Investigating neuronal networks that underlie behaviour

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- B.A. in Microbiology and Physiology, Southern Illinois University at Carbondale

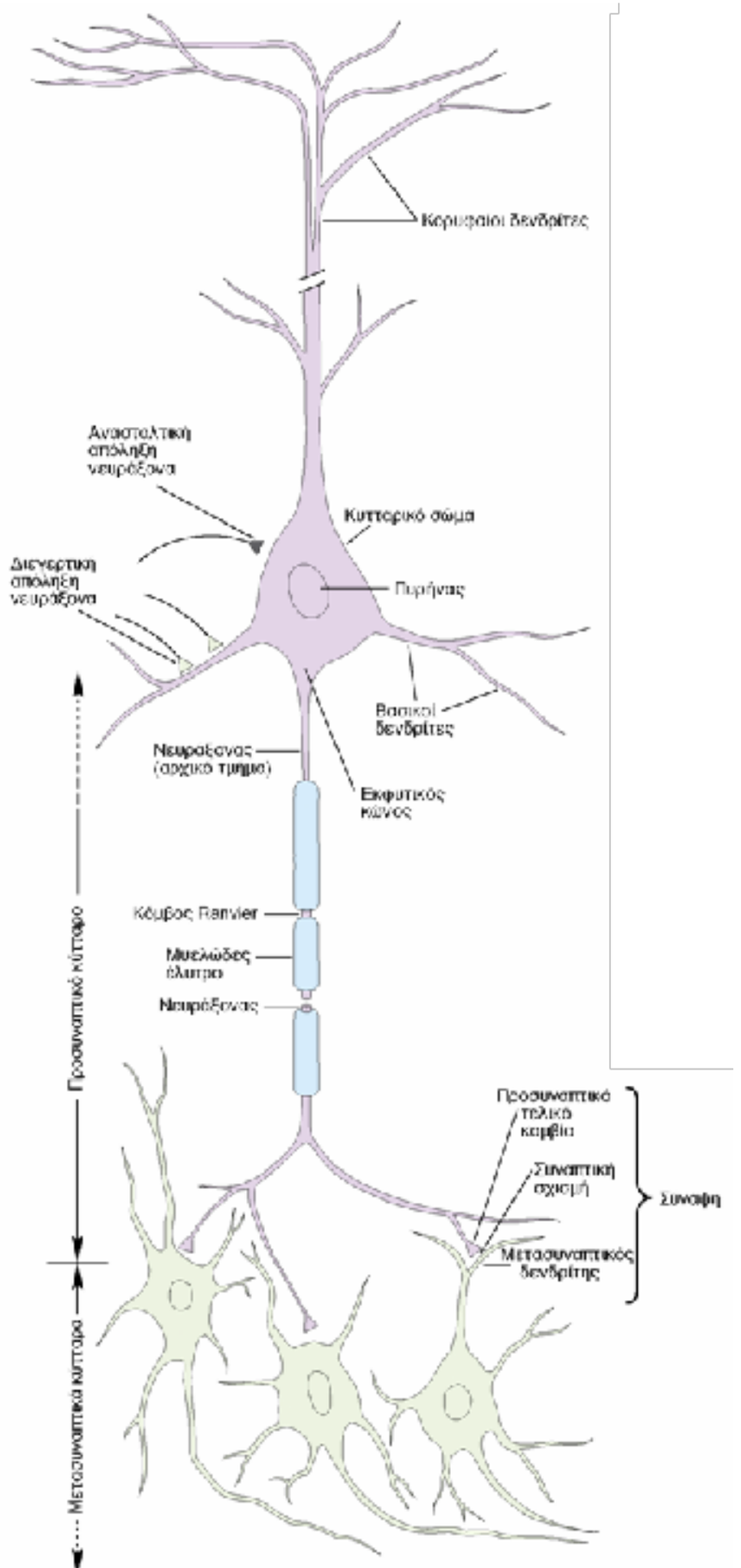
- Ph.D. in Neuroscience, Rosalind Franklin University of Health and Sciences/The Chicago Medical School

- Post-doc in ‘Computational Neuroscience’ - IMBB-FORTH

Neurons, neuronal networks and brain

Neurons are the building blocks of the brain

- The human brain consists of 100 billion neurons



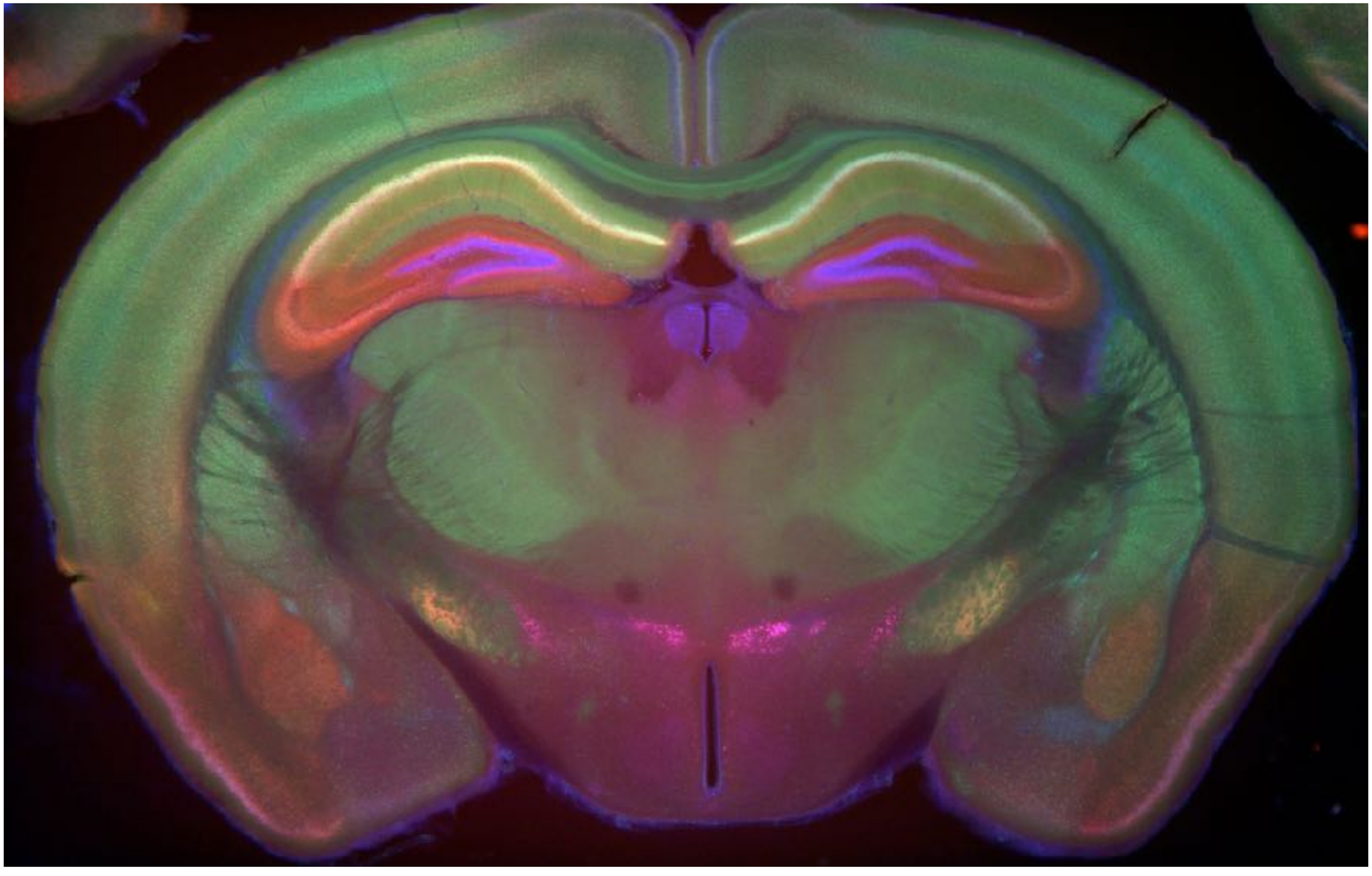
Kandel, Νευροεπιστήμη και Συμπεριφορά, ΠΕΚ

