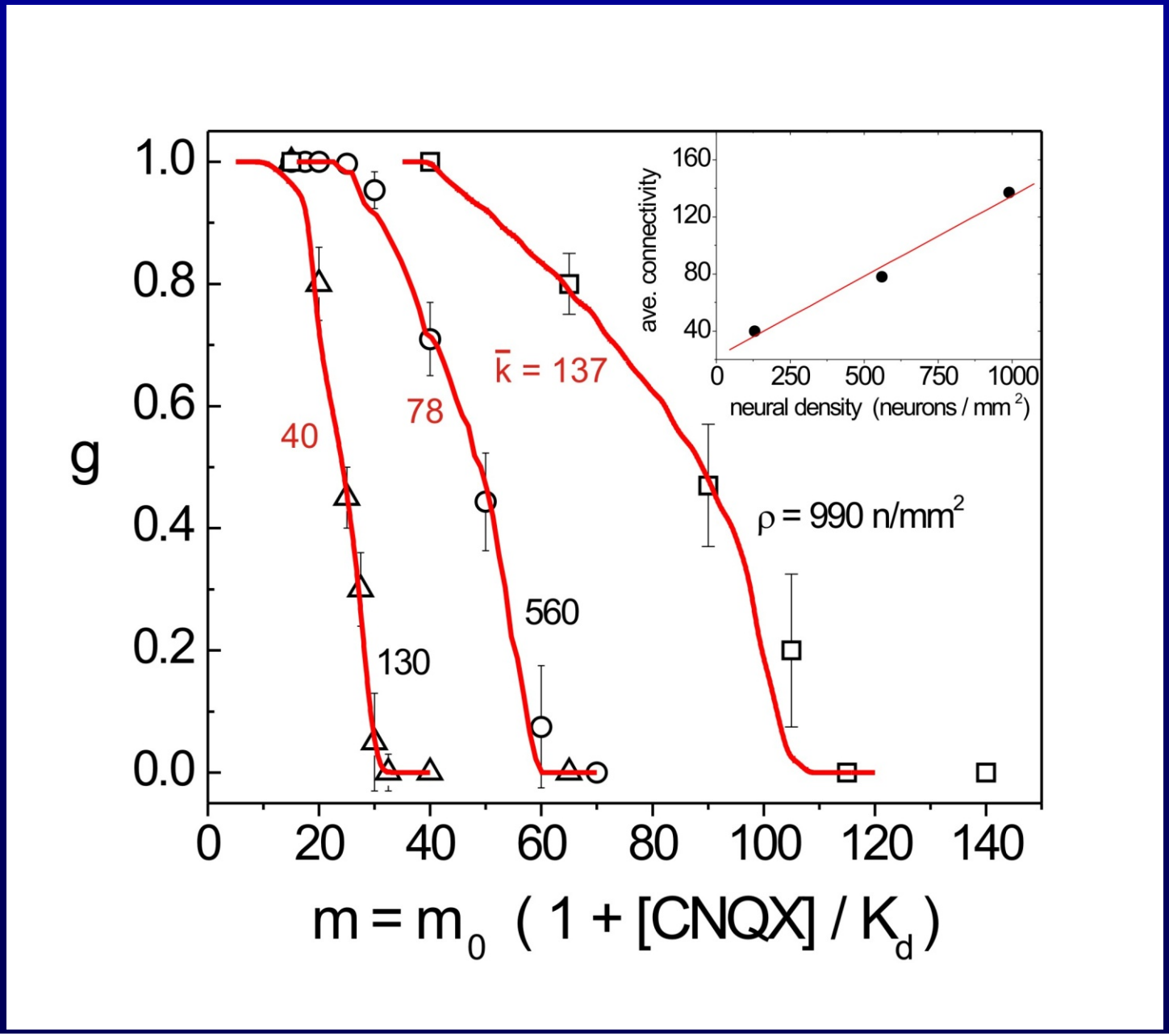
NOT scale free graph! → locality is important



•CAVEAT: the theory only deals with the input graph, the ouptut degree distribution may be different!

Changing neuronal density –

Model accommodates by adjusting gaussian of degree distribution

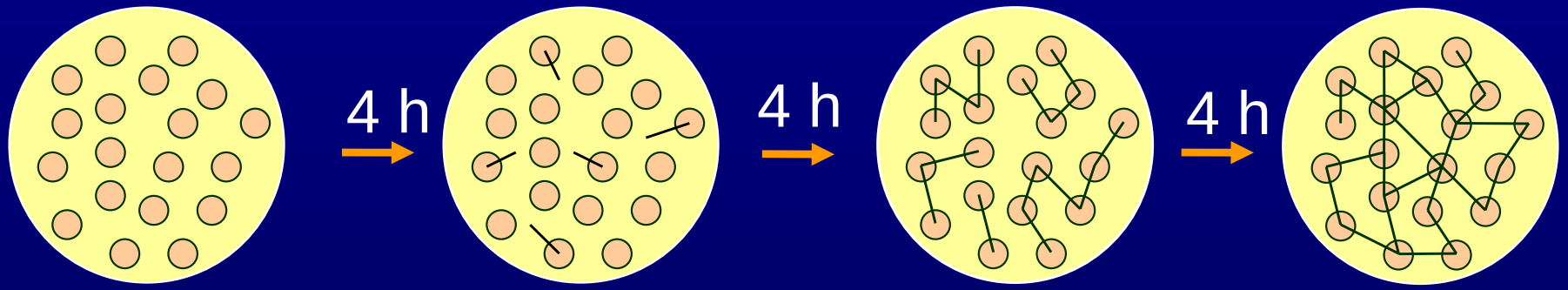


DEVELOPMENT OF THE NETWORK: Experiment

g(m) is a good experimental handle.

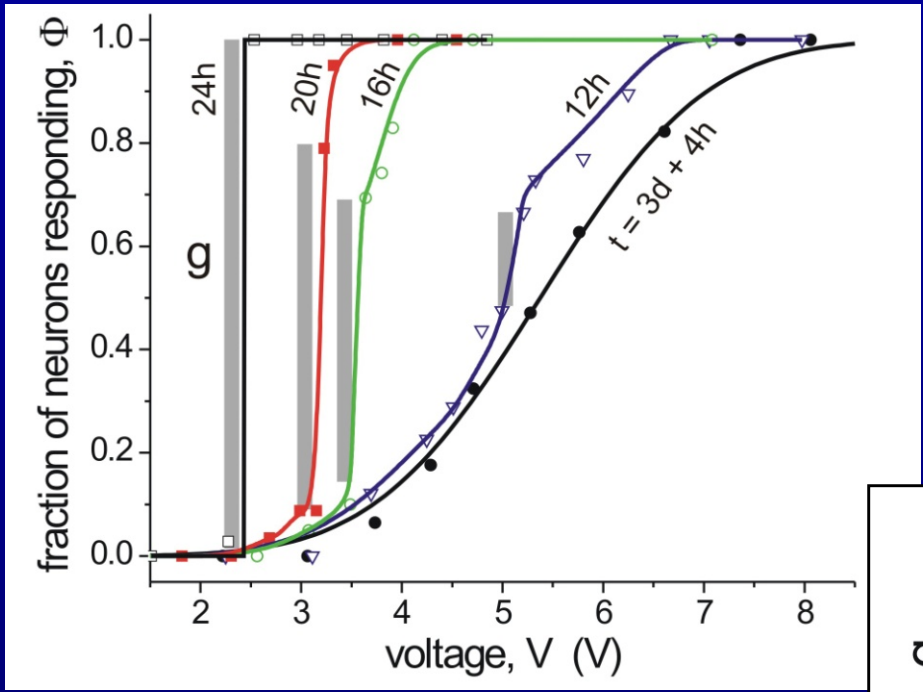
But note that connectivity increases as the culture matures!

→ Monitor *g(time)* as neurons connect after plating



Take cultures at days 17, 19 of pregnancy and at day of birth

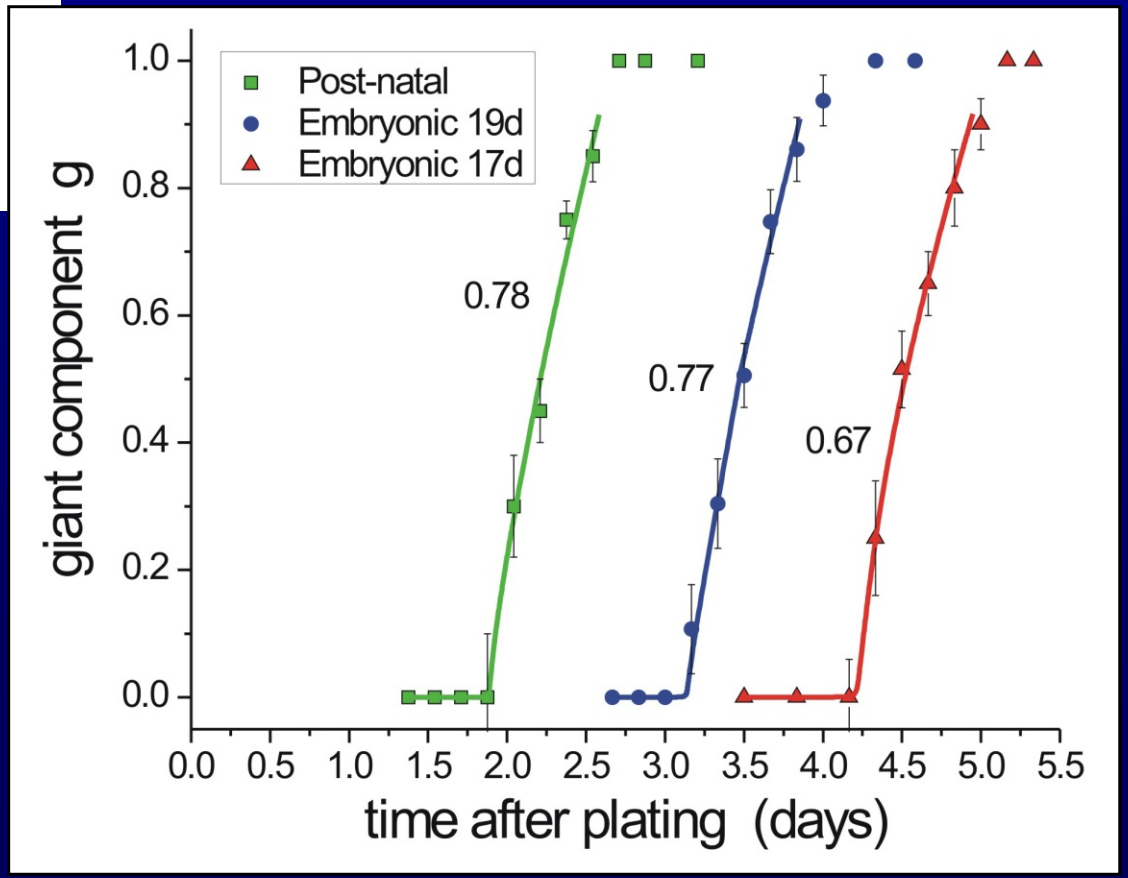
DEVELOPMENT OF THE NETWORK



Time of Birth 21-22 days

~17+4 ~19+3 !

Similar to the network's disintegration with CNQX.



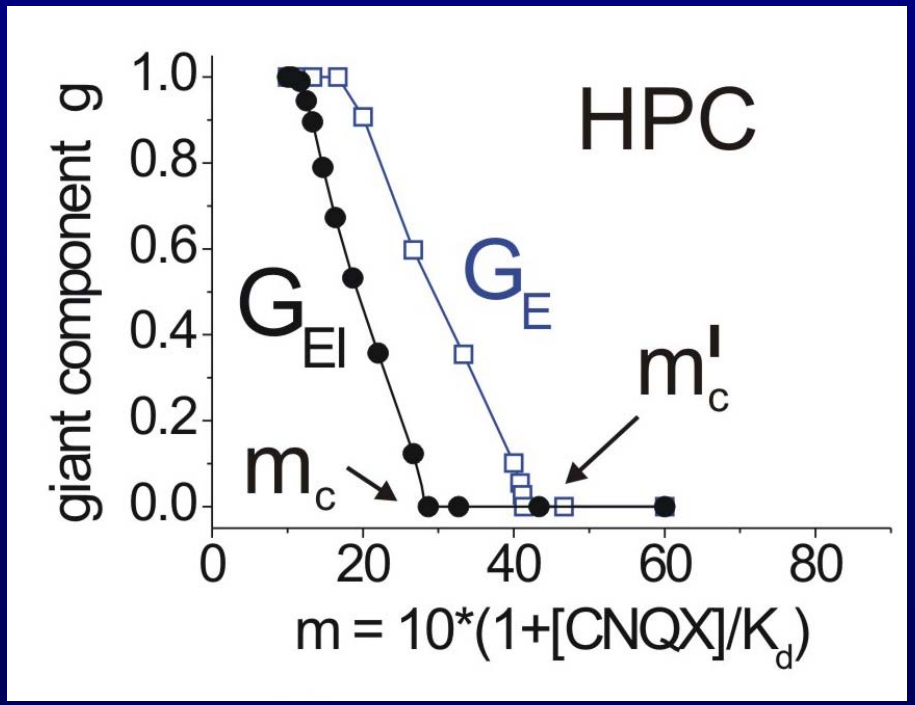
•EXTRACTING CONNECTIVITY

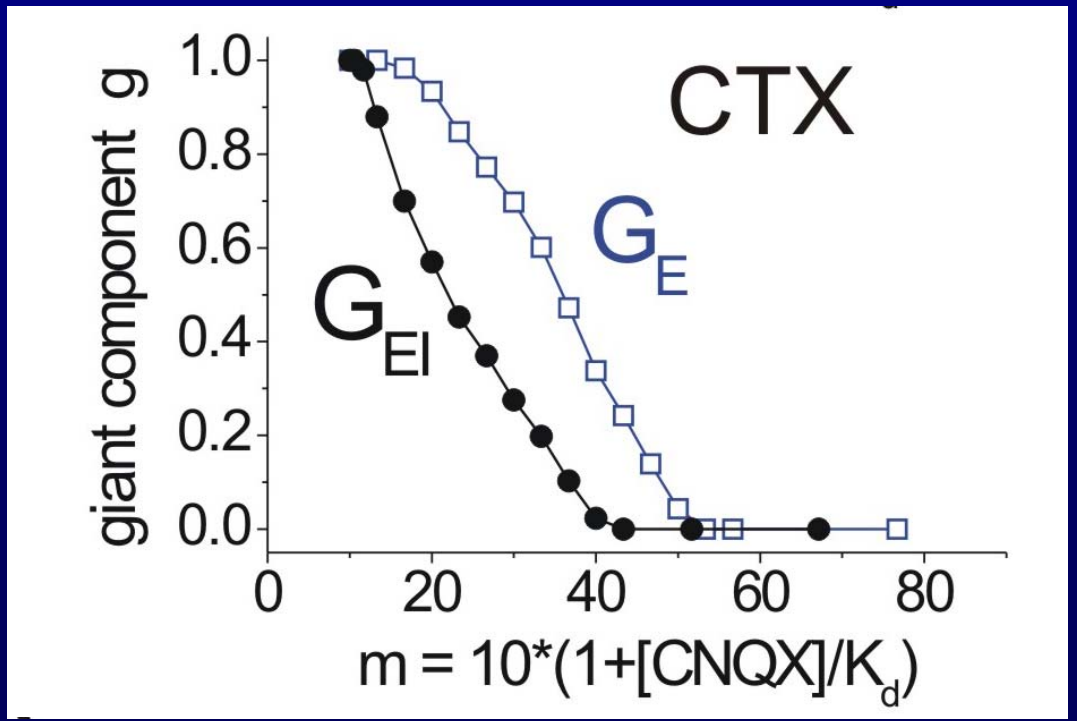
Disintegration of the network:

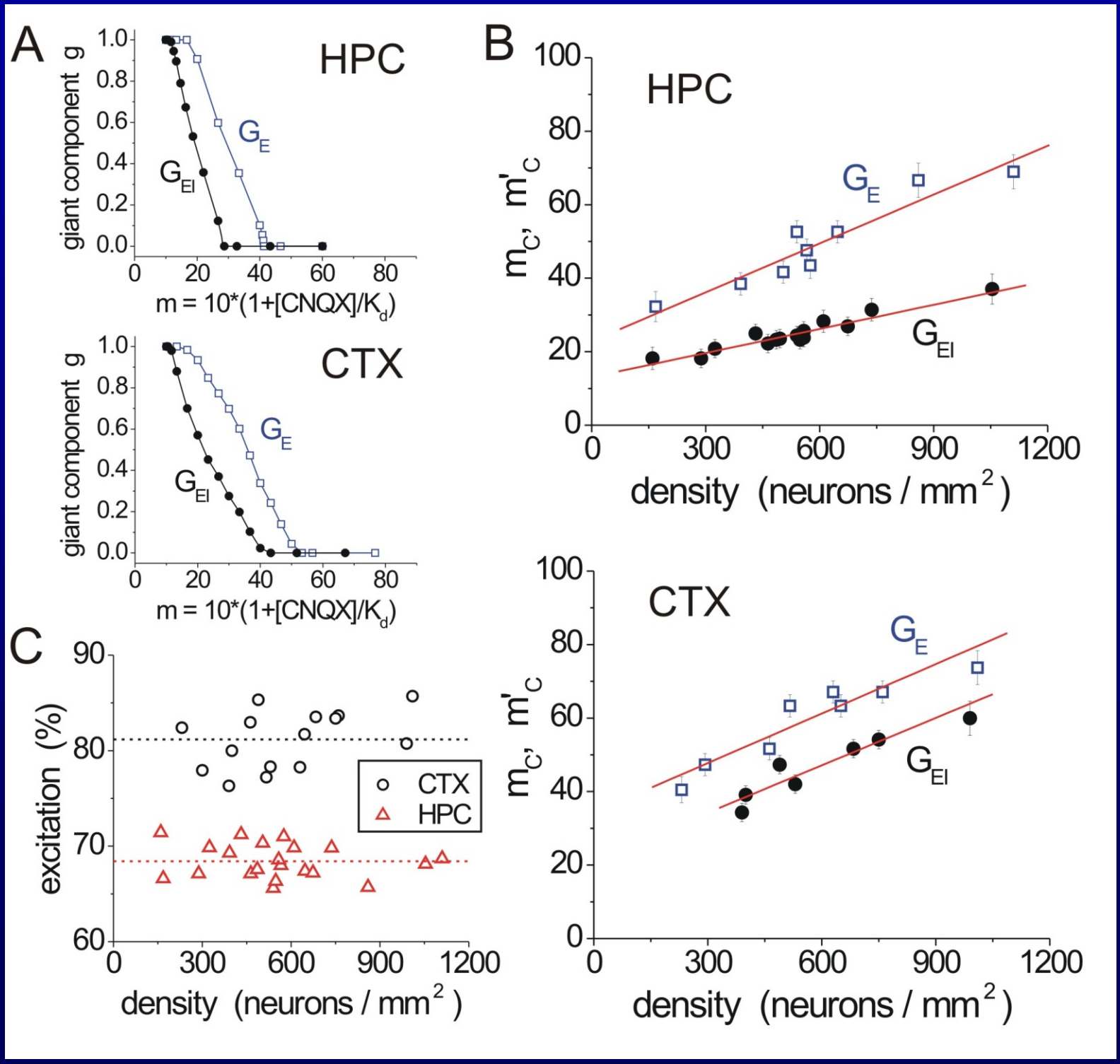
$$m_c = \frac{m_{EI}}{m_0}\ , \quad m'_c = \frac{m_E}{m_0}.$$