There are many different types of neurons

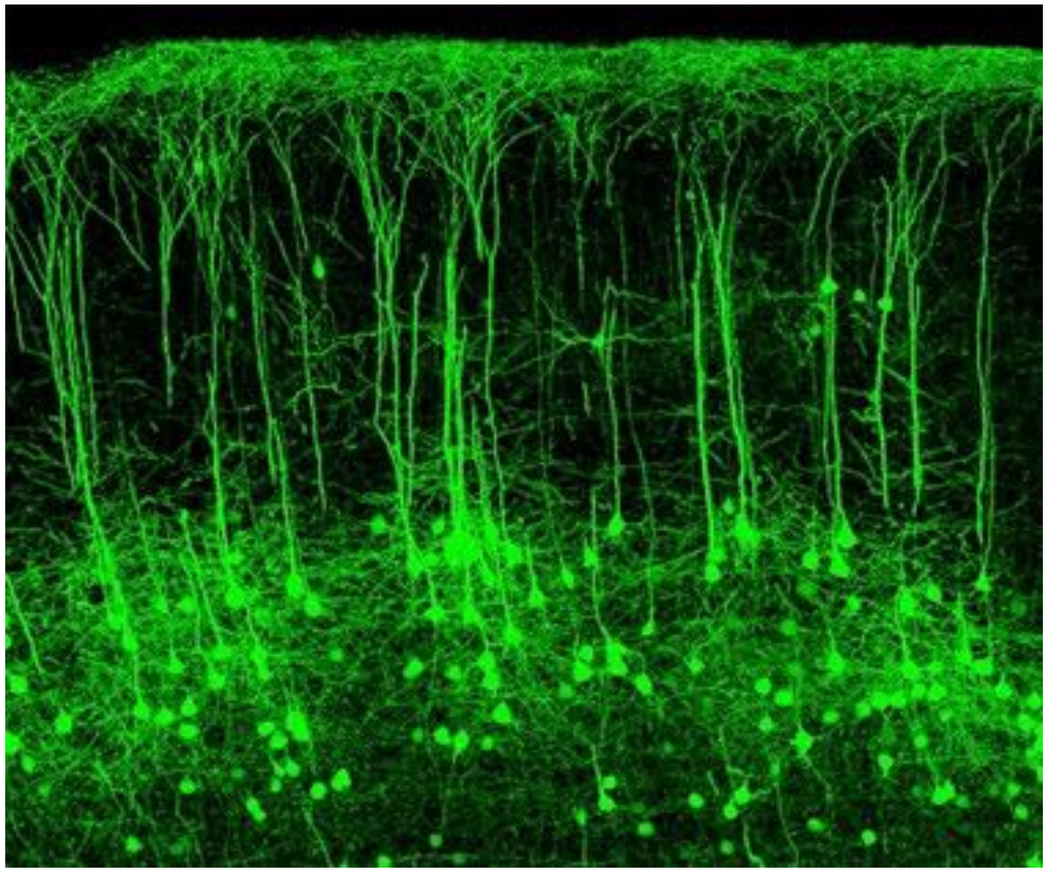
- According to the neurotransmitter
  - Glutamatergic, GABAergic, Dopaminergic, etc