$$\frac{m}{m_0} = 1 + \frac{[\mathrm{CNQX}]}{K_d}.$$

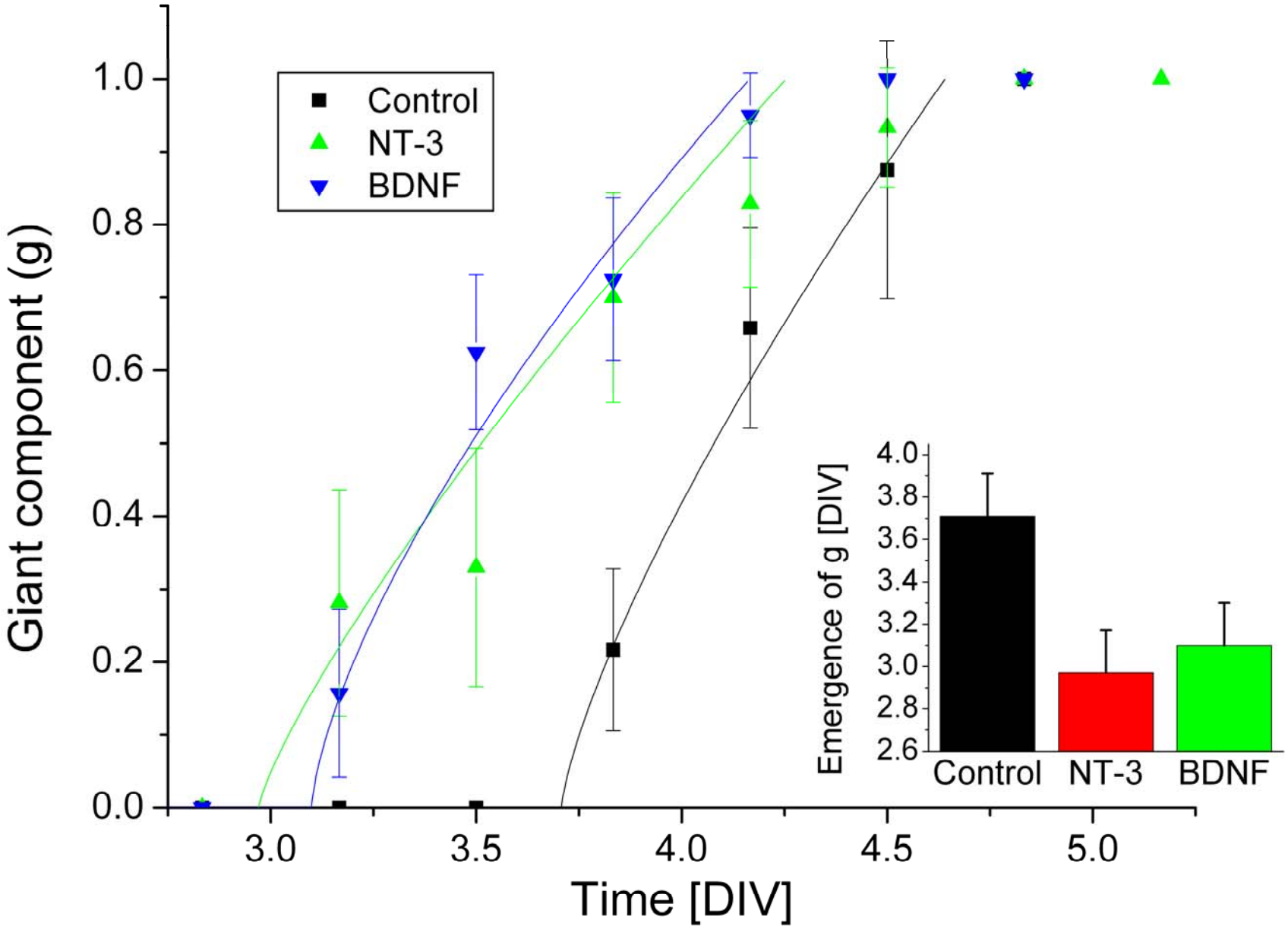
$$m_{EI} \simeq \bar{k}_E - \bar{k}_I\ , \quad m_E \simeq \bar{k}_E.$$



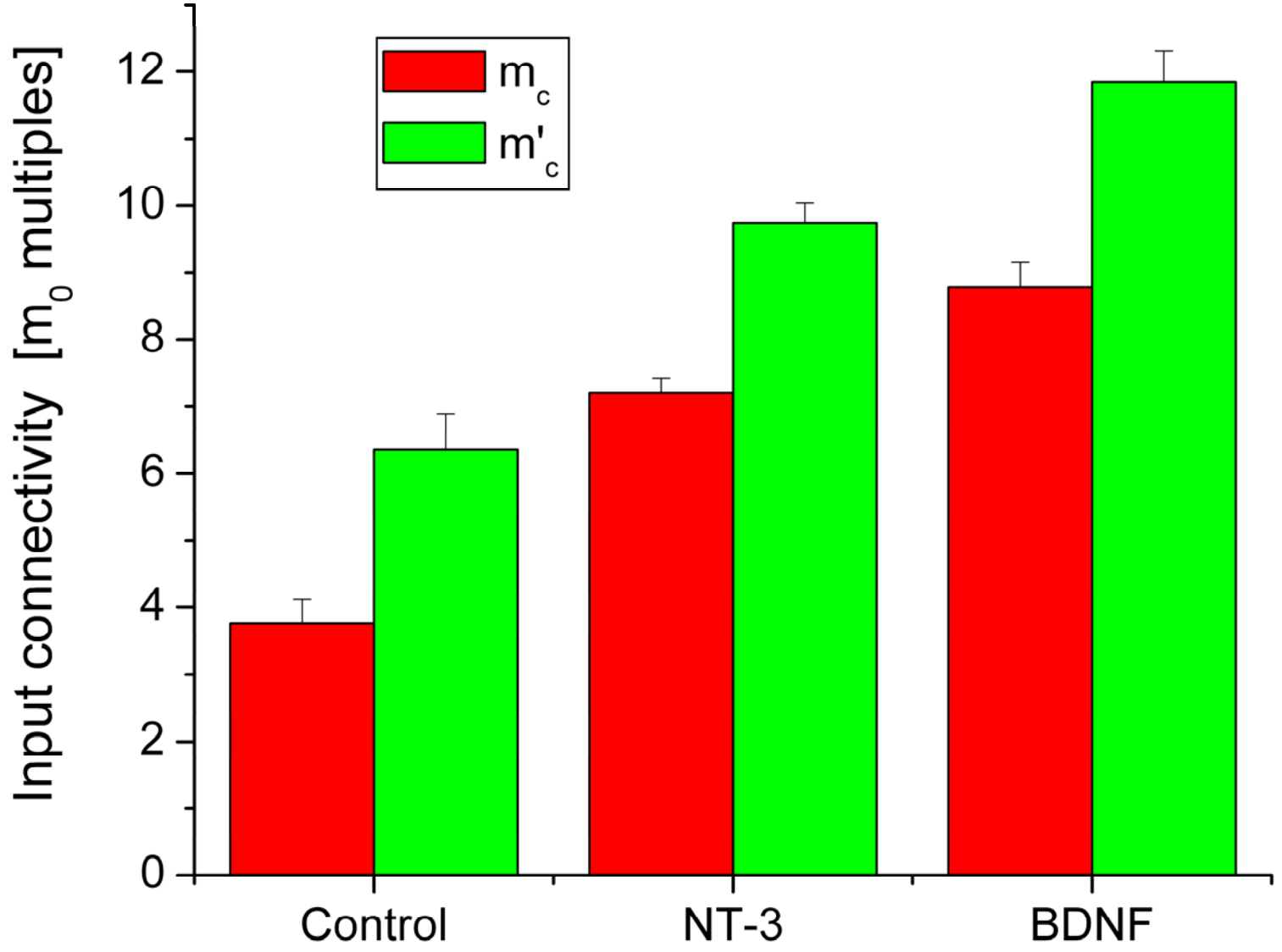



A
HPC
giant component g
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
G_E
G_EI
m = 10*(1+[CNQX]/K_d)
CTX
giant component g
m = 10*(1+[CNQX]/K_d)
B
HPC
m_C, m'_C
100
80
60
40
20
0
0
300
600
900
1200
density (neurons / mm^2)
CTX
C
excitation (%)
90
80
70
60
CTX
HPC
density (neurons / mm^2)

•Effect of neurotrophic factors in development



•Effect of neurotrophic factors in connectivity



Conclusions from Percolation

- 2d cultures fire together because they are highly connected.
- The connectivity is mostly random, with a gaussian like input degree distribution
- Geometry → IN degree distribution ~ Dendrite length distribution

  while OUT degree distribution ~ Axon length distribution

How do neurons decide to fire?

Burst Initiation in 2D Cultures

Leaders of Activity in 2D Cultures

Data from PotterLab/MaromLab/EMLab

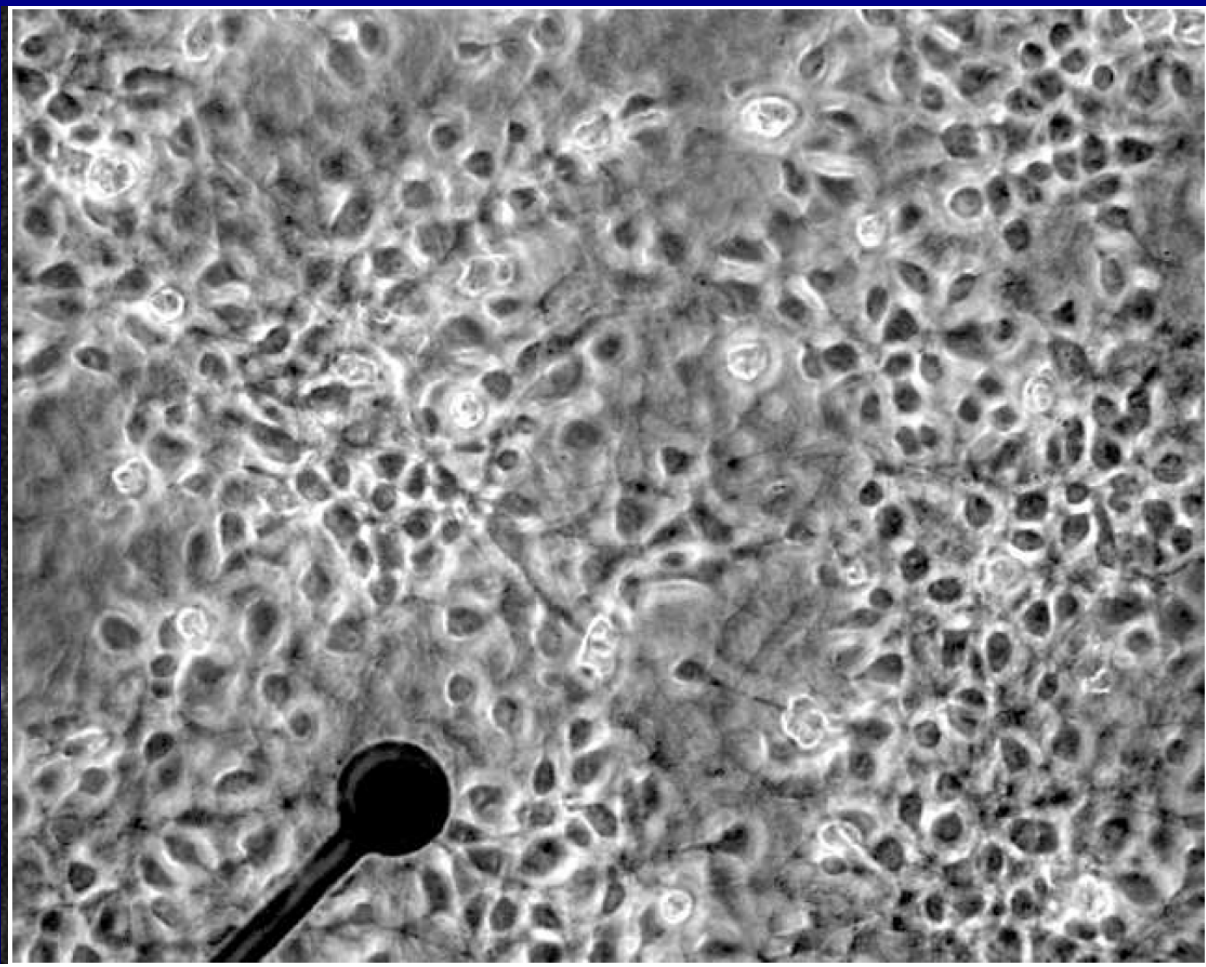
Culture self-organizes randomly

Spontaneous population bursts – pacemakers, or special connectivity ?

First-to-fire neurons (Eytan and Marom 2006)

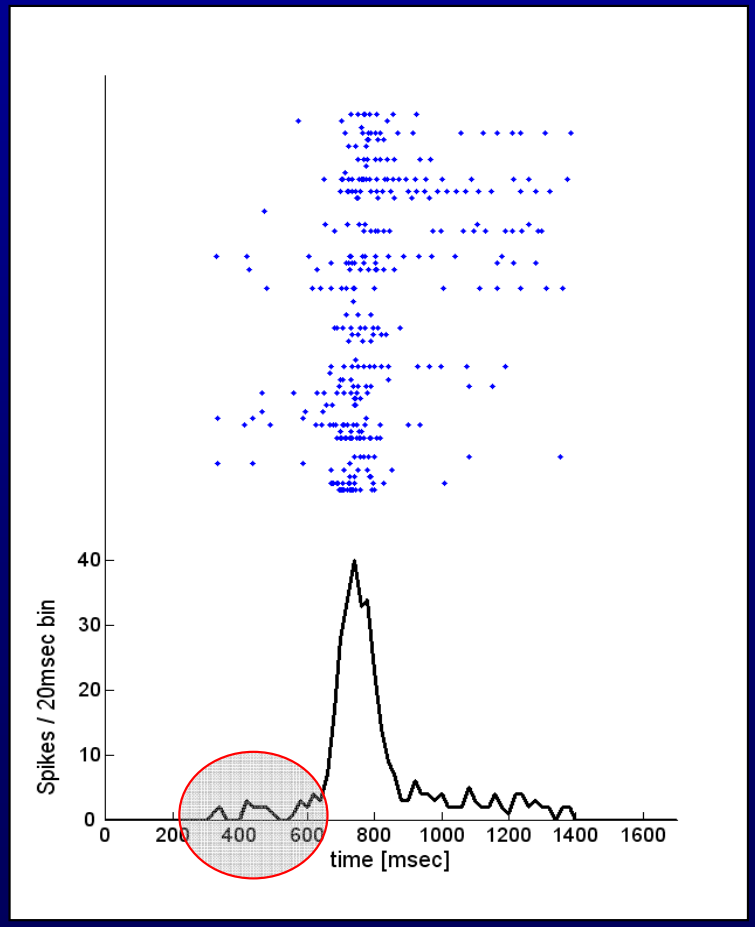
‘Firing patterns’ or ‘songs’ formerly detected in

- behaving monkey cortex, Abeles et. al (1993)
- brains slices, Ikegaya et. al (2004)



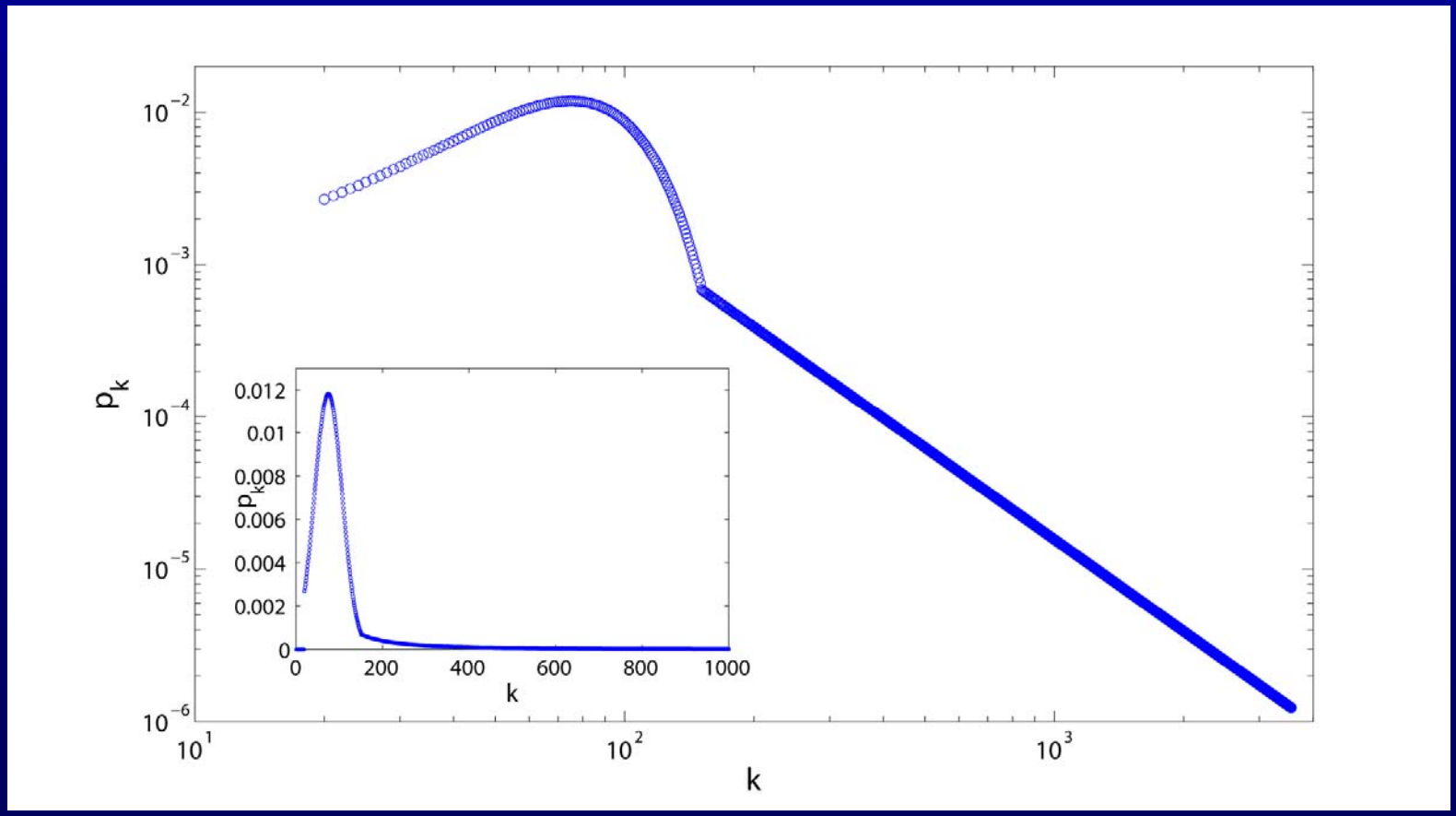
Spontaneous population burst

- Data from three labs analyzed
- Daily recording in ages of 5-40 days
- Population bursts detected ~1000 bursts / day
- First-to-fire neurons detected in any part of culture examined and found to be stable
- Marom and Eytan: Initial growth is exponential



Quorum percolation suggests that exponential is created because:

Leaders are a highly connected minority



And… they are everywhere

Conclusions from ‘Leaders’ study

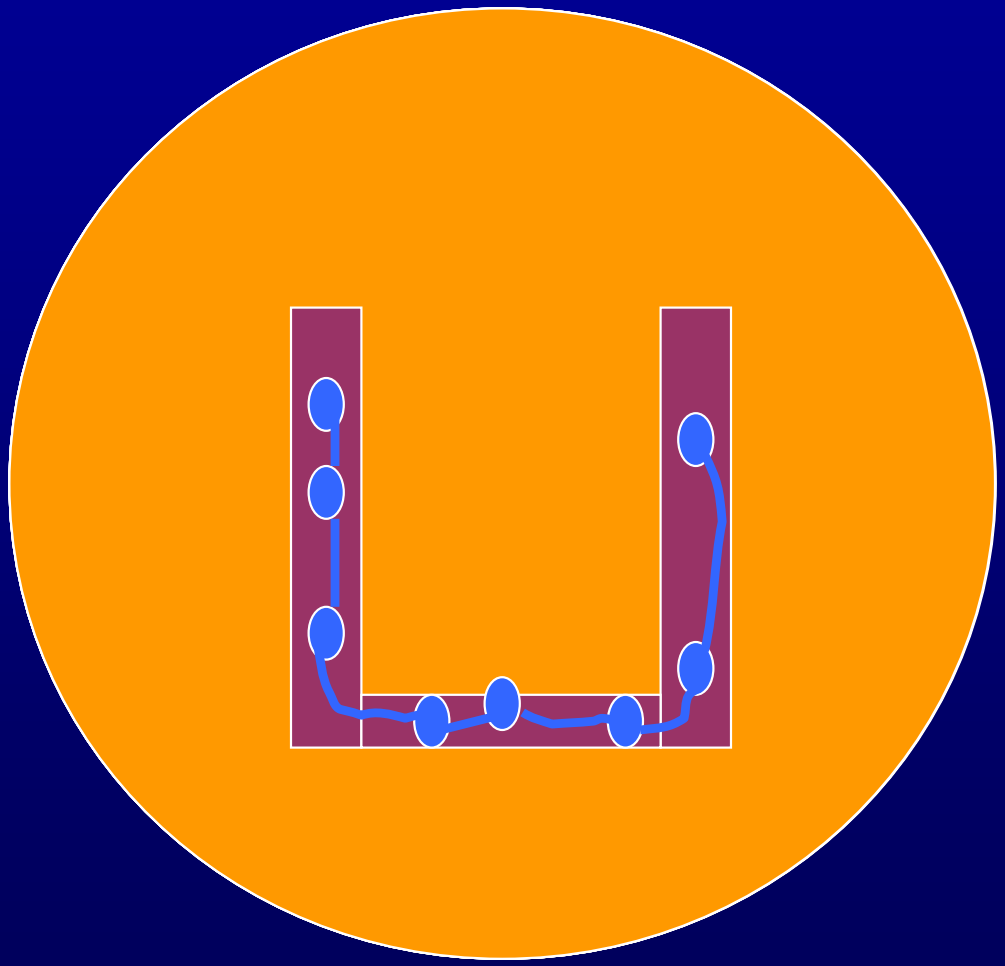
- First-to-fire neurons *lead* burst initiation
- Leader neuron sub-network is culture-wide
- Leaders are highly connected, belong to tail of distribution (k^2→ exponential rise time)
- → 2D cultures pose difficulties for study of fundamentals - burst initiation, information production and computation