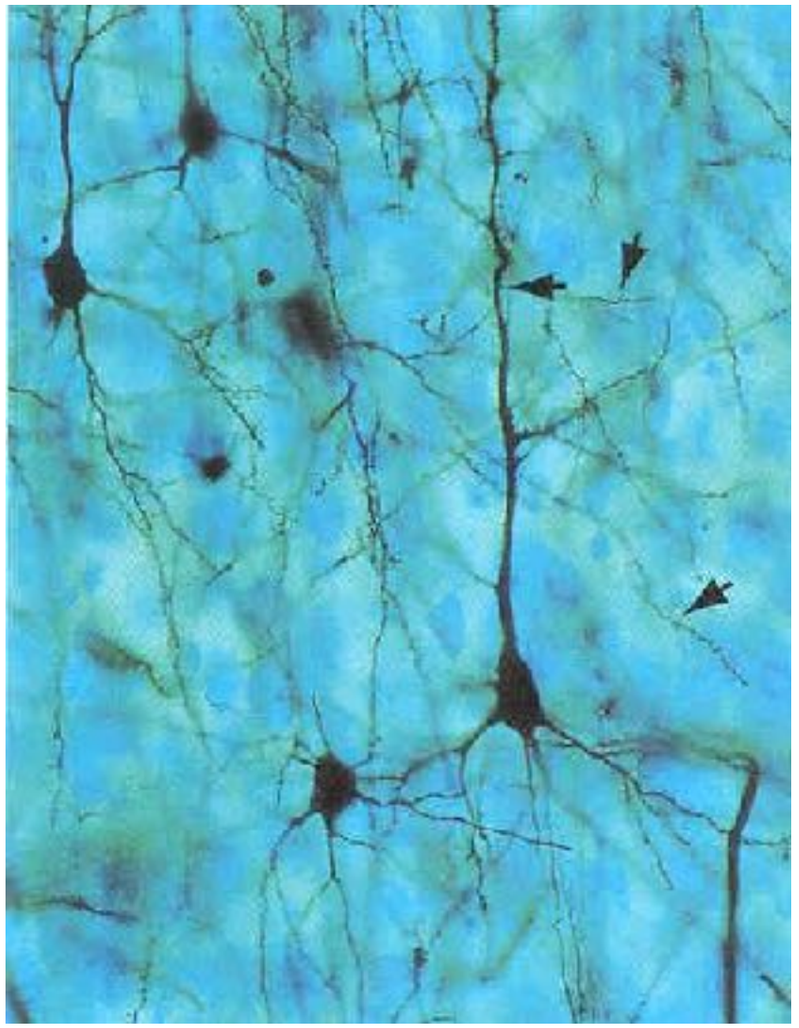
Brain slice

Cortex, Hippocampus, thalamus, hypothalamus



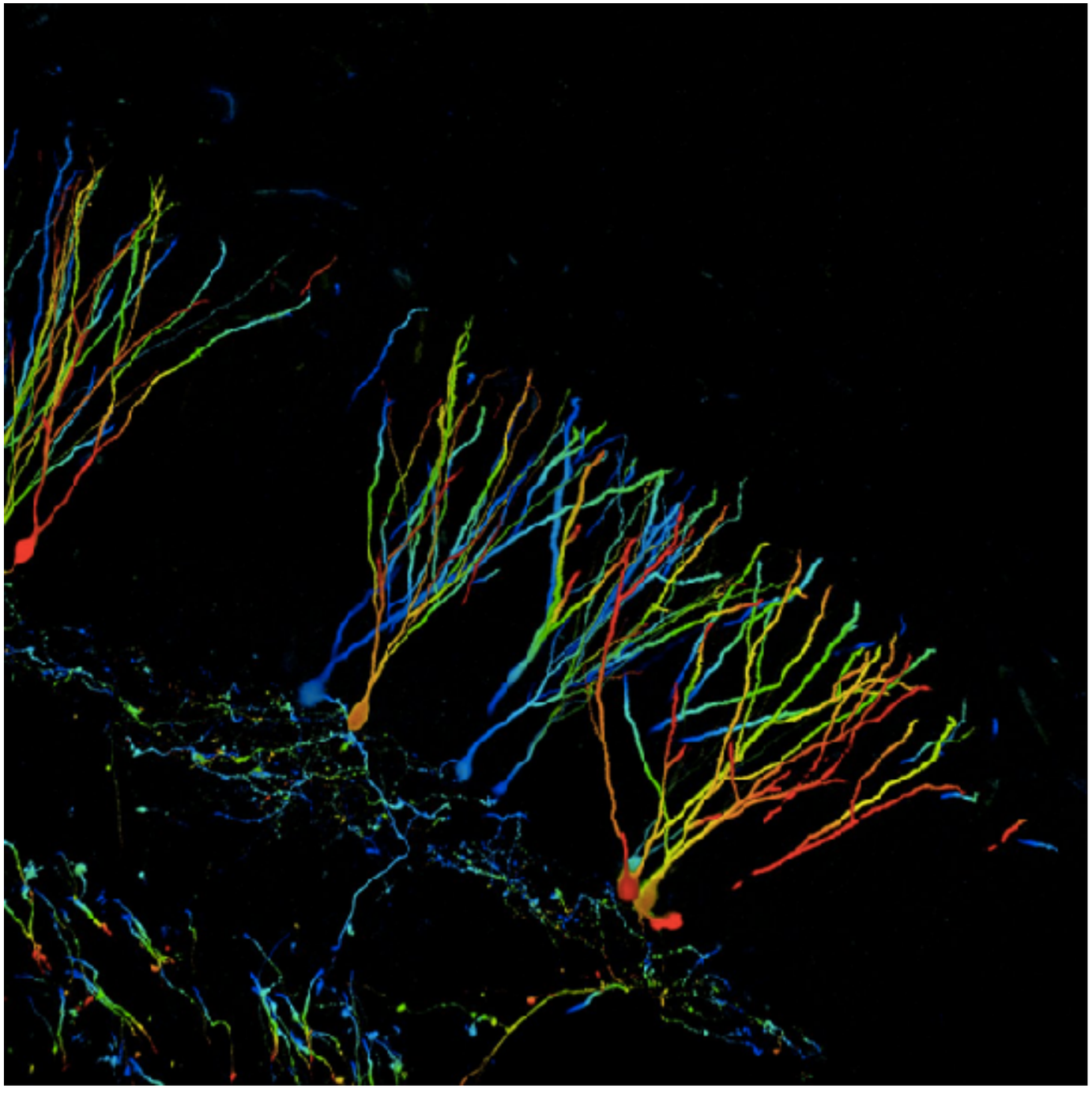
- Pyramidal neurons
  - Cortex, hippocampus, amygdala
  - Glutamatergic neurons
  - Projection neurons