A Physicist's Simplification
Go to 1D

- Must occur in brain - channels end up as linear decision making processes

- Funneling of information from high dimensional space into 1D

Sample Preparation



Glass Coverslip

Protein Repellant Coating

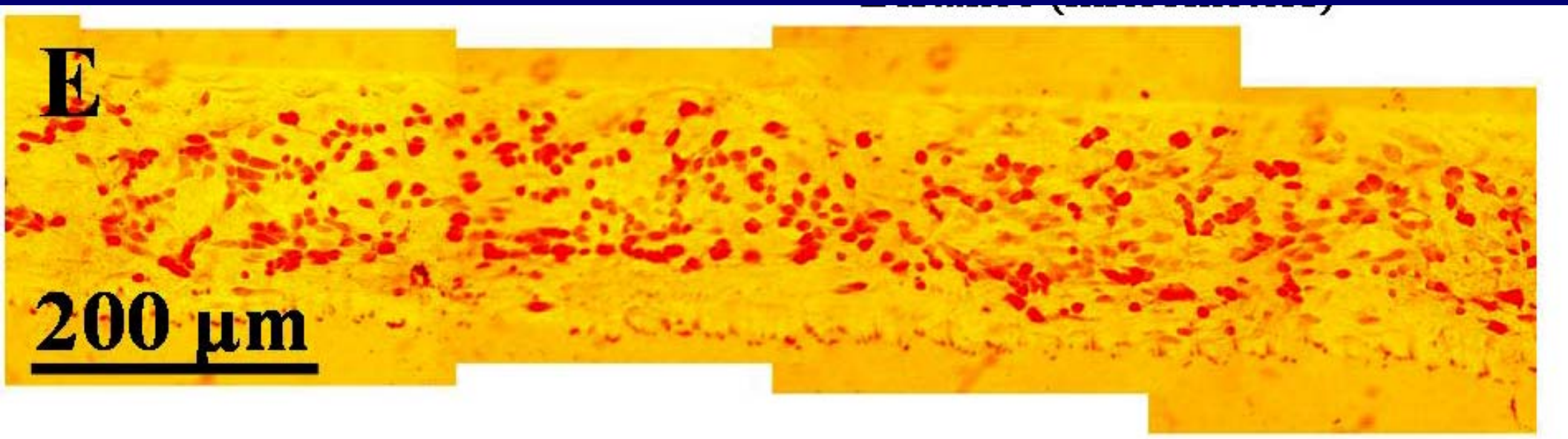
Adhesion Proteins

Neurons

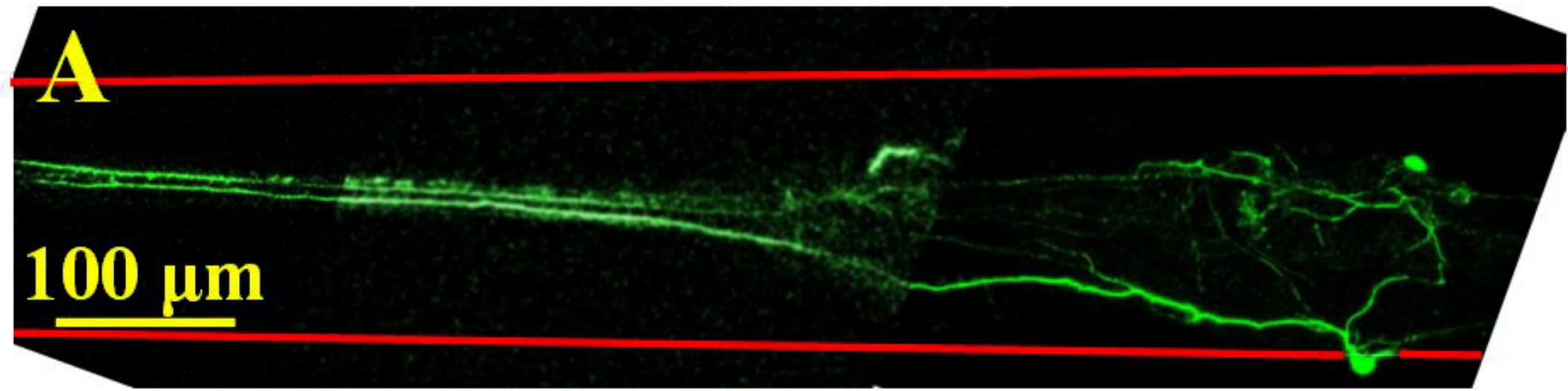
Neurons are stained with Neu-N (neuron-specific nuclear protein) to demonstrate density and distribution.

Linear density of 0.31 ± 0.02 neurons per µm

On thin parts of the line (50 µm width) neurons have difficulty attaching and preferably migrate to wider lines.

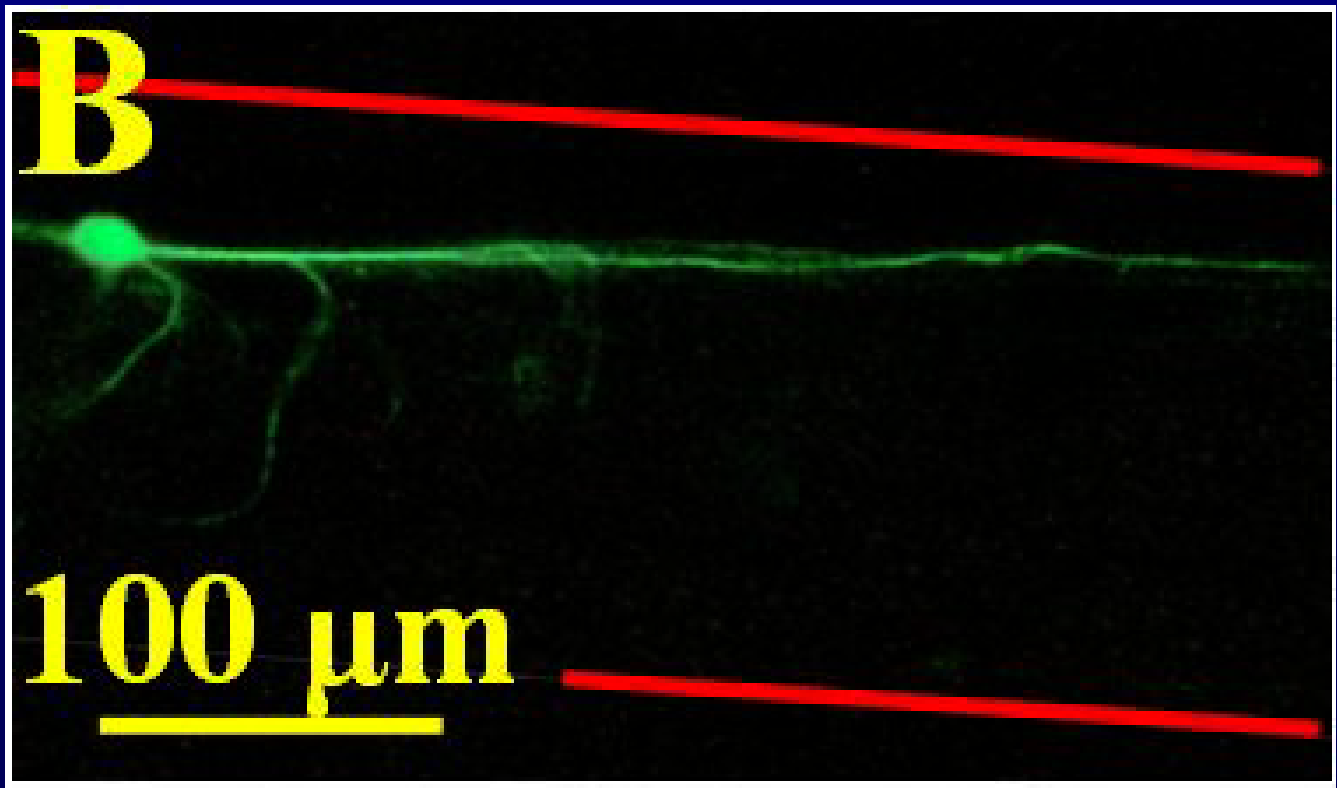


•τGFP - mark axon with fluorescence



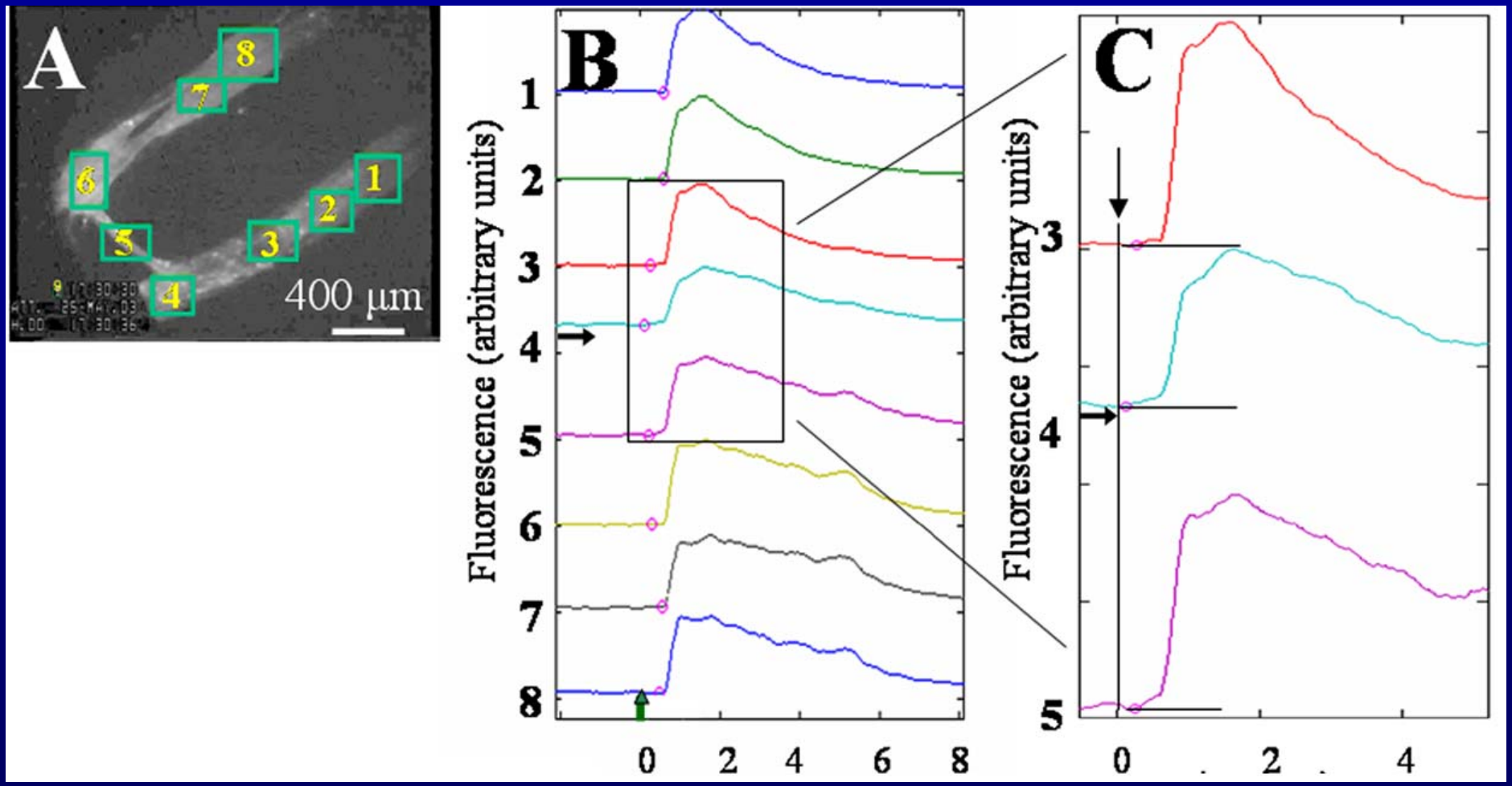
Mean length 360 ±18 µm, <6% reach 800 µm

Projected mean distance 308 ±16 µm, < 3% reach 800 µm



1. Propagation of firing activity

Response of a linear network to glutamate stimulation



A) Topology of the network in fluorescence (fluo-4). Fluorescence measured every 20 milliseconds.

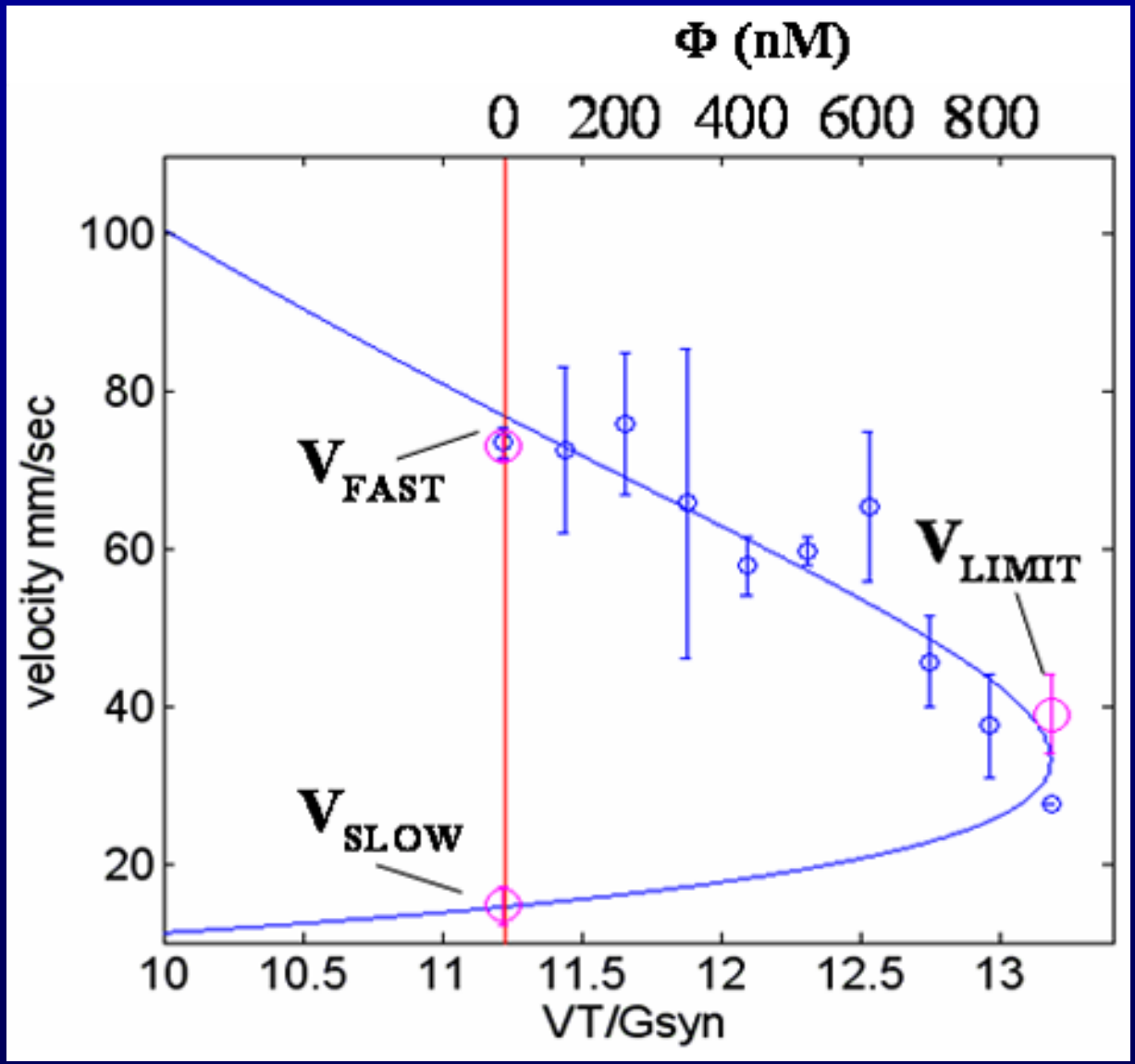
B) A typical event.

C) Enlargement of B). The two stages are evident, a slow asynchronous one that transits into a synchronous (synfire) one.

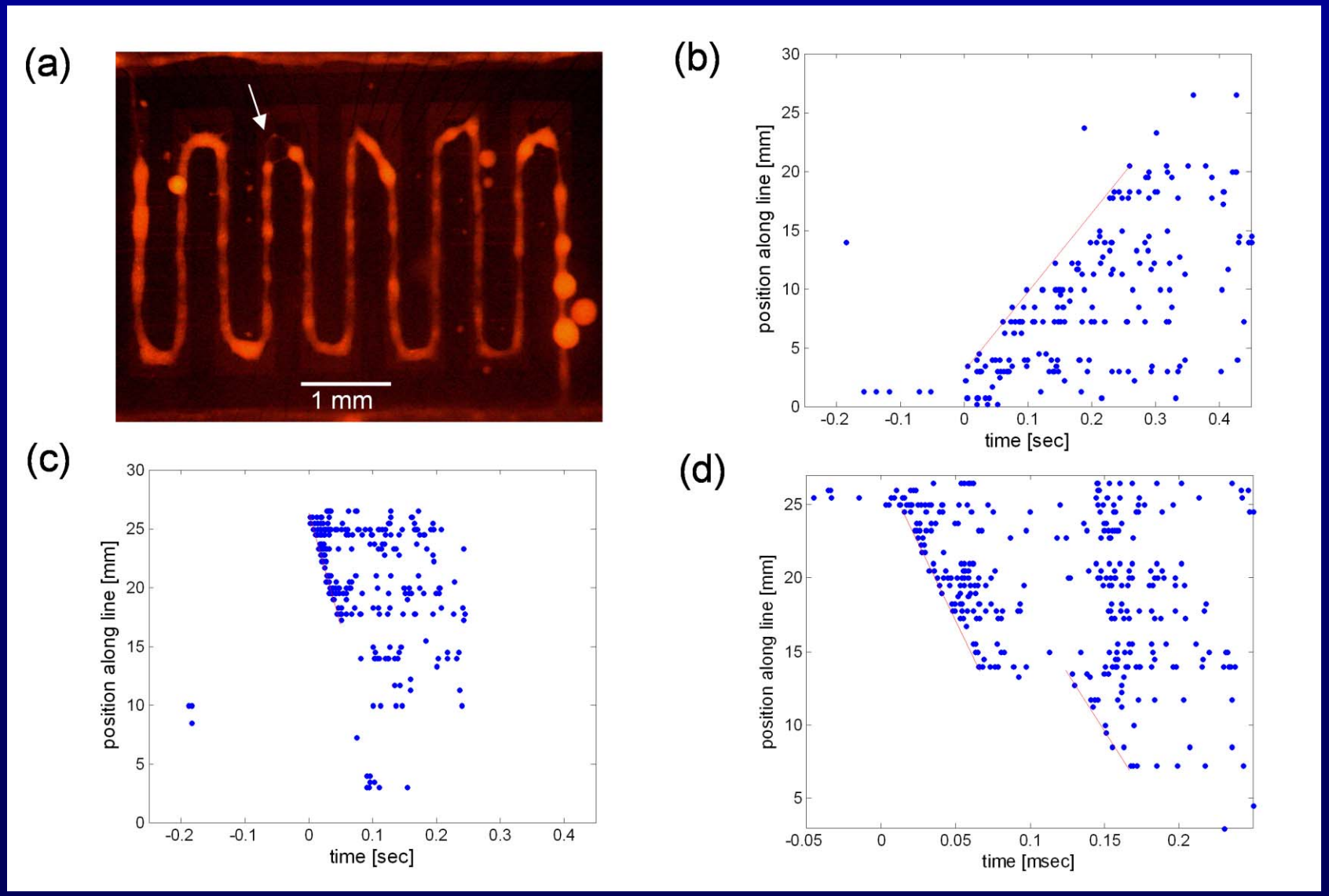
Compare to model of synaptic transmission (Osan and Ermentout)

Synaptic accumulation delays the advance in the initial stage and decrease its amplitude.

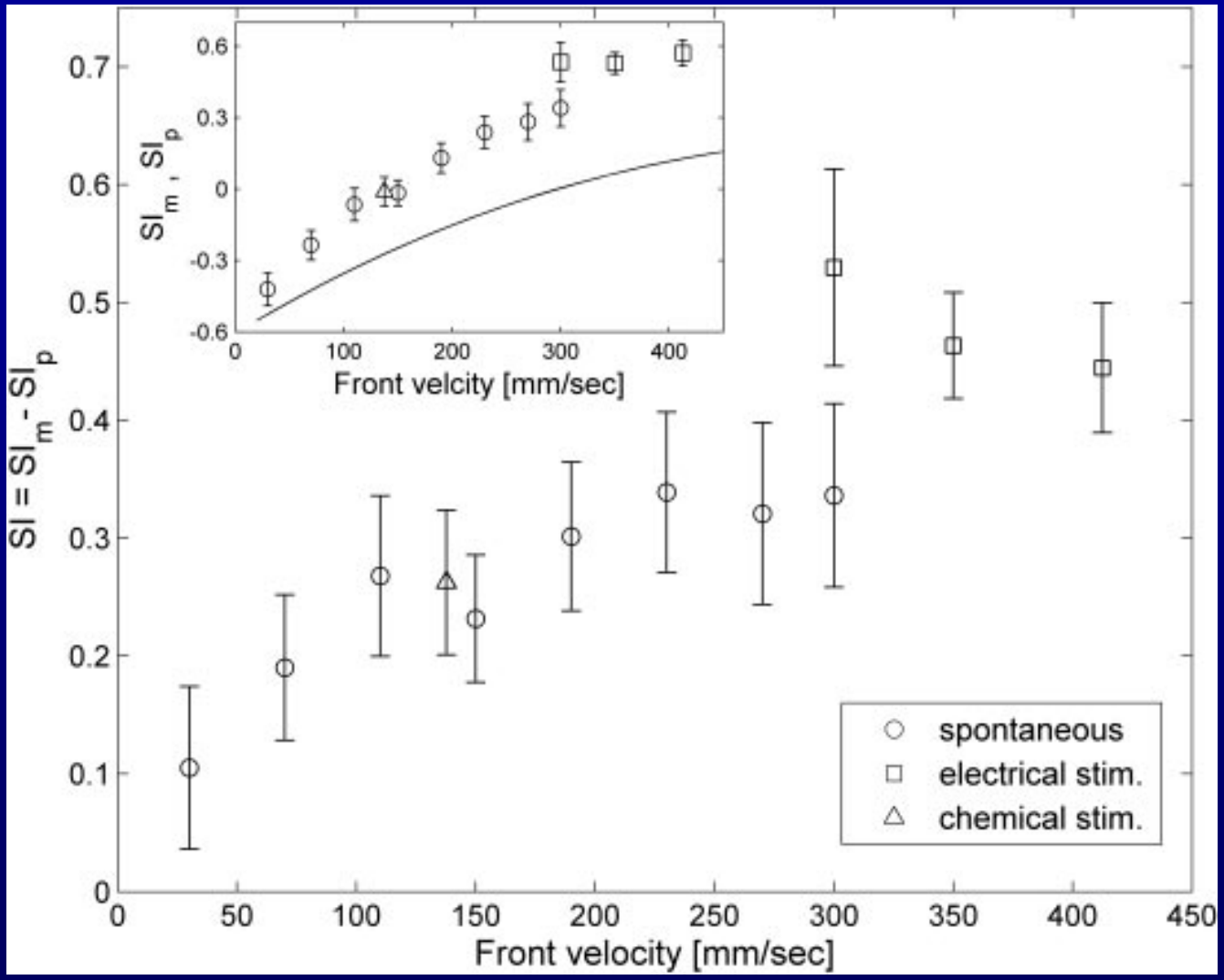
In the second stage **synchronization** occurs, and the wave accelerates significantly



Linear Patterning on MultiElectrode Array



Measure of Synchrony



SI is the fraction of electrodes that spike within 3 msec of the time that the front of excitation reaches them

2. Transmission of Information

Information constitutes the physical quantity (just like energy or momentum) that is actually transported in neural networks and in the brain.

Moving that information around the brain, manipulating and storing it are issues that lie at the basis of the study of neural activity.

Estimating Mutual Information

Once the synchronized burst is propagating, it can go for at 8 cm in our system.
How reliable is this signal?

==> Measure MI, and see how it withstands the passage of distance.

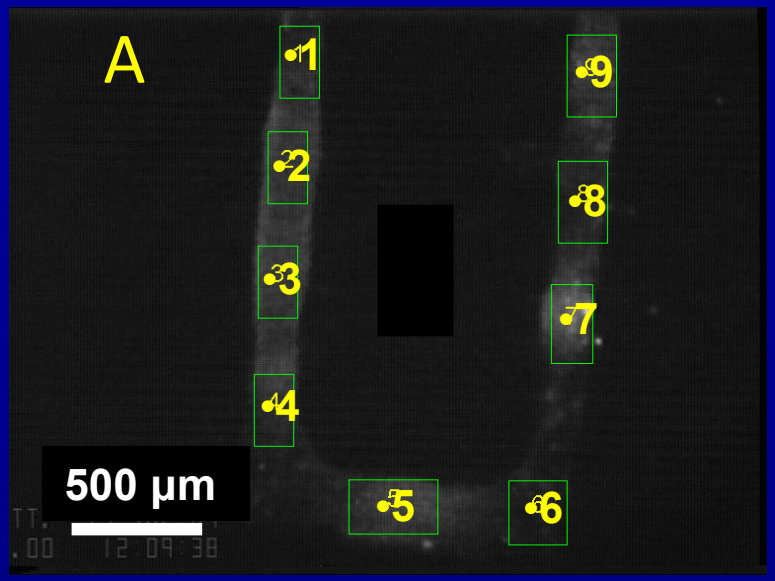
$$I(X,Y) = \iint dxdy\ \mu(x,y)\ \log \frac{\mu(x,y)}{\mu_x(x)\mu_y(y)}\ .$$

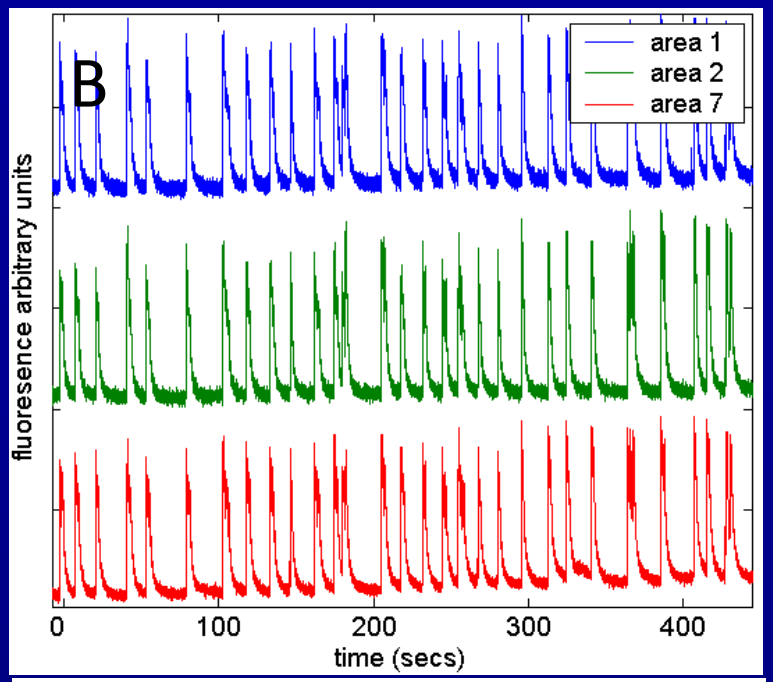
$$I(X,Y) \approx I_{\text{binned}}(X,Y) \equiv \sum_{ij} p(i,j) \log \frac{p(i,j)}{p_x(i)p_y(j)}$$

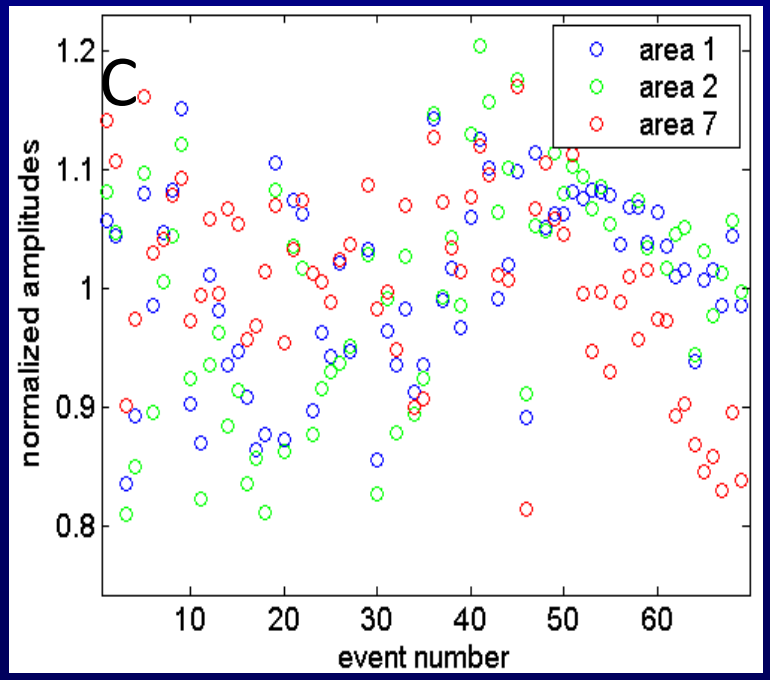
MI tells us how much information about X is obtained by measuring Y.

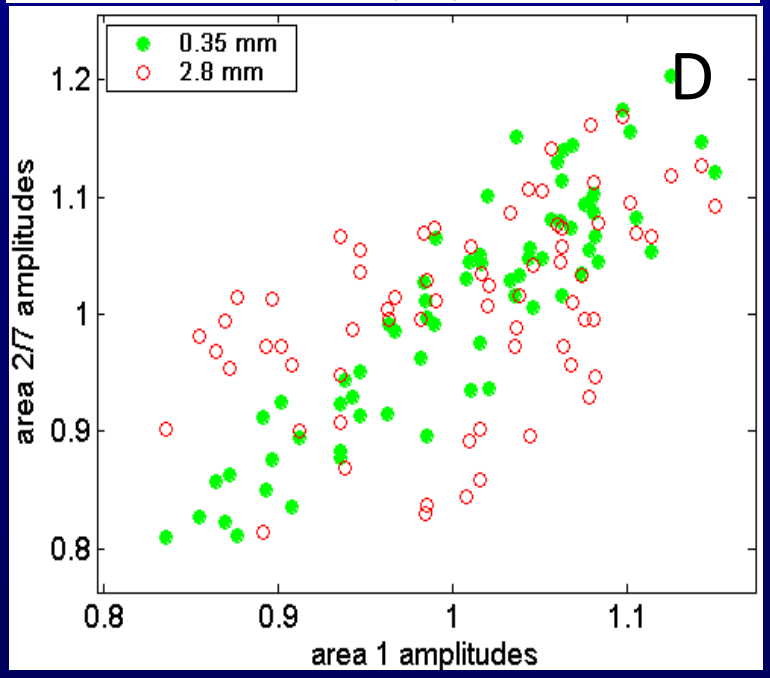
X,Y independent ==> MI=0

X=Y implies MI = regular information (or entropy)





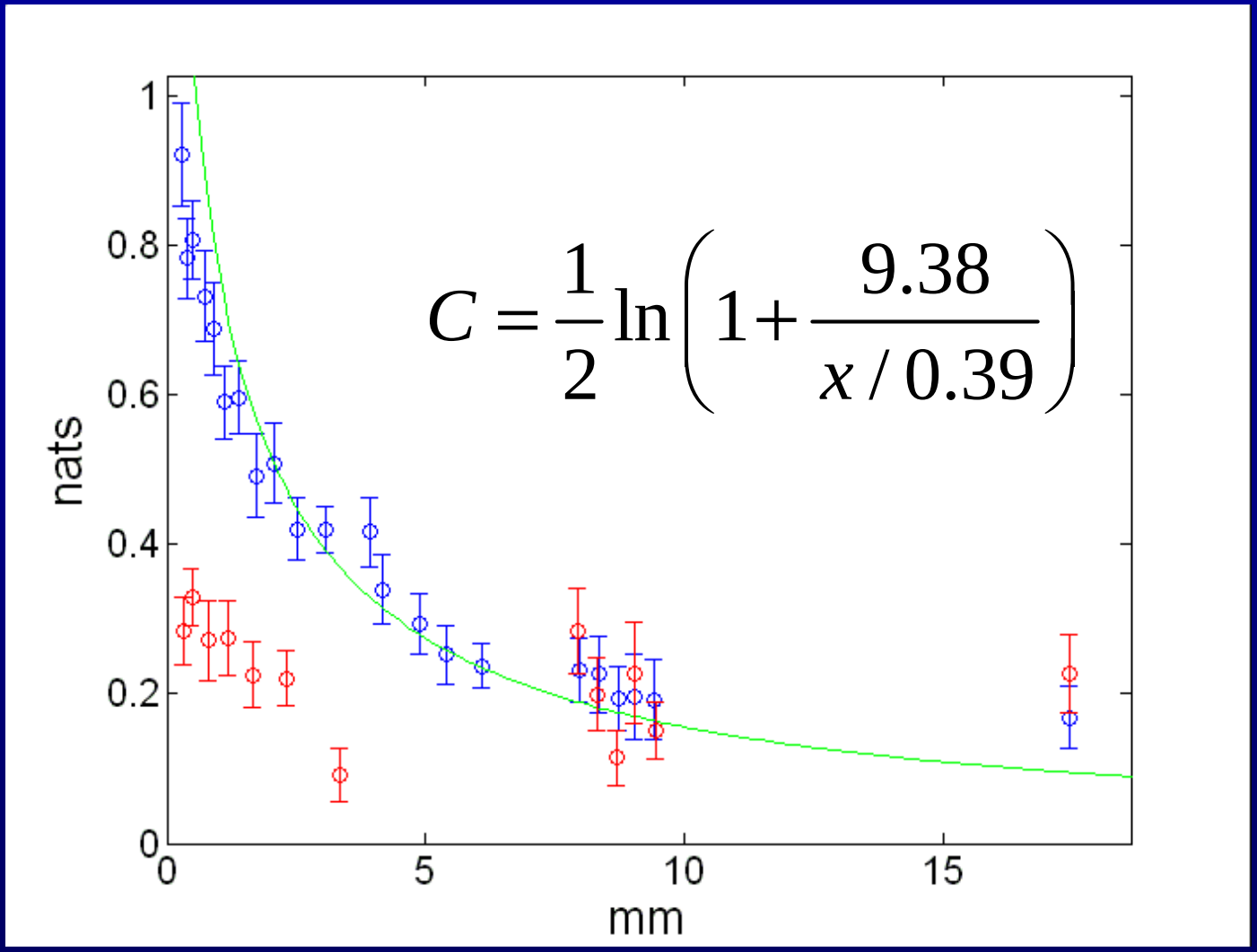




- Gain = 1
- Information in amplitude, not in 'time' = existence of spike

A) Regions of interest 1-9 **B)** Regions '1','2' and '7' **C)** 69 consecutive events
D) Amplitudes of '1' vs, same event in area '2' (green). Red dots for areas '1' and '7'.

Decay of Mutual Information as a function of distance



Mutual information between amplitudes at different distances along a 170 µm wide culture.

Green line - theoretical capacity of a **gaussian chain**, determined by measured signal / noise ratio of adjacent areas.

Red - 40 µM bicuculline ==> **inhibition** is important for production and transmission of information.

Can the brain use the amplitude (=spike rate) as a tool for coding messages on long pathways?

“Rate code” versus “Temporal code”.

Measured amplitude =number of neurons X number of spikes per neuron (=spike rate). The strong decay of MI indicates that this is not a reliable code.

The signal always manages to pass. The span between two signals may survive.

Temporal coding may work.

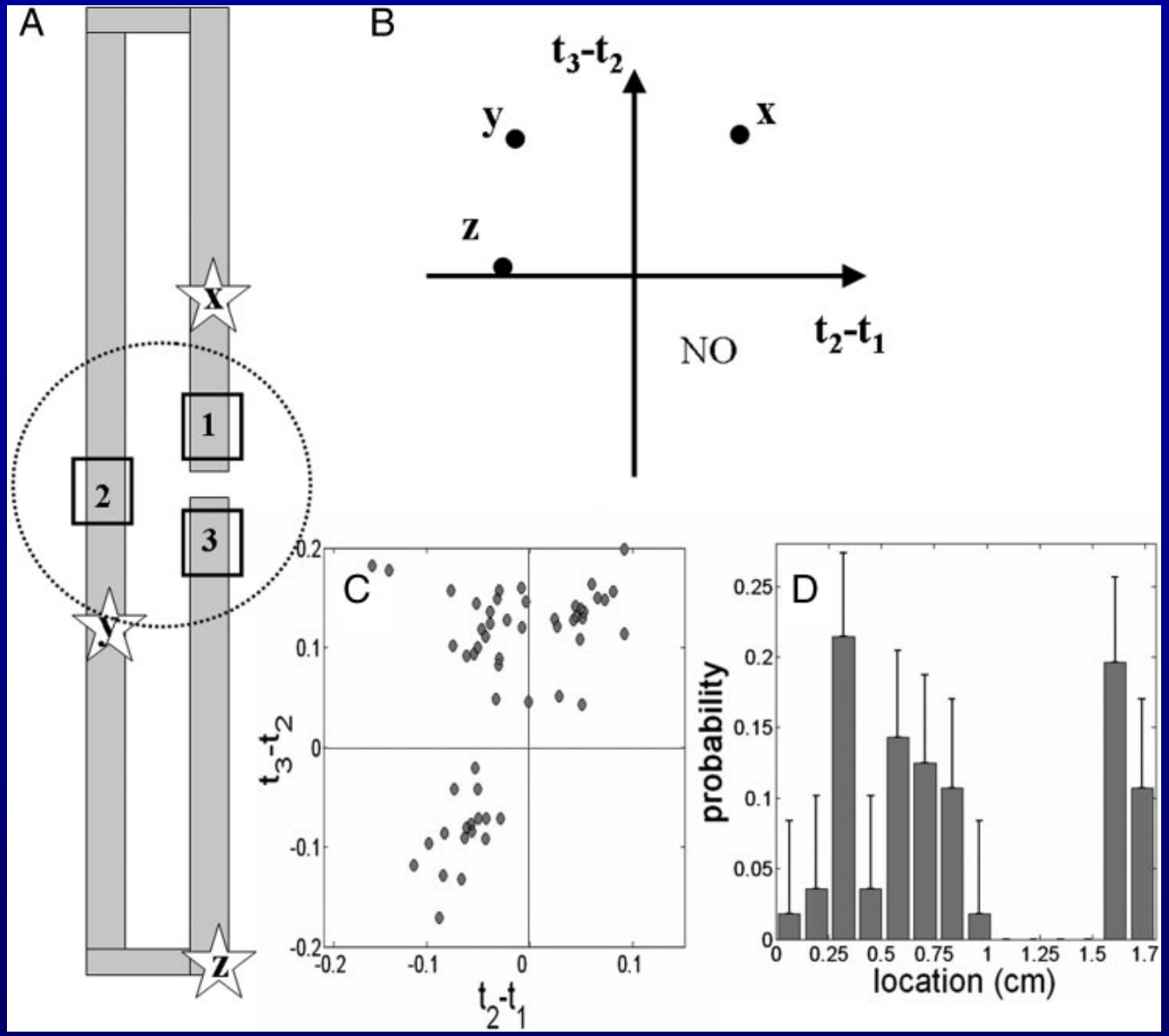
Amplitude or rate coding simply will not survive long distances and the multiple synaptic transmissions associated with it!

3. Pacemaking Regions in Spontaneous Activity

The Initiation Problem (Epileptiforms??)