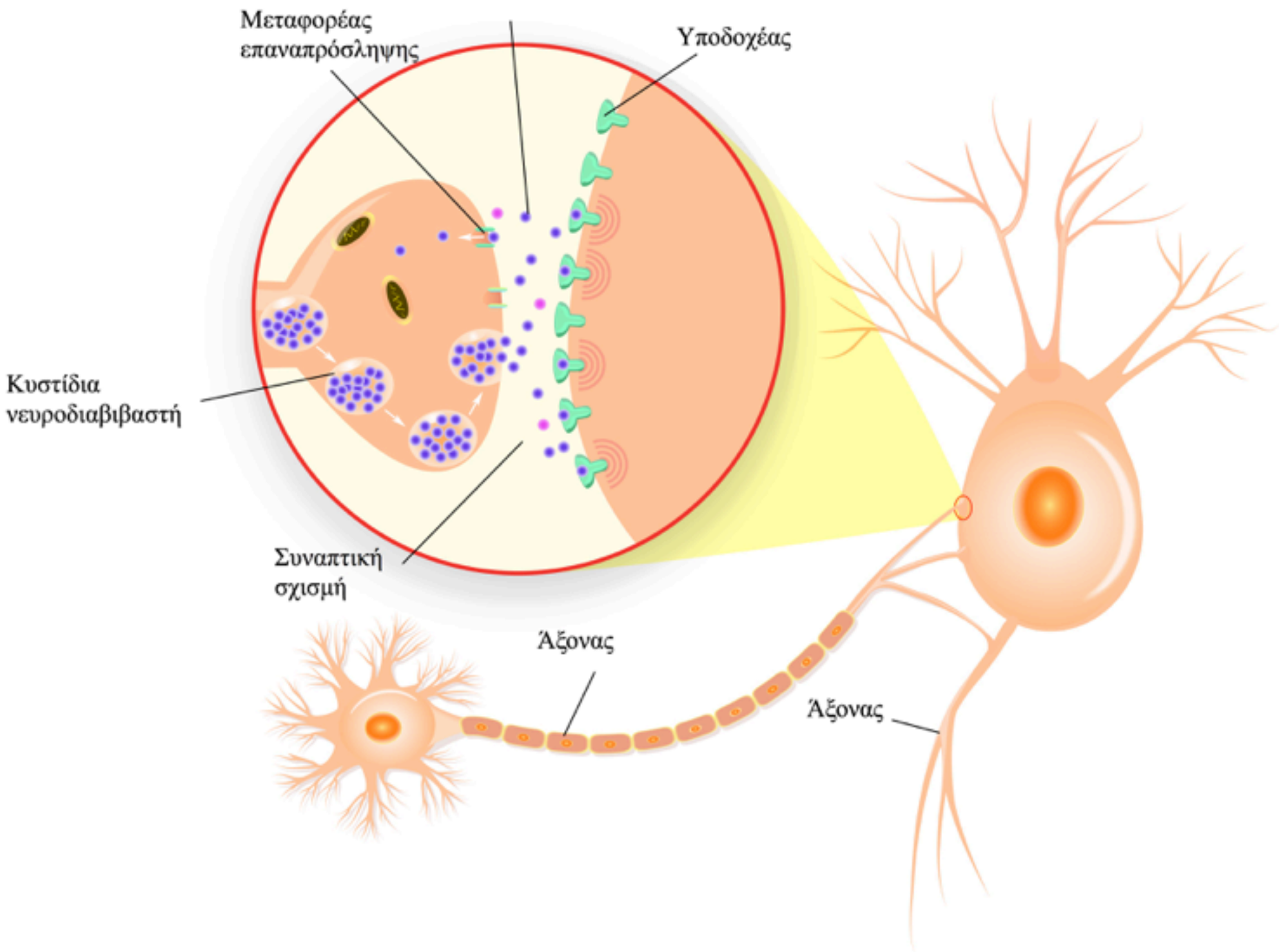
https://www.uthsc.edu/neuroscience/imaging-center/images/pyramidal.jpg

Granule cells in the dentate gyrus



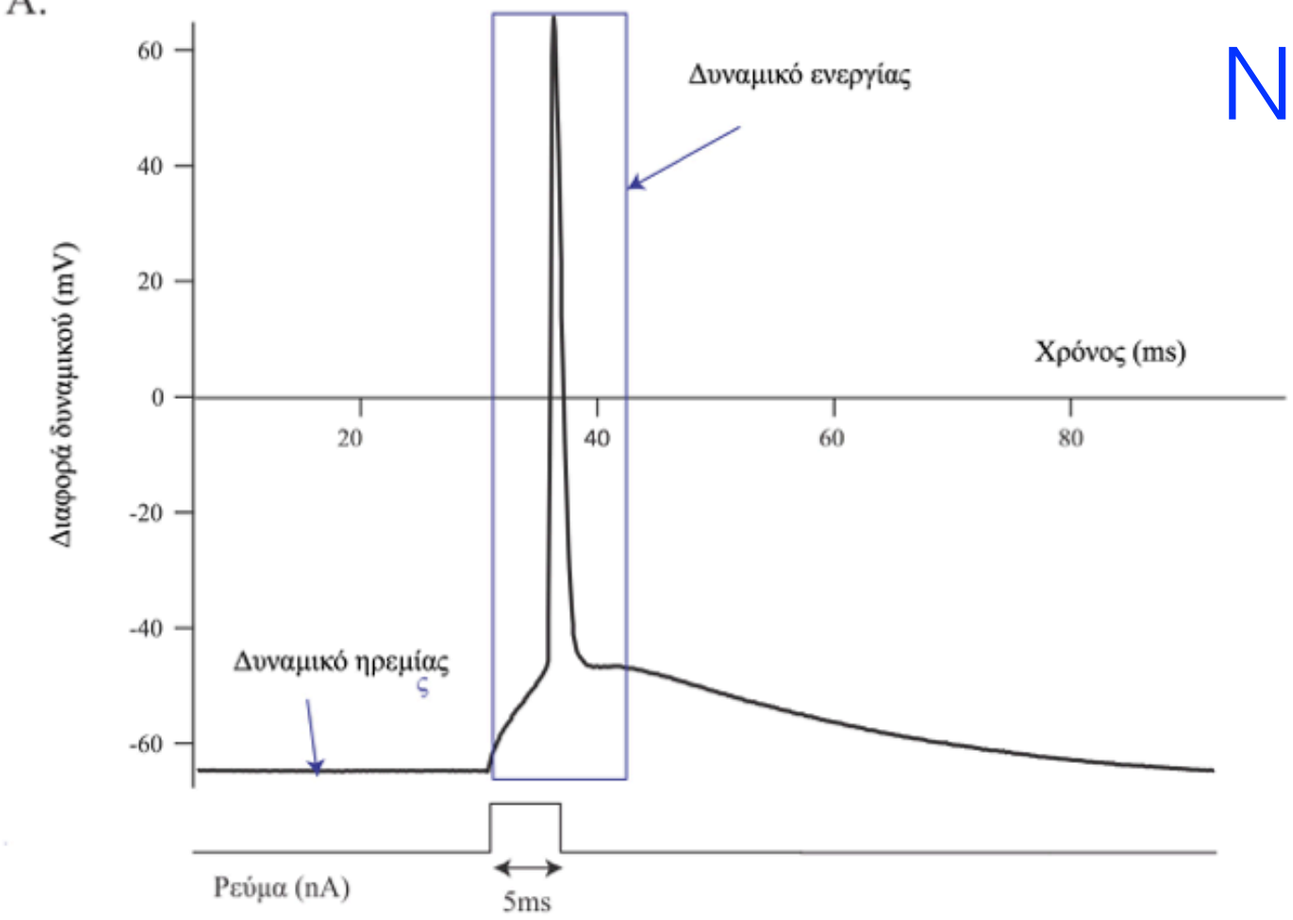
Cerebral Cortex
Pyramidal neurons and basket cells
Greg Dunn - microetchings