Synchronization of large scale areas - Distribution of pacemakers

Up to now - not known if and how pacemakers are distributed - advantage of 1D over 2D.



Pacemakers on a 17mm long line.

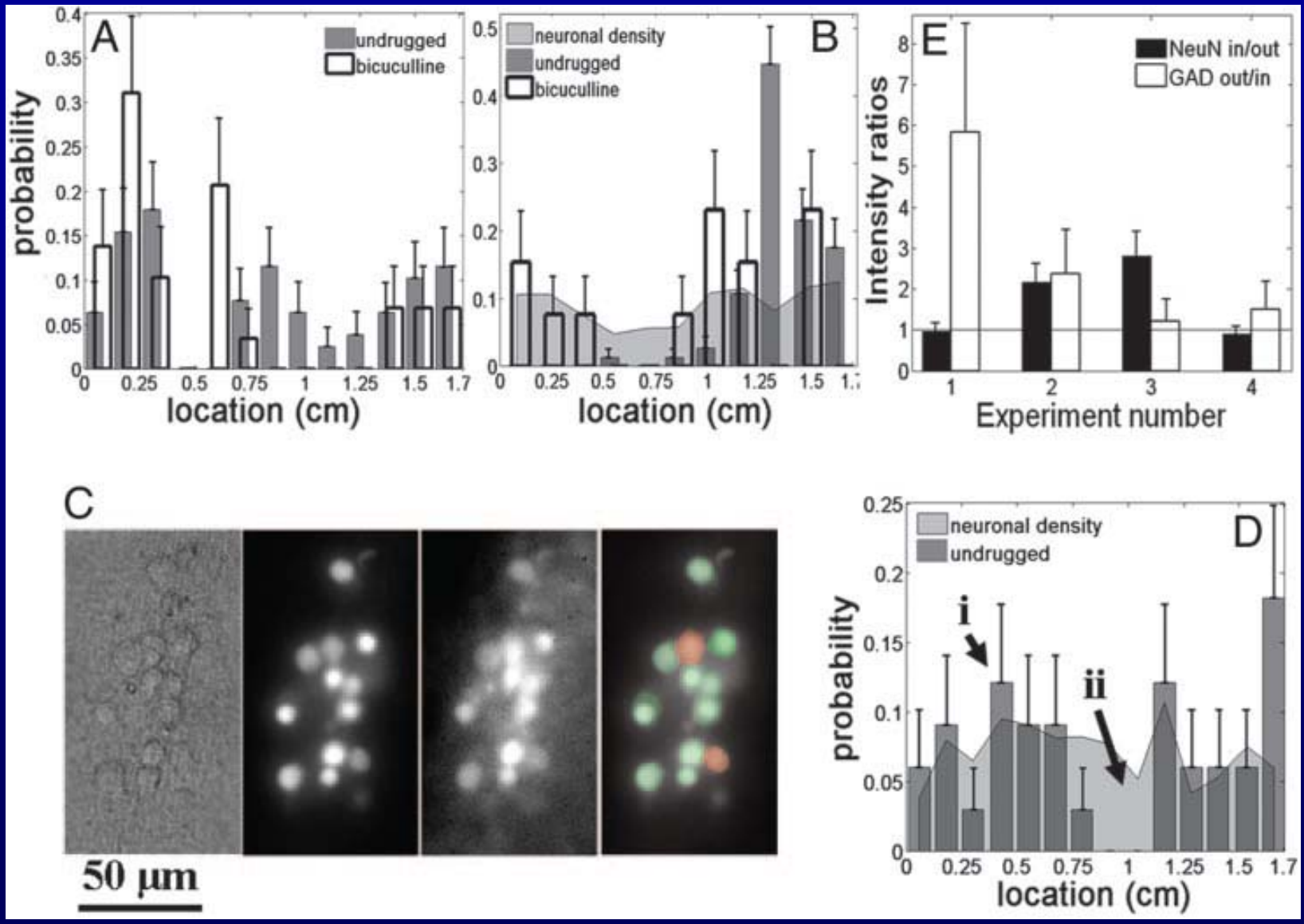
A. A schematic sketch of the line.

B. Allowable time delays

C. Time delays measured for a specific experiment.

D. The probability of each area to initiate a burst, as calculated from the 57 time signatures in C. Three sources are evident.

The effect of network properties on the distribution of spontaneous sources



Correlate density of excitatory or Inhibitory neurons with BIZ location.

Initiation zones are associated with high neuronal density and low inhibitory neuron density.

What we have learnt up to now:

- Information is the central quantity we should measure, equivalent to mass, energy or force in the standard physics system.

- To control the flow of information we do not have to control every neuron, but rather it can be done by geometric means (constriction in space). Given the right spatial constraints computation may be a self-emergent property of the network.

Using what we have learned from 1D and 2D to build Neuronal Devices

Use the threshold m_0 to manufacture logical devices.

Attain ‘serial’ logic by going to ‘linear’ cultures.

Problem: Neuronal transport is unreliable at the synapse. Need more than m_0.

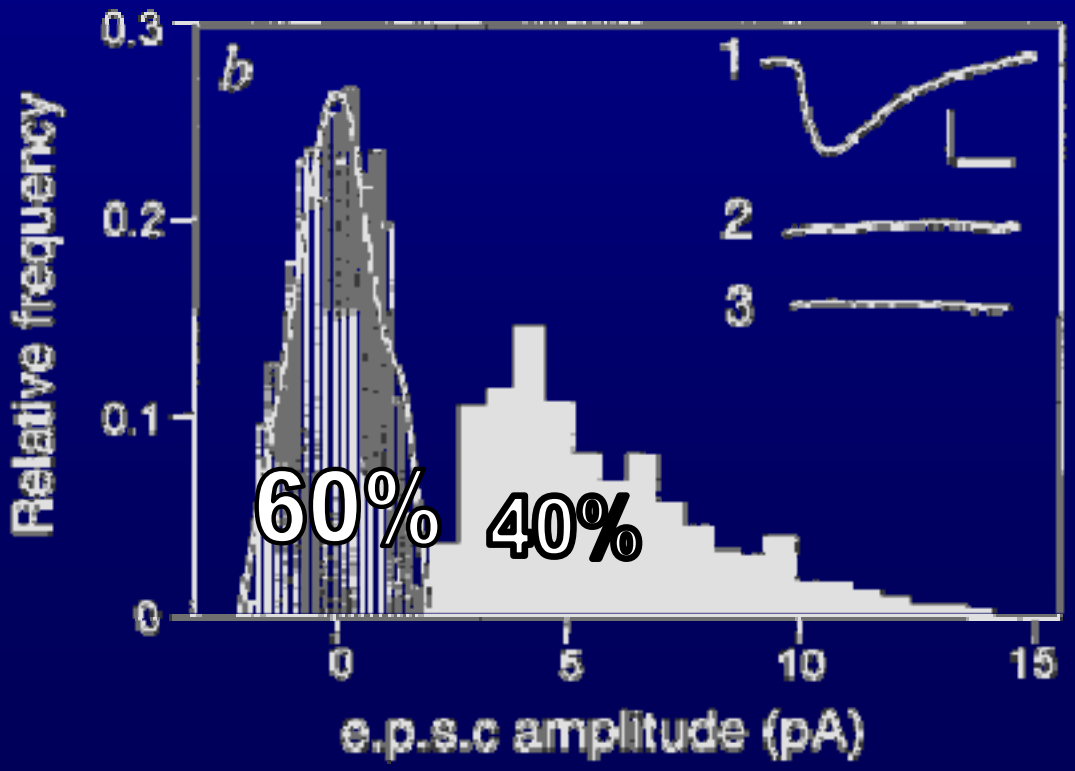
Synaptic connections are unreliable

Kleppe & Robinson, Phys Rev E 2006

Probability of failure:

$\eta = 0.6$

(Stevens & Wang, *Nature* 1994)



Min # of inputs for a spike:

$m_o \sim 15$

(Thomson et al. J Neurophysiol 1993)

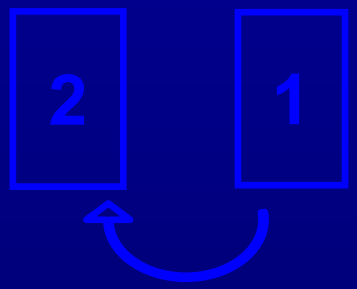
Using *geometry* to guide neurons and build Neuronal Devices

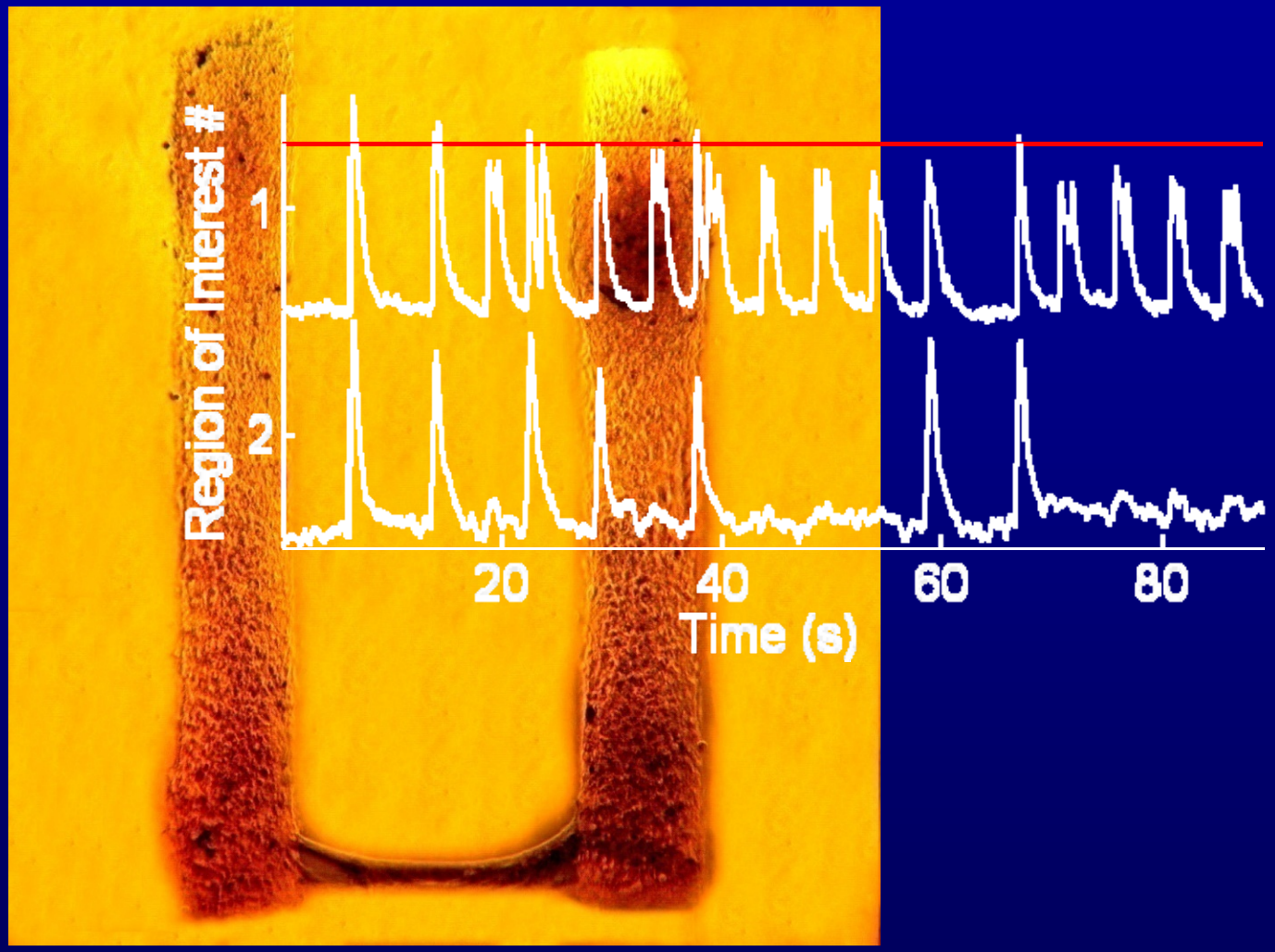
1. Funnel information by geometric constructions

2. Use the threshold m_0 to manufacture logical devices. Attain ‘serial’ logic by going to ‘linear’ cultures.

Note: Neuronal transport is unreliable at the synapse. Need more than m_0.

Threshold device

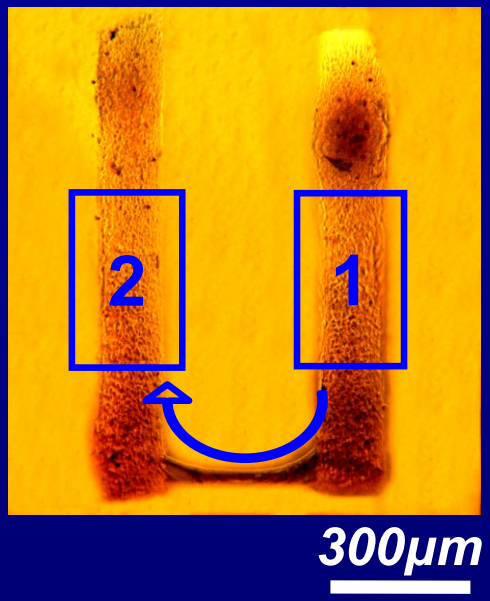


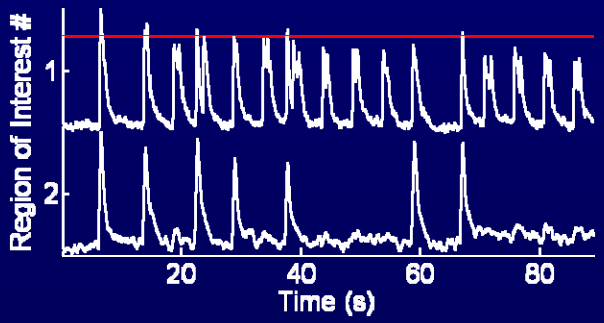


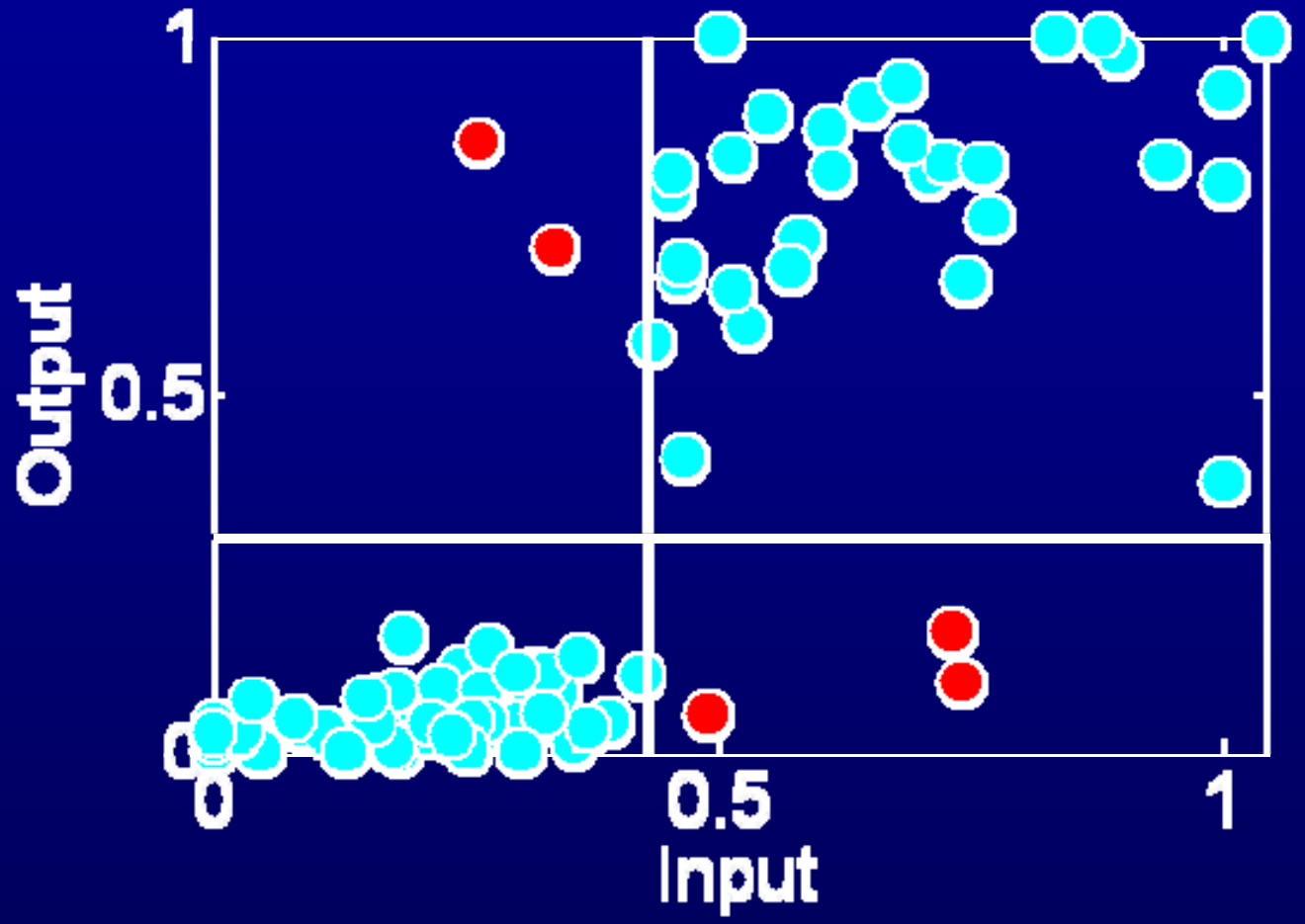
300µm

Thin connection serves as a ‘bottleneck’ through which only a limited number of axons (and no neurons) pass