Neurons communicate with each other to form networks through synapses

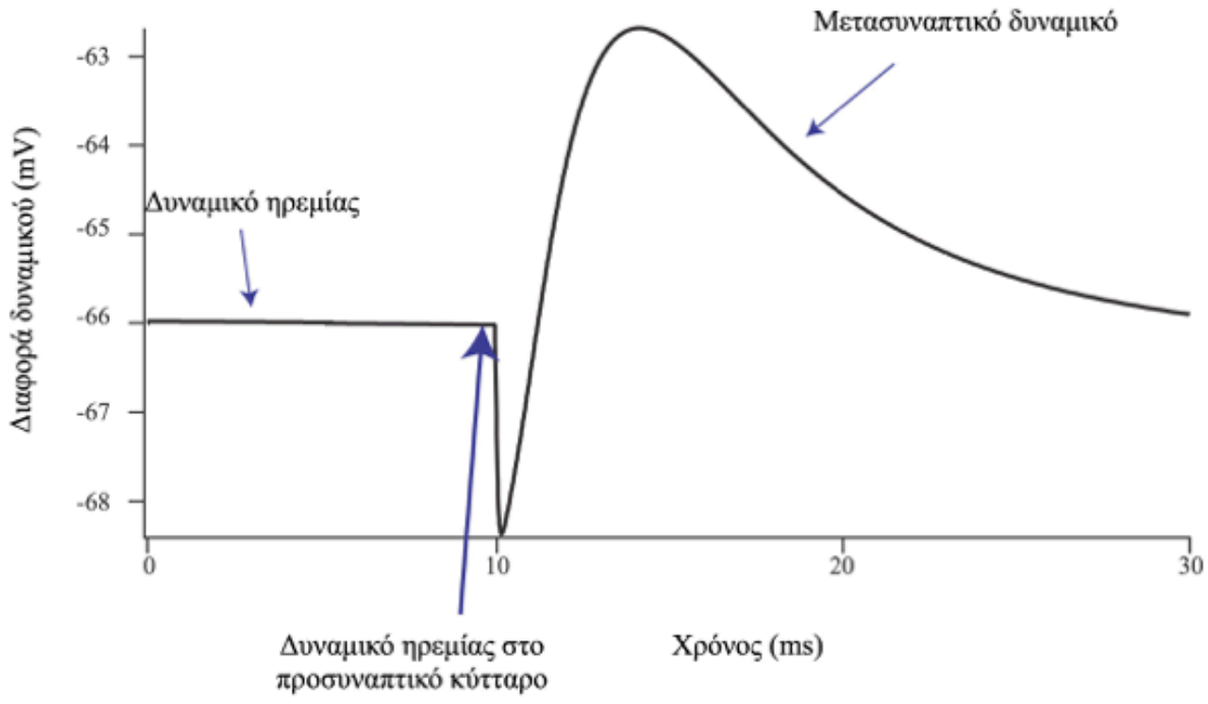


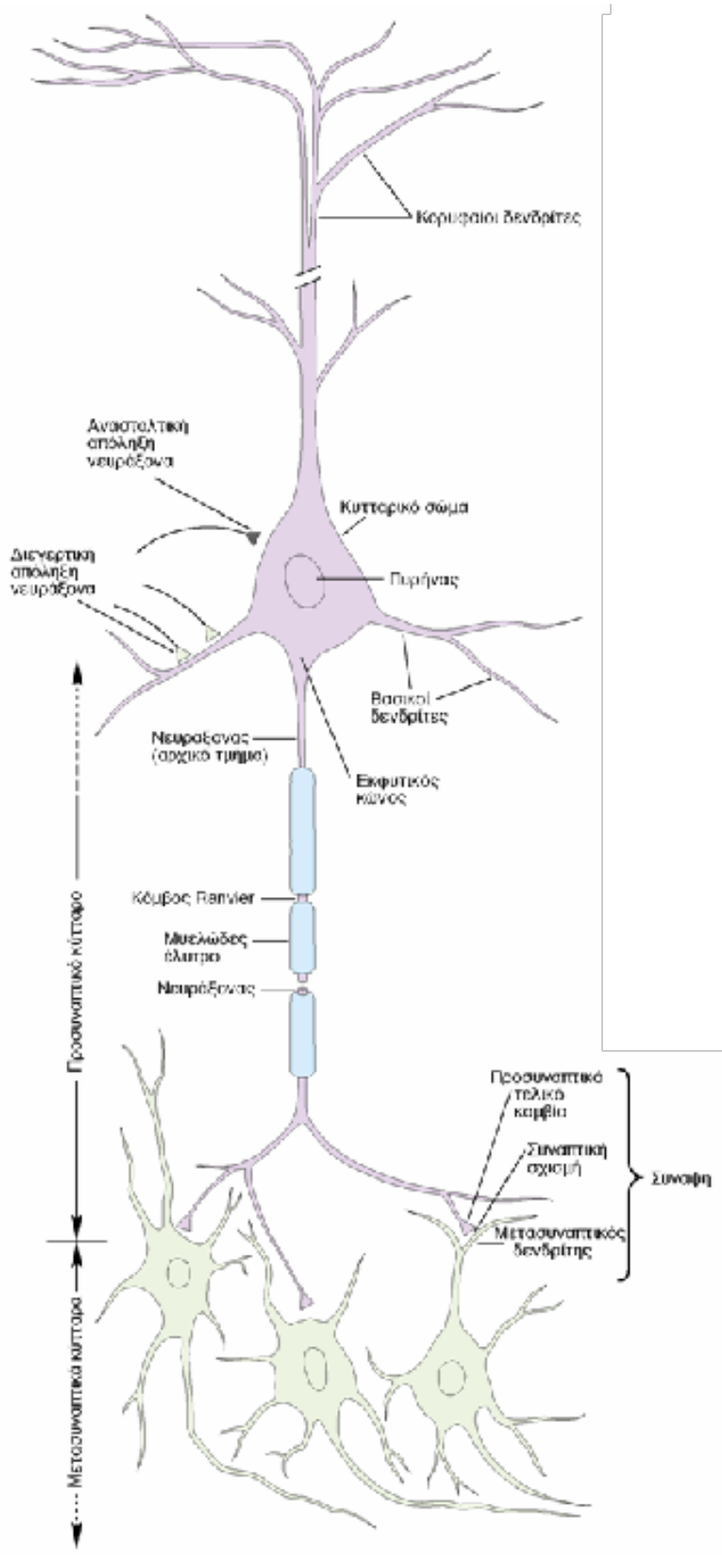
Neurons communicate with electrical signals