Threshold device

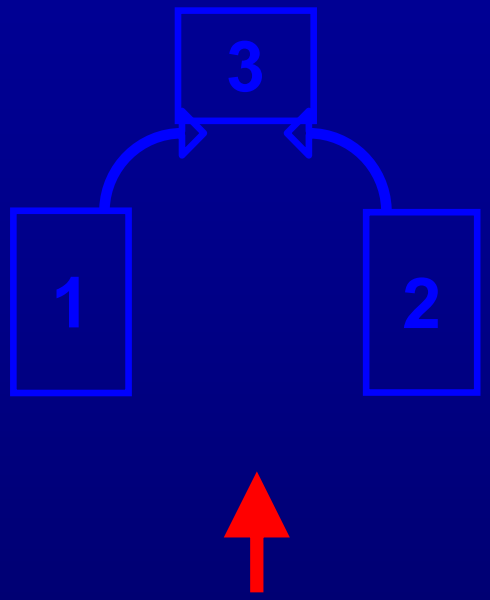


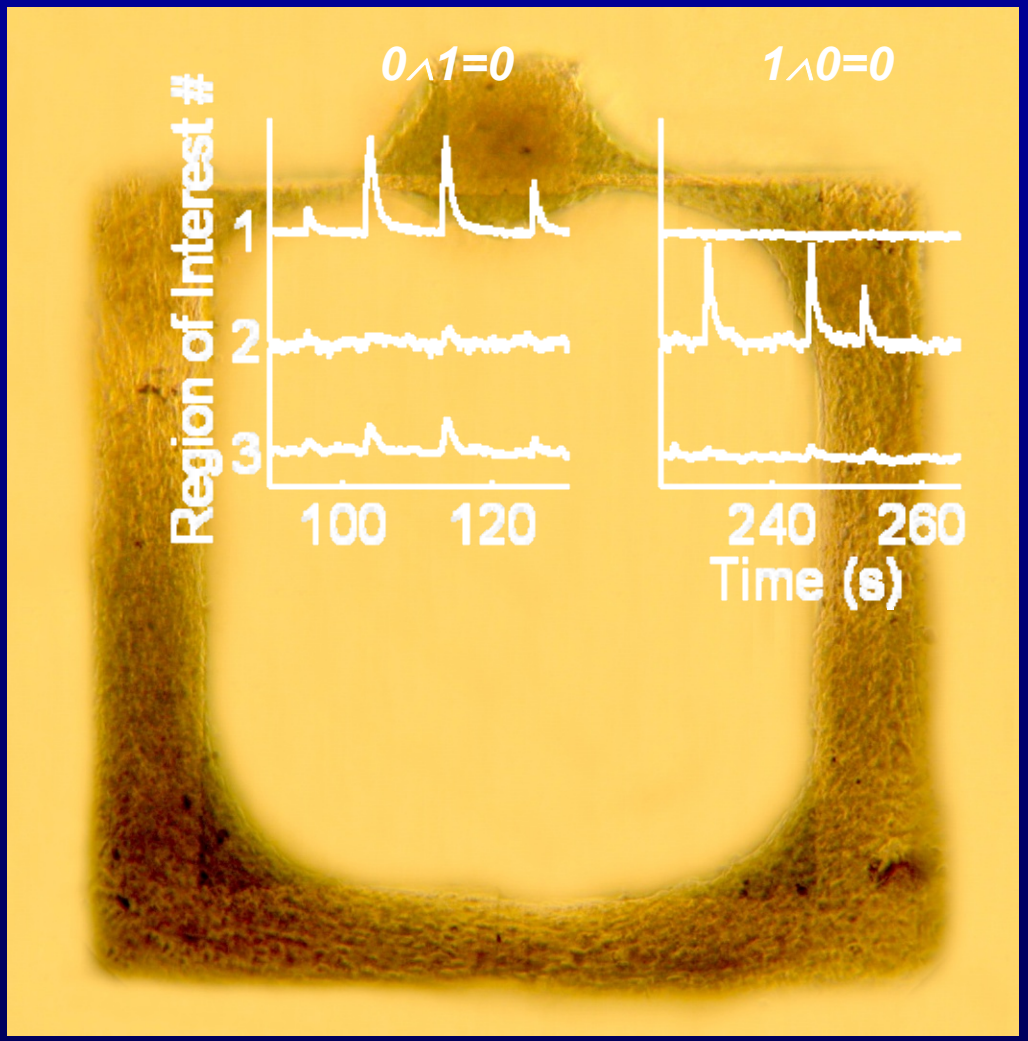




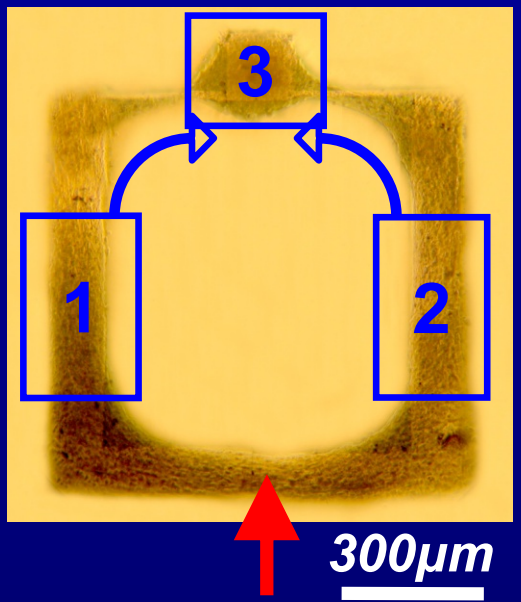
Reliability > 94%

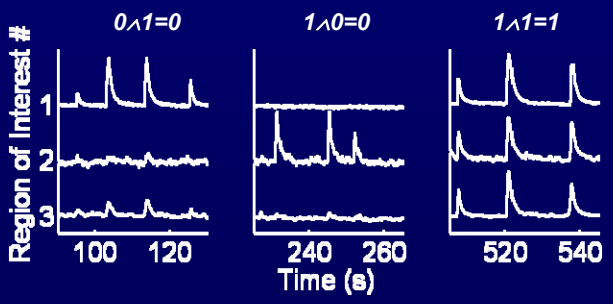
AND gate

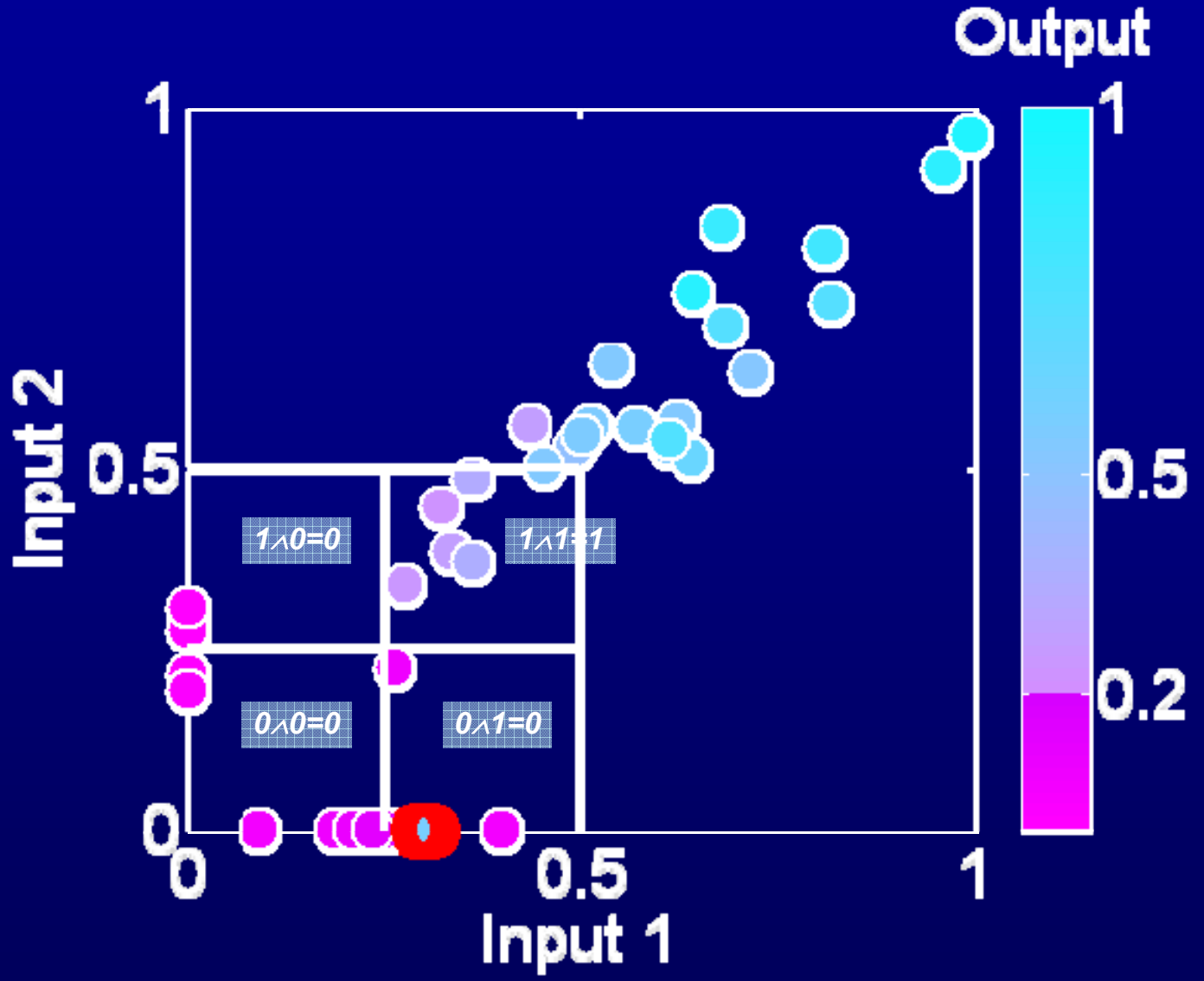

3
1
2


0∧1=0
1∧0=0
1∧1=1
Region of Interest #
1
2
3
100
120
240
260
Time (s)
520
540
300μm

AND gate



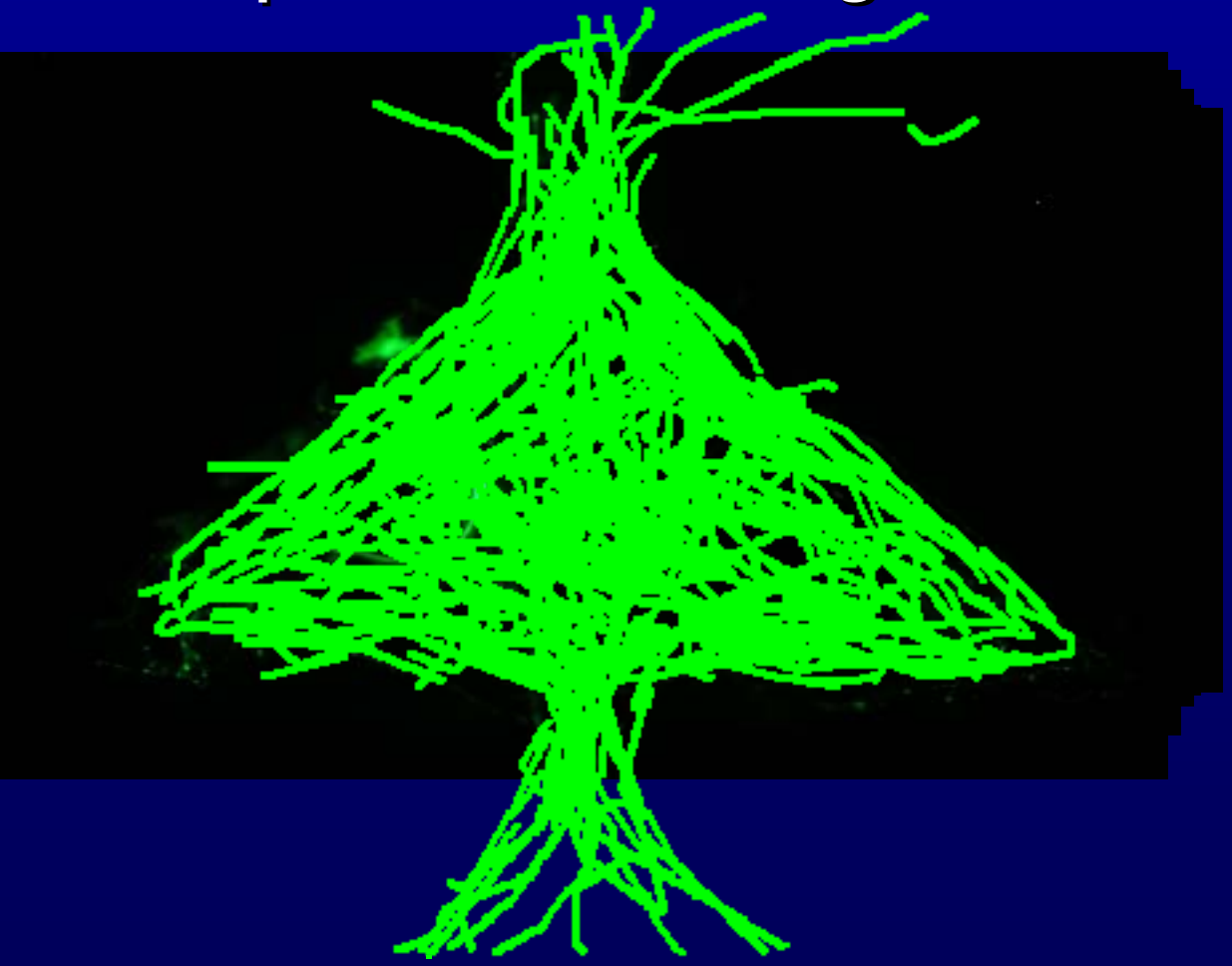


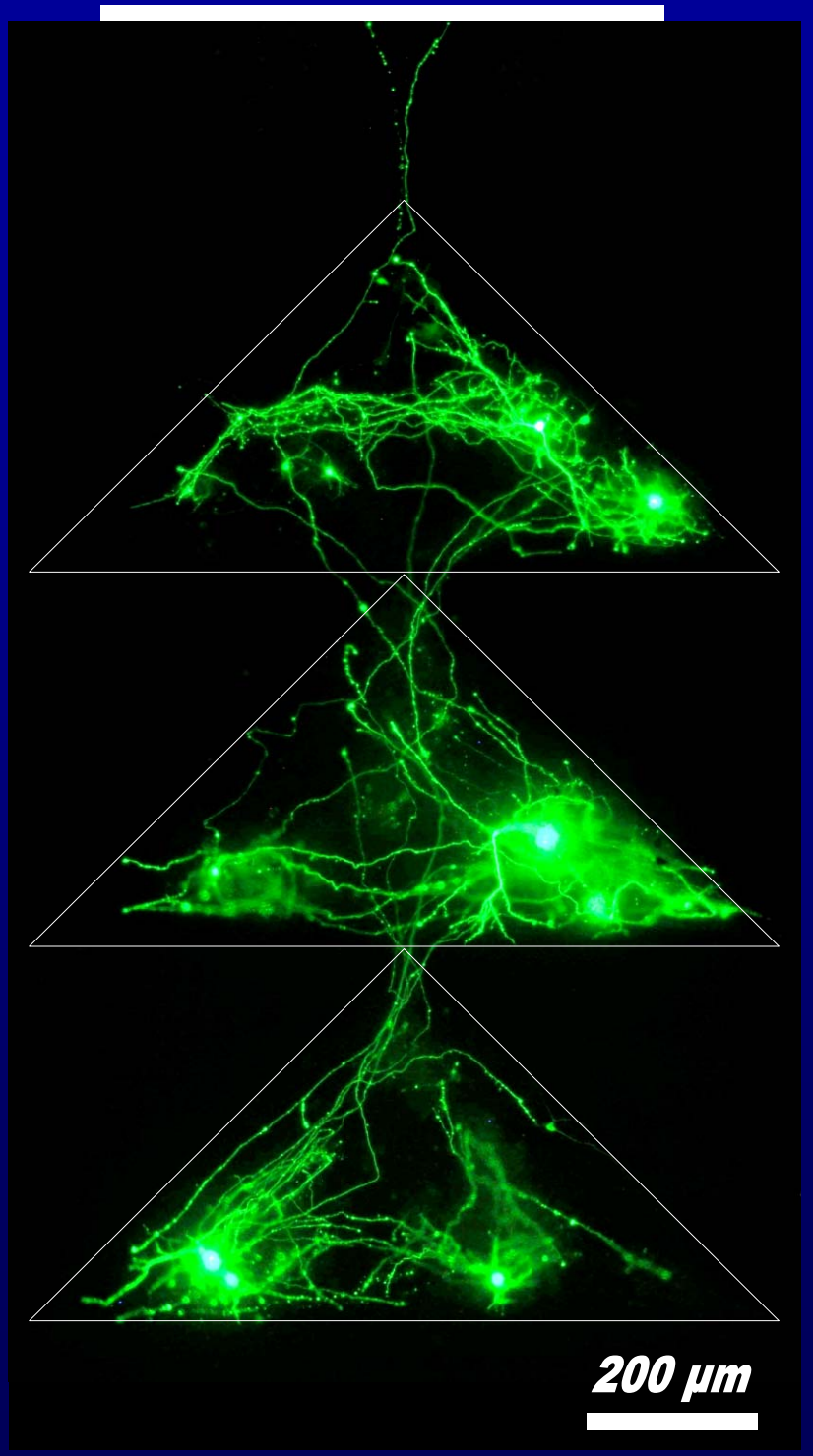


Reliability > 94%

Directed informational flow

Axonal guidance by spatial funneling

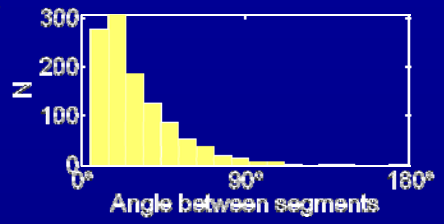




Simulation of axonal growth

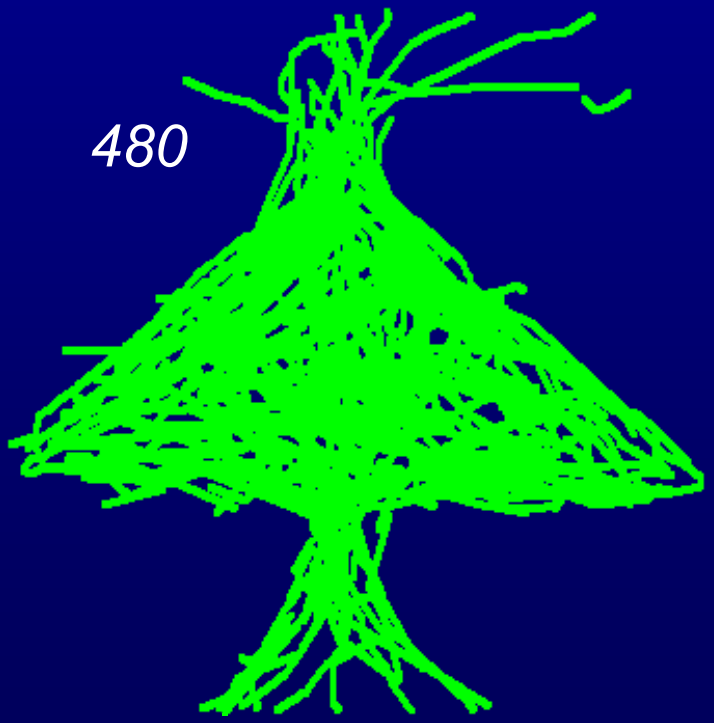
Axon propagation rules:

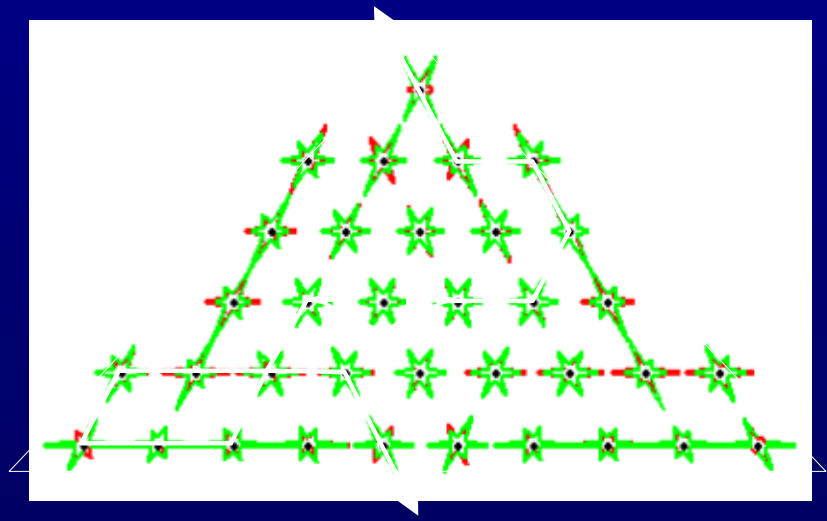
- Stays in the lines
- Rarely turns



360±120

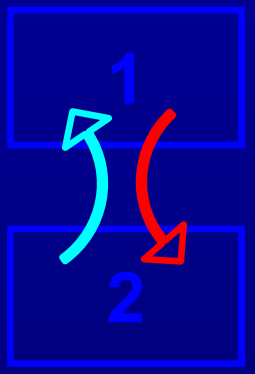
480

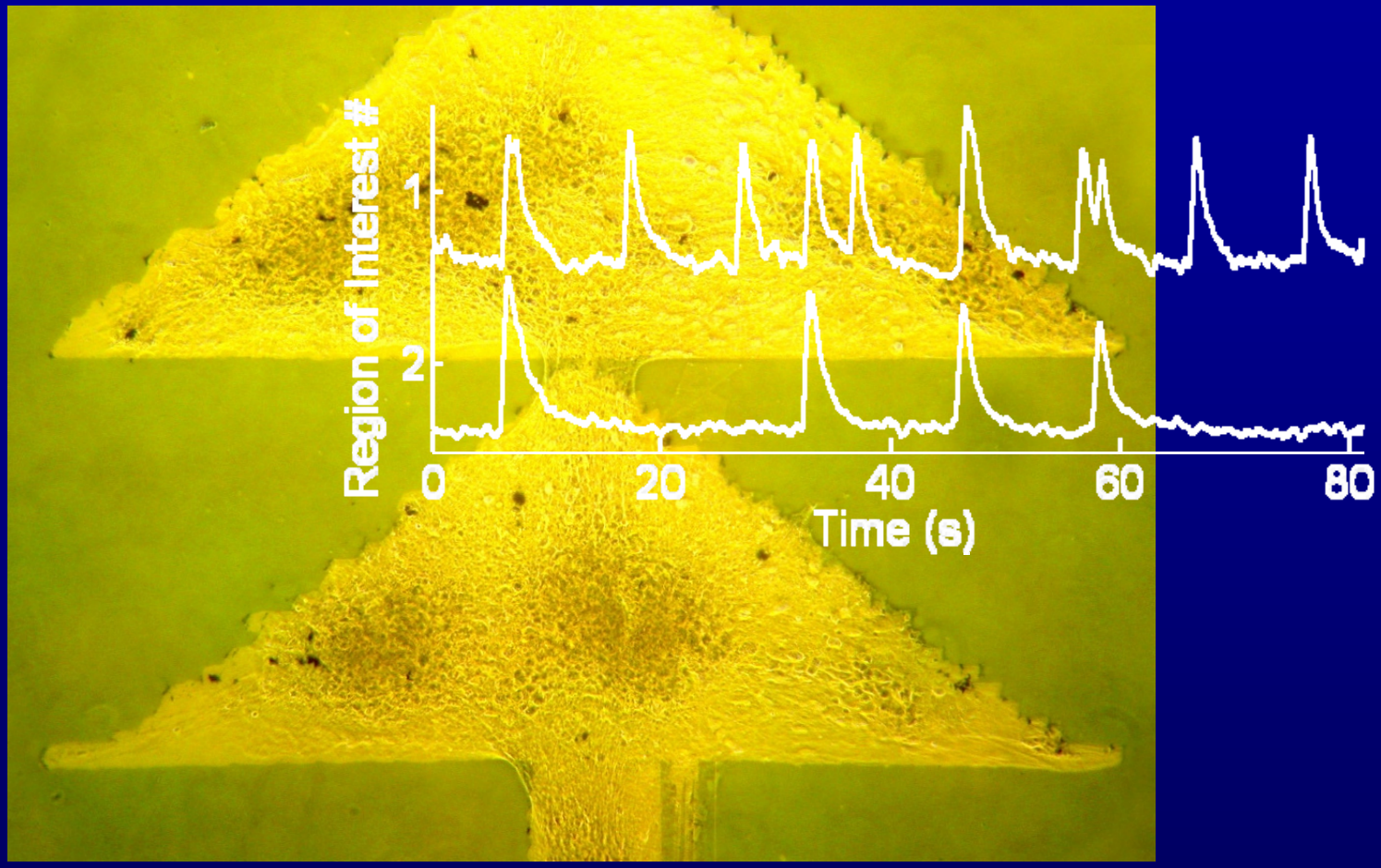




120±40

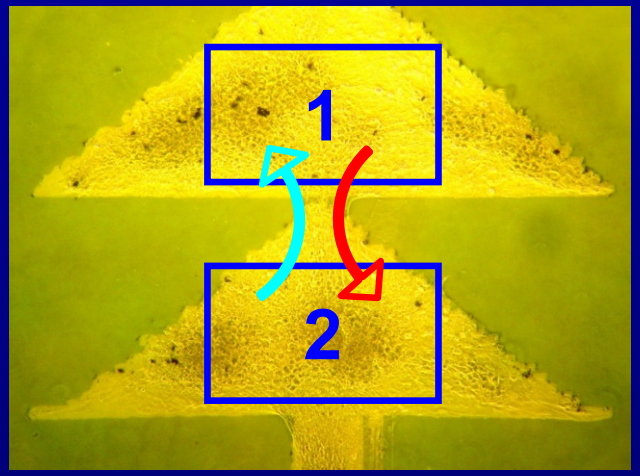
Neuronal Diode



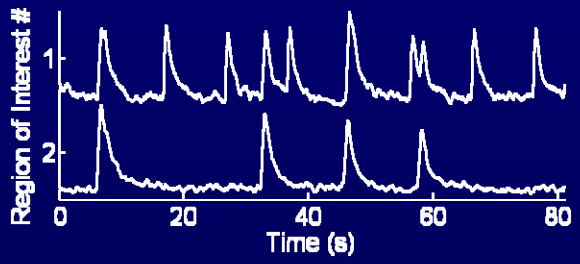


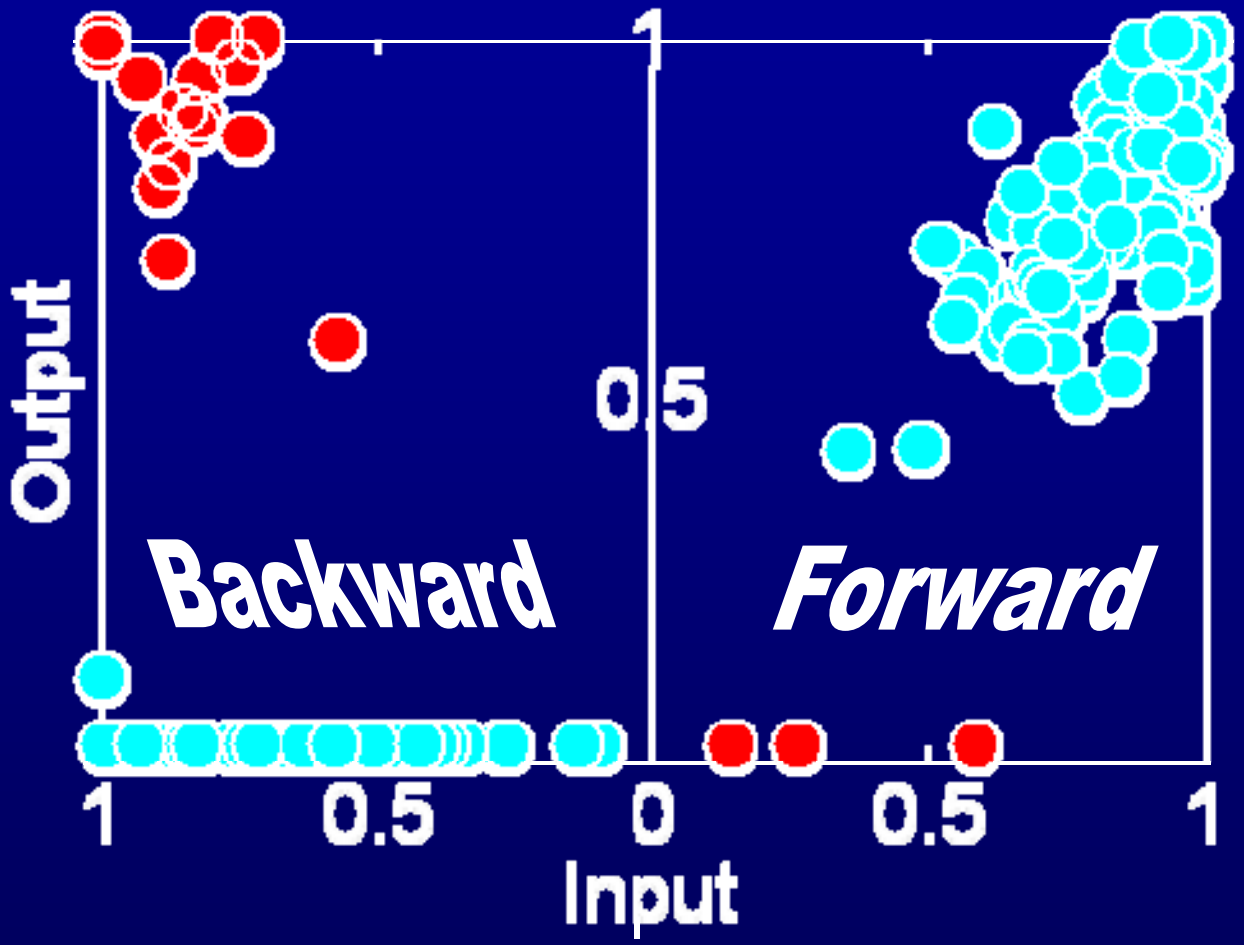
300µm

Neuronal Diode



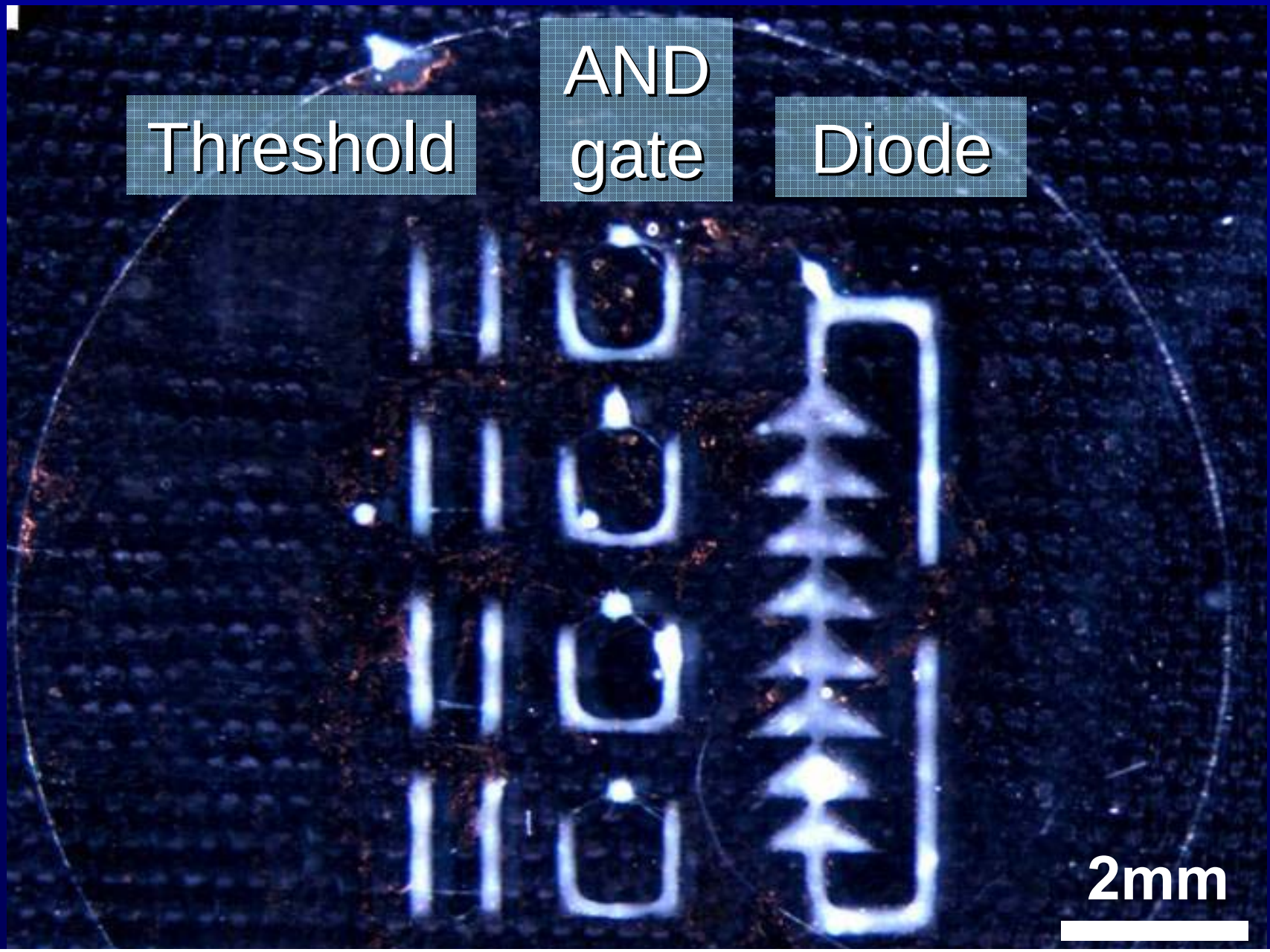
300μm



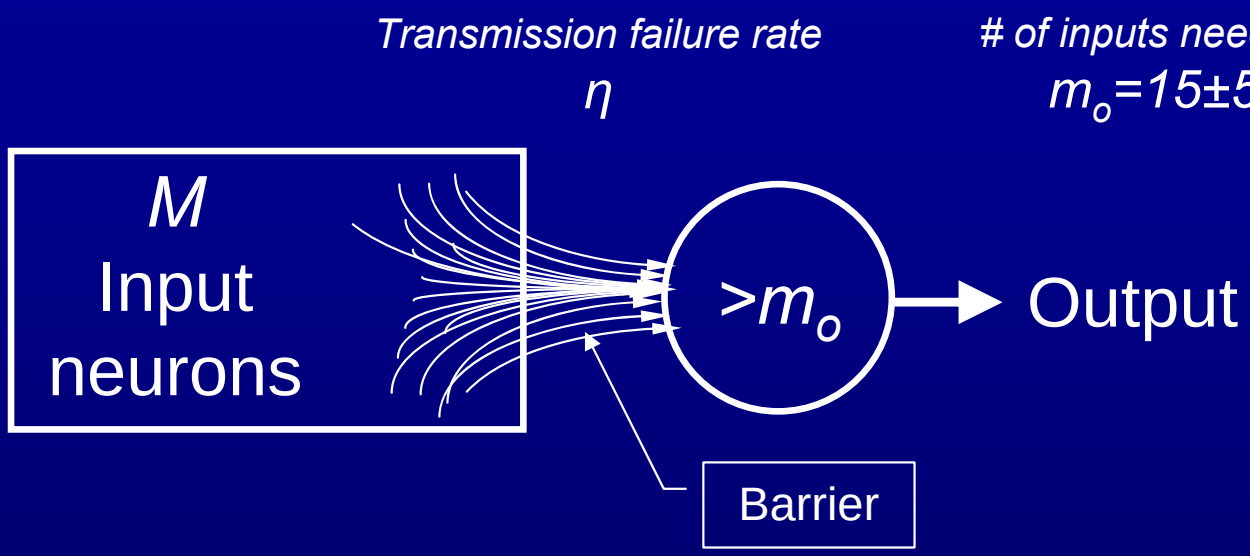


Reliability > 92%

Neuronal Devices

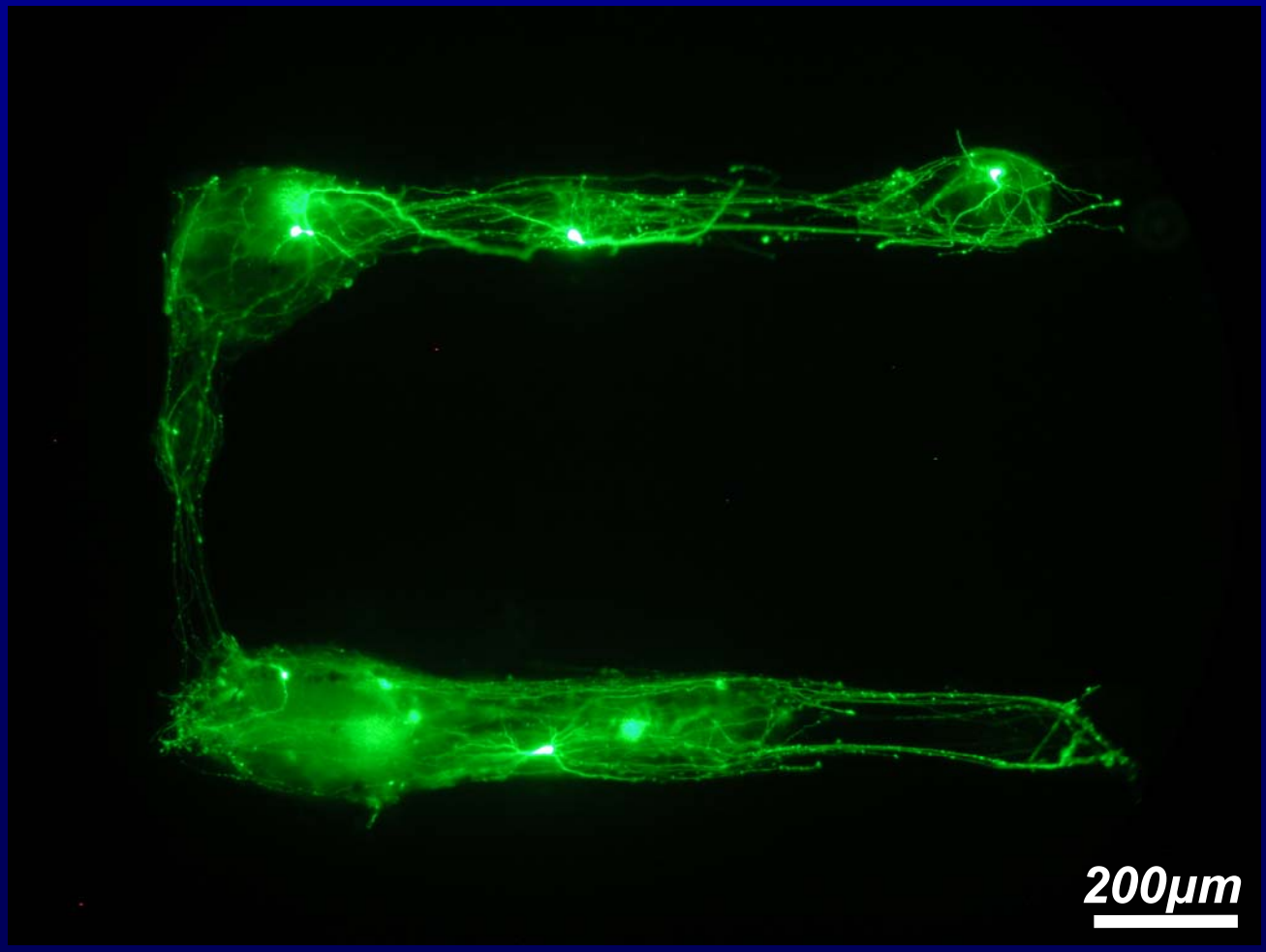


The model (von Neumann 1956)