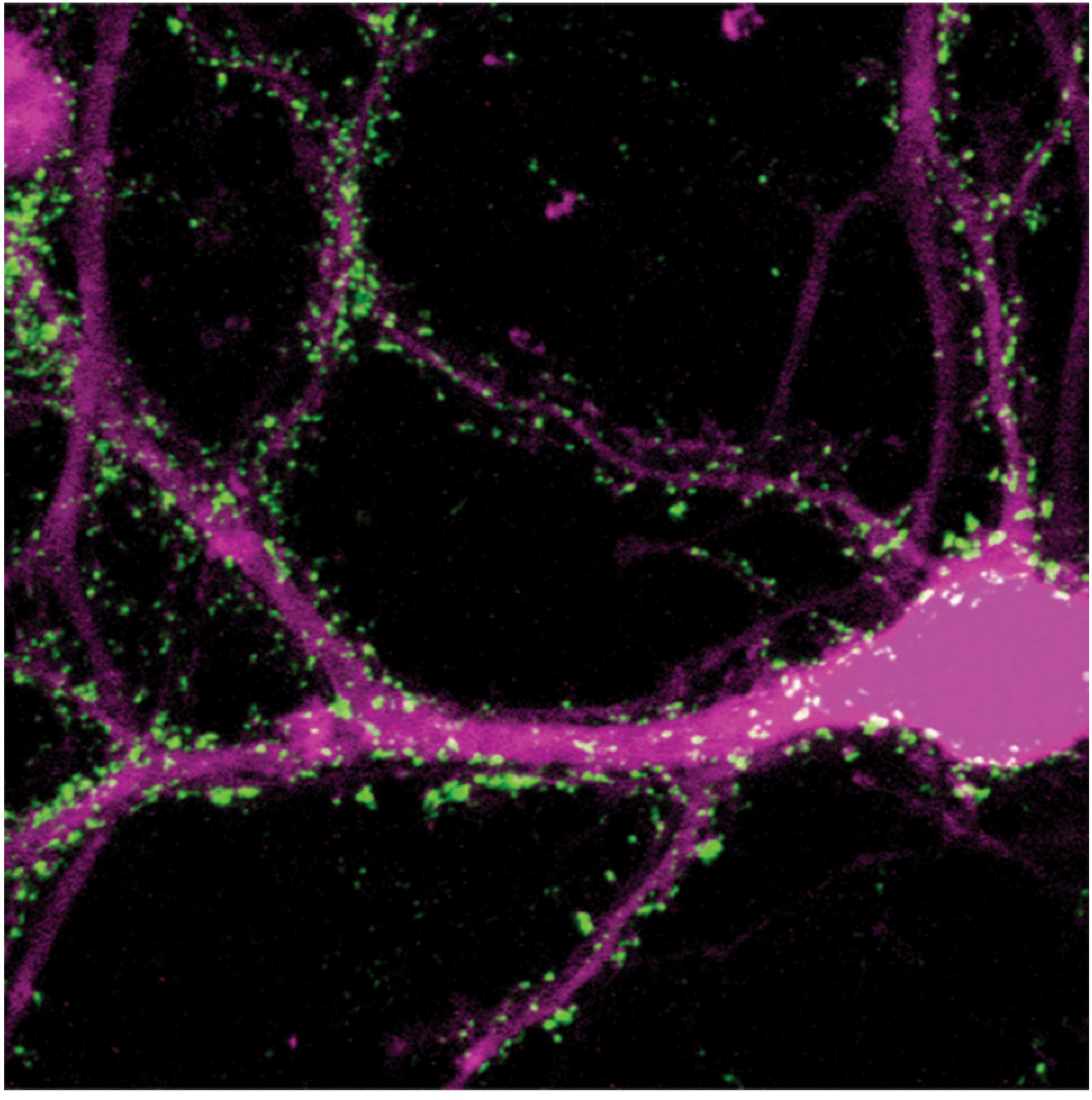
A.



B.





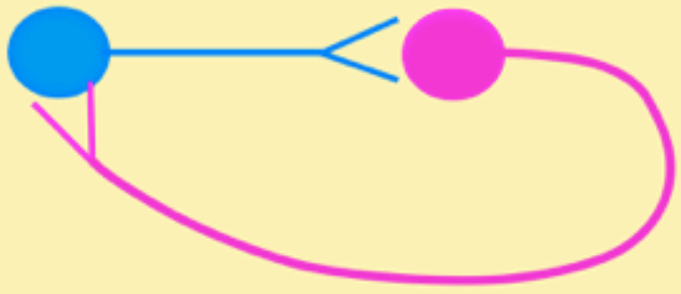


Neuronal networks in the brain

Ορθόδρομη διέγερση

Ορθόδρομη αναστολή

Οπισθόδρομη ή Ανατροφοδοτούμενη αναστολή



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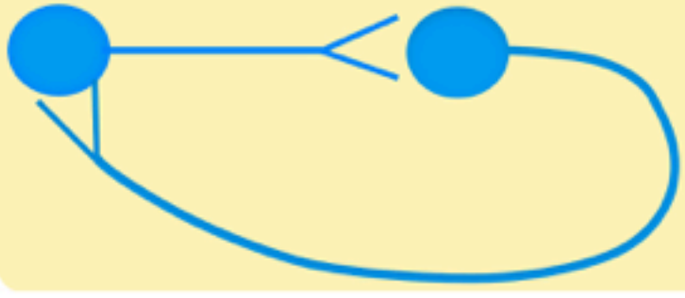
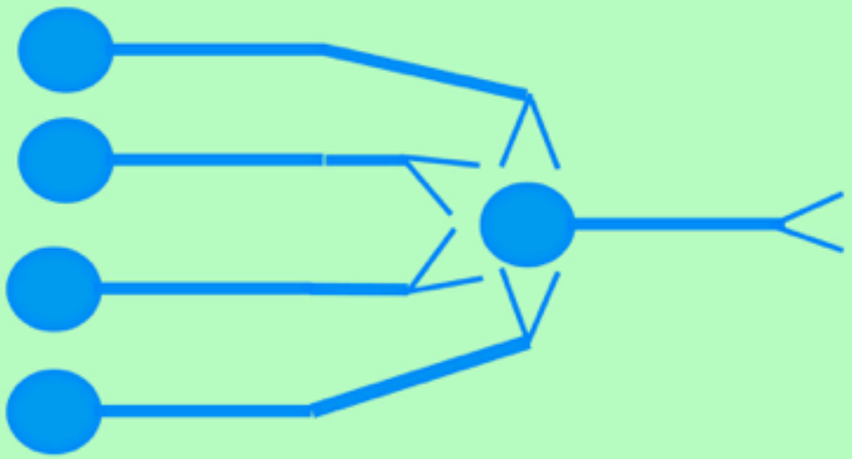
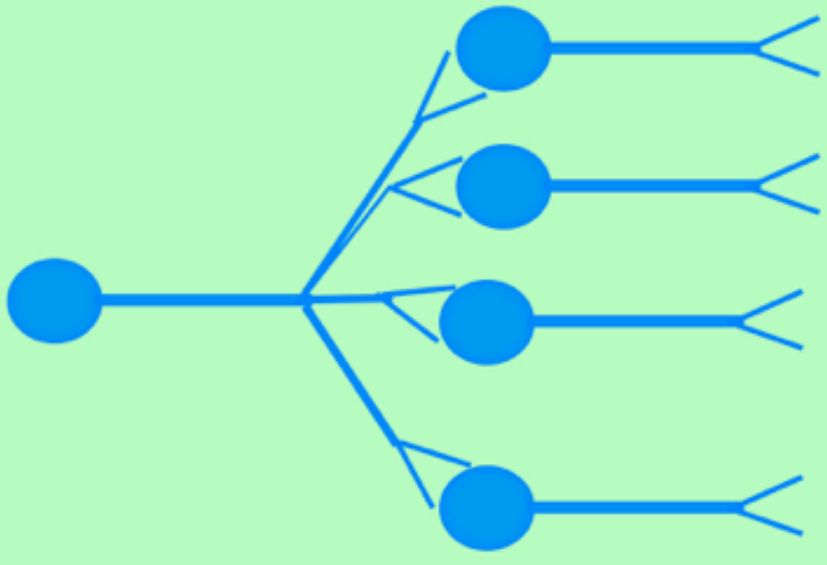