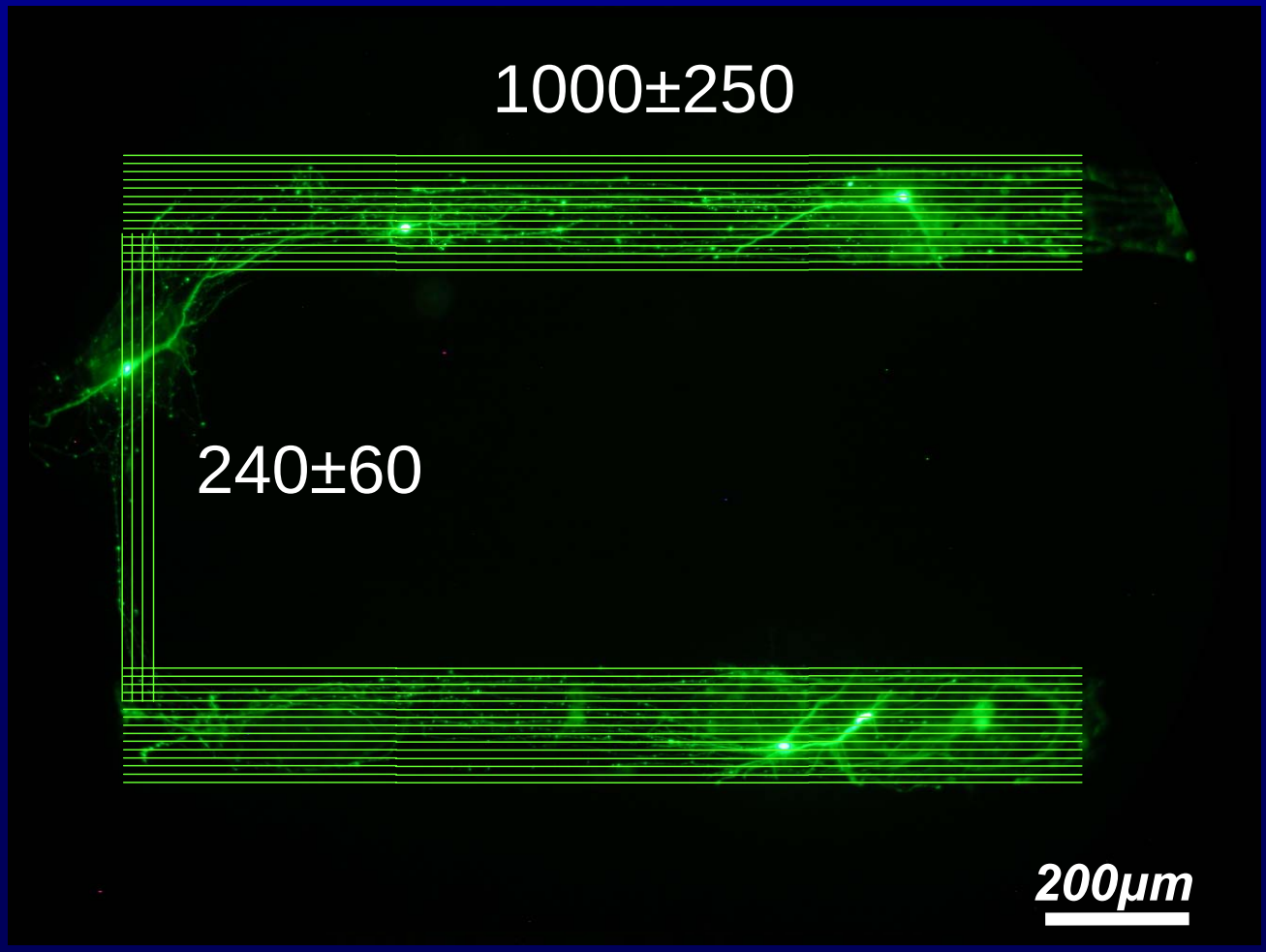


Structure of devices

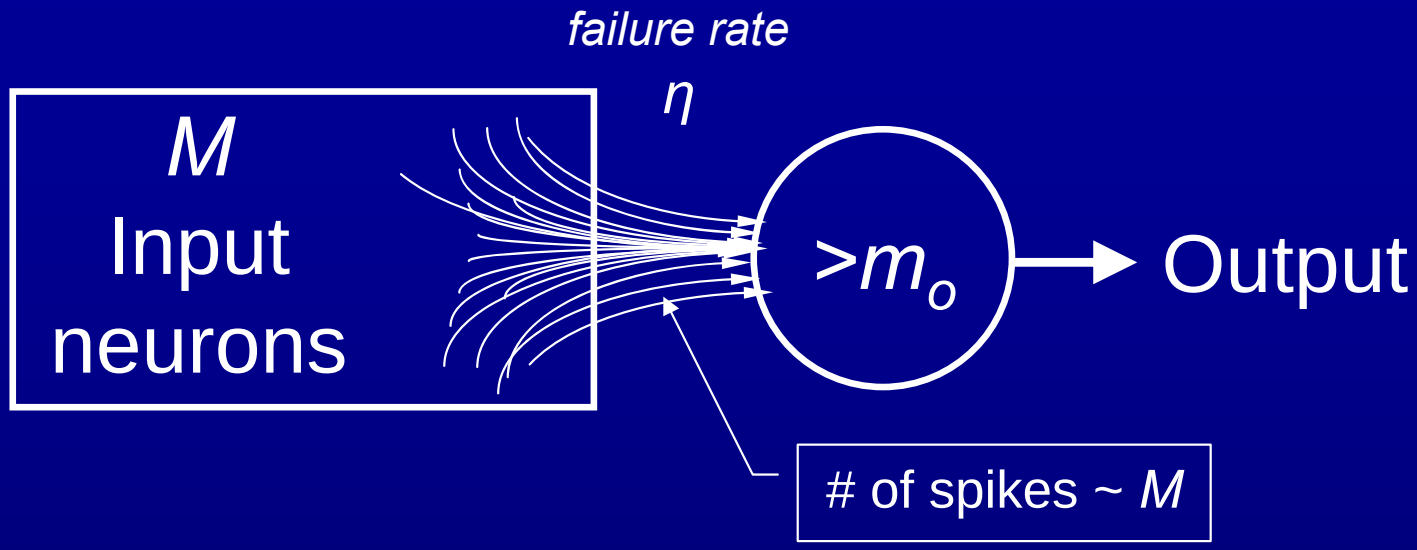
- Geometrical constraints



Structure of devices



Rescale Input by *M* = *# of neurons crossing*

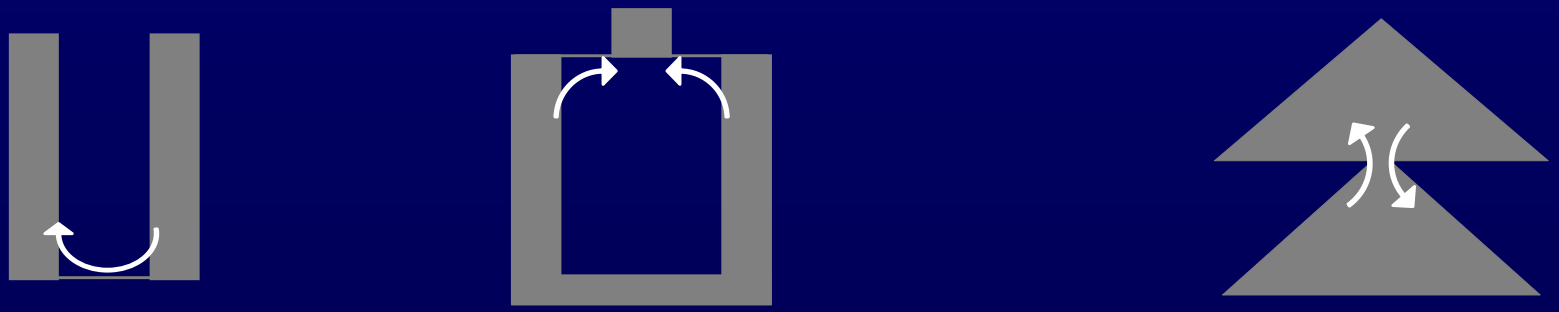


M=120 Threshold

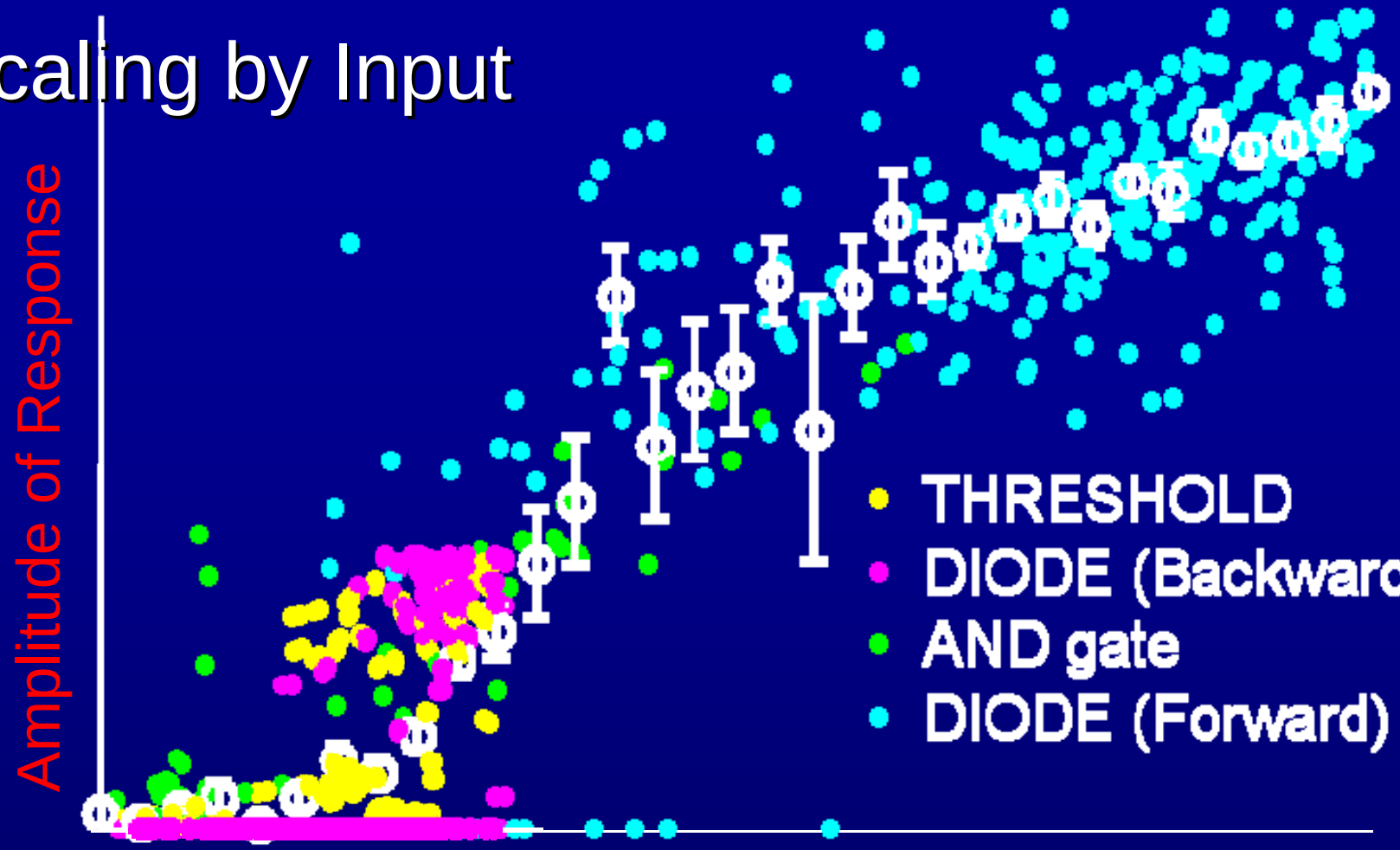
M=240 AND

M=120 Backward

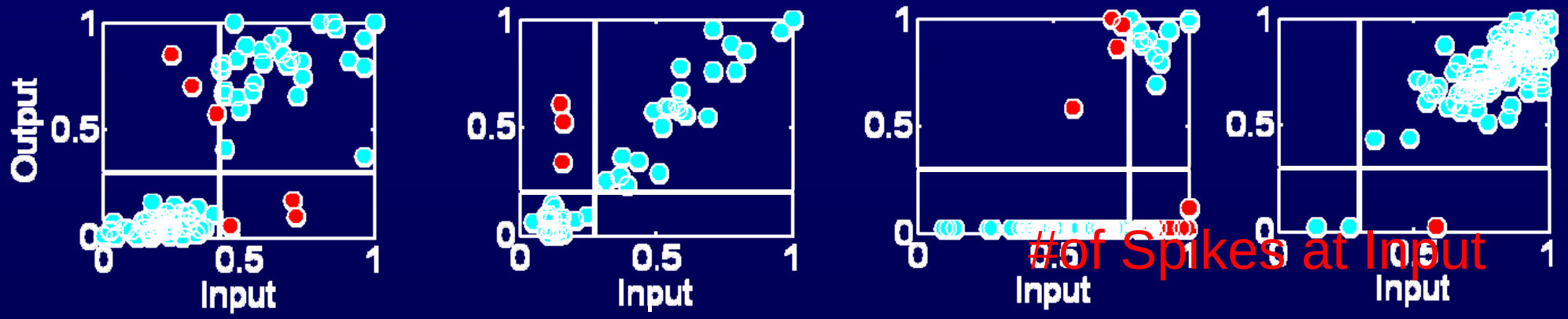
M=360 Diode Forward



Rescaling by Input

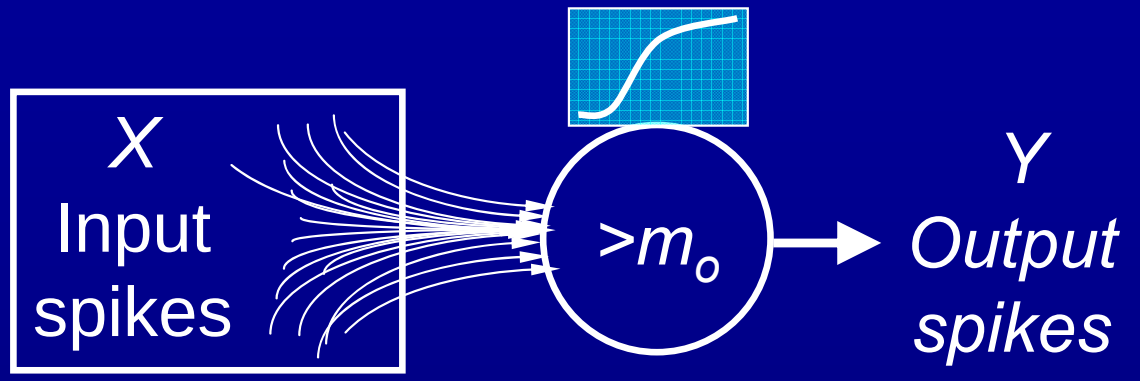


120 *240* *120* *360*



Leaky Integrate & Fire model

Transmission failure rate	η
Correction factor	$0.4C$
LIF max # of spikes	A
Minimal # of inputs	$m_o=15\pm5$
LIF time constants ratio	$B=\tau/t_{ref}=3$



Probability of n failures

$$P(n)=\binom{X}{n}\eta^{n}(1-\eta)^{X-n}$$

Effective input

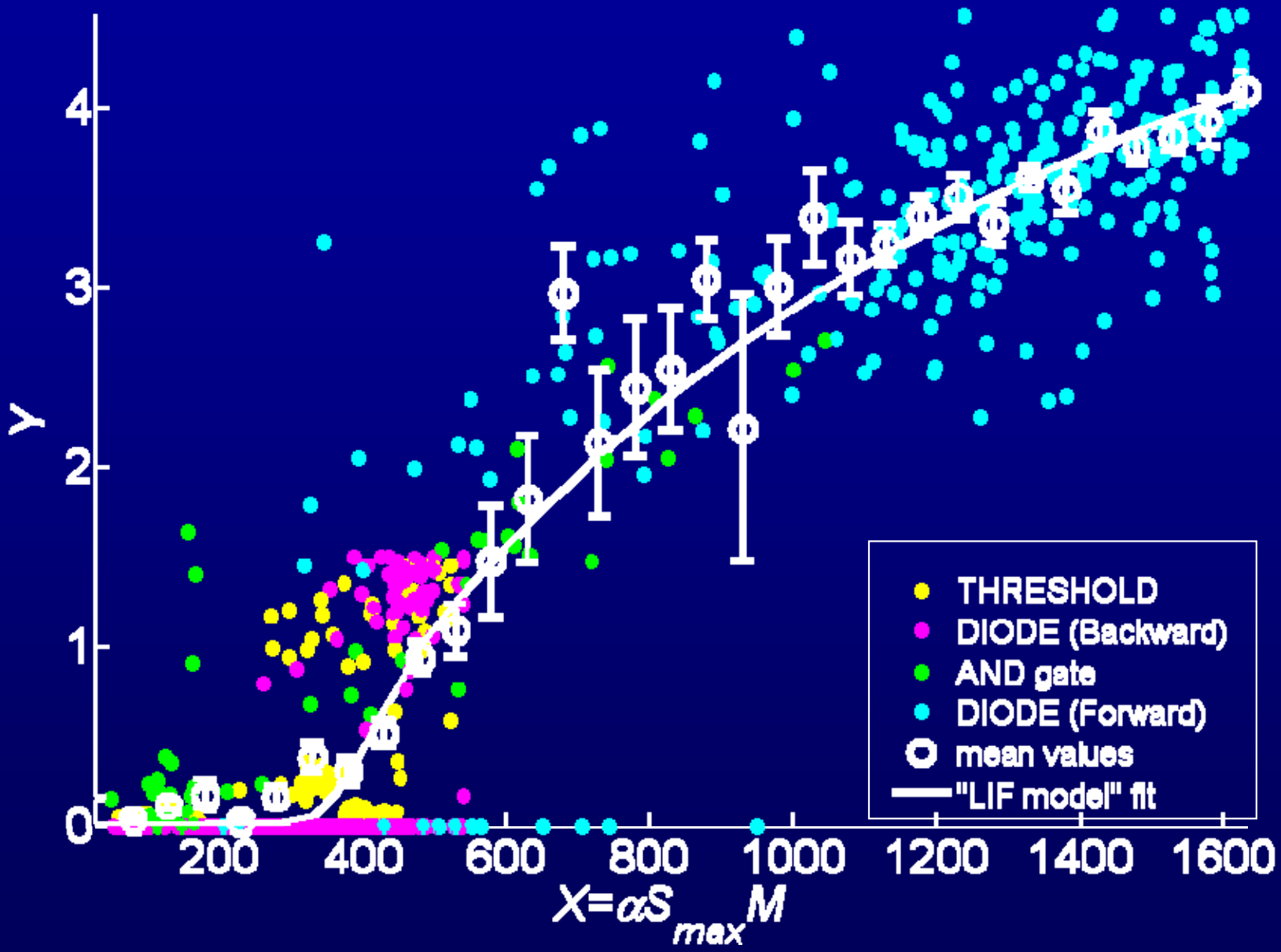
$$X_{in} = 0.4C(X-n)$$

Output model: Leaky Integrate & Fire

$$y=\frac{A}{1-B\ln\left(1-\frac{m_o}{X_{in}}\right)}$$

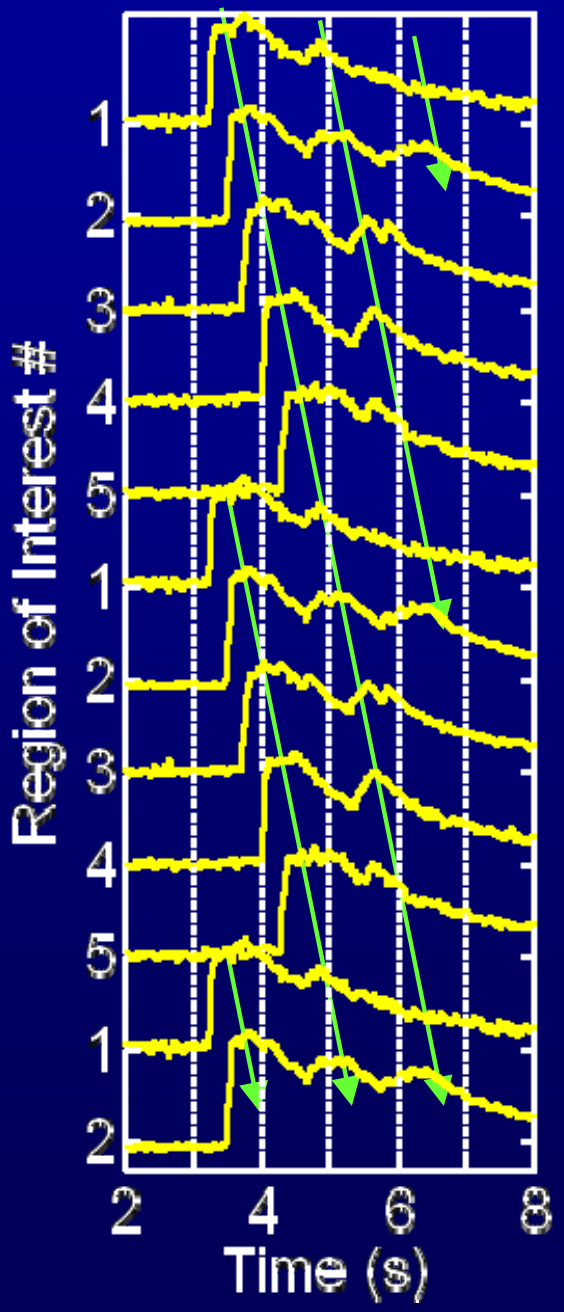
$$Y(X)=\sum_{n}\left[P(n)\,y(X,n)\right]$$

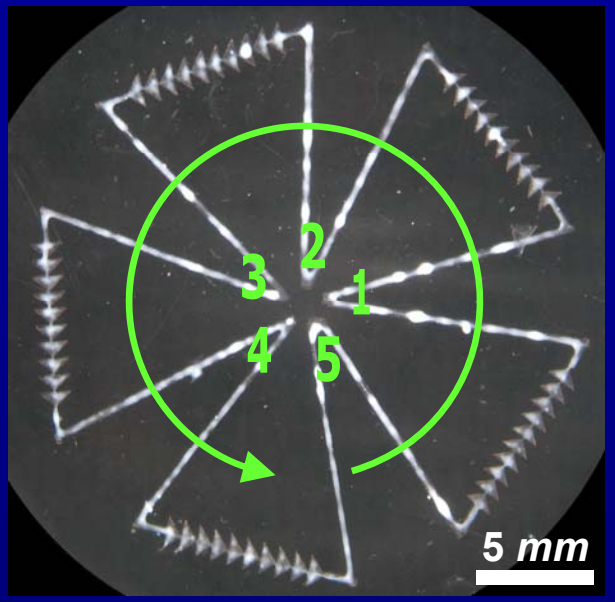
Leaky Integrate & Fire model



$\eta = 0.64 \pm 0.08$ $C = 0.25 \pm 0.1$ $A = 7.3 \pm 0.8$

Composite Devices - The Oscillator





Summary

- Even fundamental concepts like ‘computation’ and its relation with ‘thought’ are unresolved (why are these cultures so limited in their computational repertoire??)
- Simple experiments that probe deep questions of information, its creation, transport and processing.
- Tools of physics prove to be surprisingly effective in unraveling very messy neurobiological questions.

Thank you for your attention

And thanks to:

Shimshon Jacobi
Nava Levit-Binnun
David Biron

Menahem Segal
Jean-Pierre Eckmann

Varda Greenberger

Clore Center for Biological Physics
Minerva Stiftung, Munich, Germany
Paedagogica Foundation
Israel Science Foundation