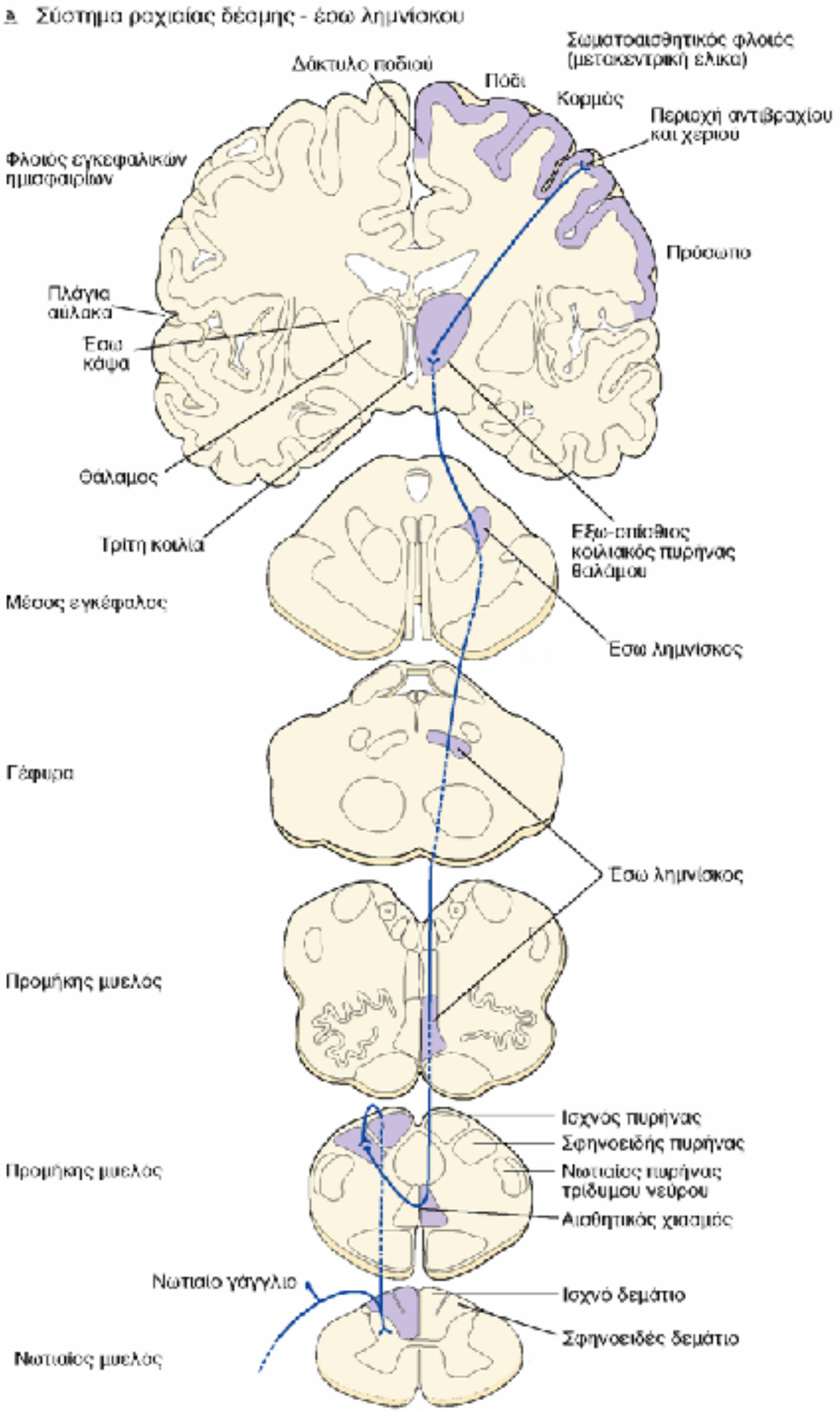
Συγκλινόμενα νευρωνικά δίκτυα



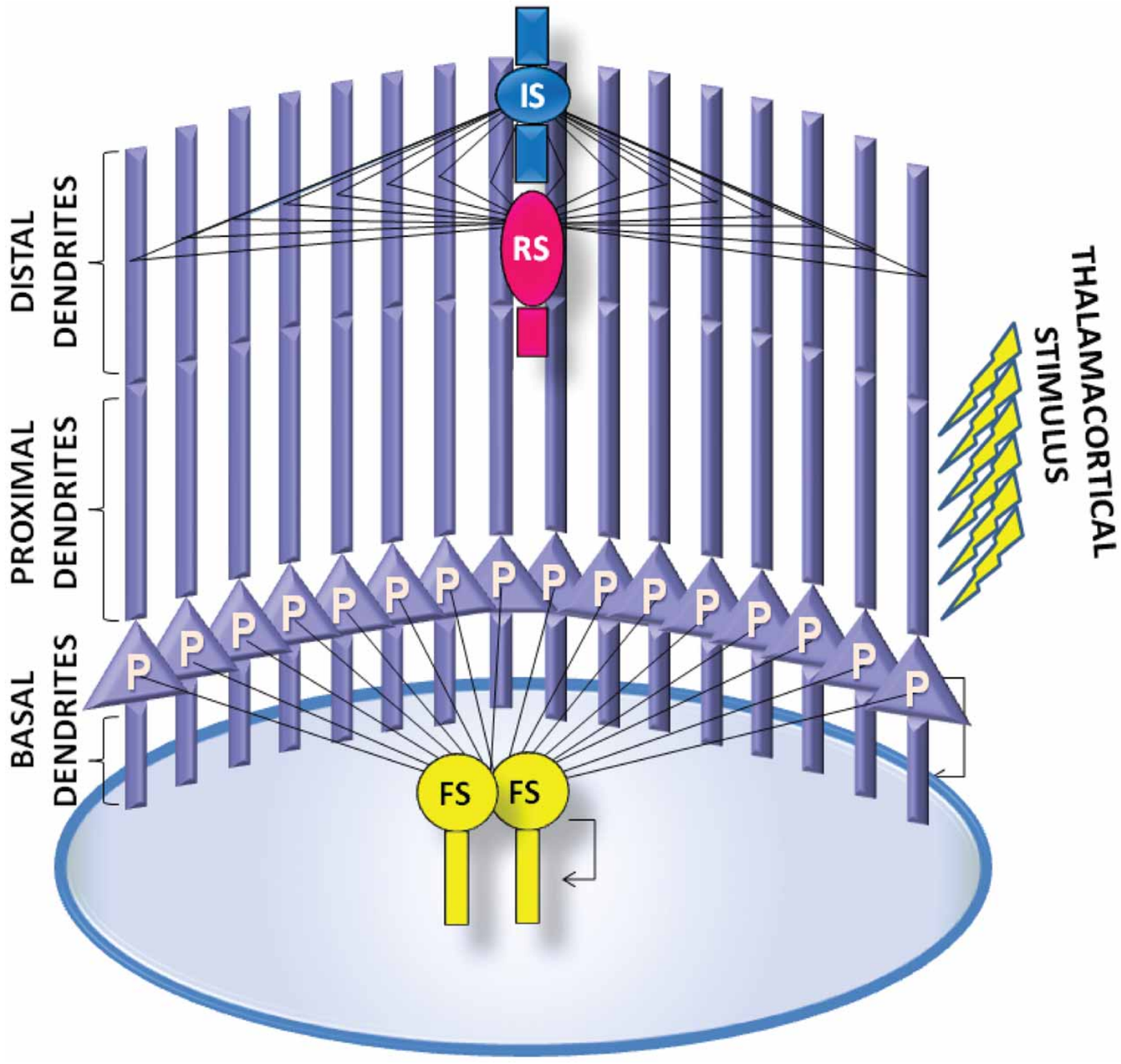
Αποκλινόμενα νευρωνικά δίκτυα



Orthodromic excitation
Somatosensory system

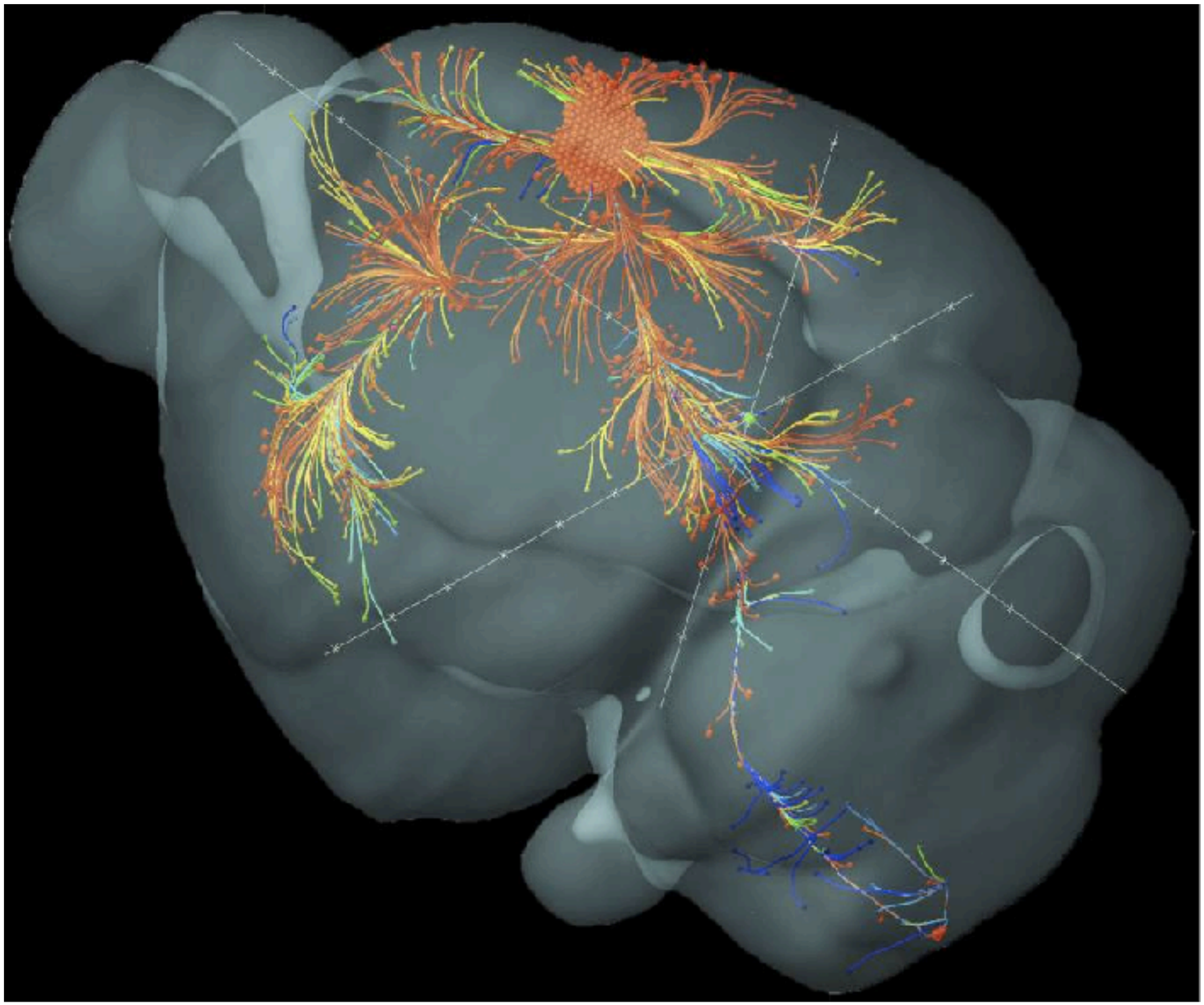


Recurrent excitation and inhibition



Konstantoudaki et al, 2014, Frontiers in Neural Circuits

Connectome



Why do we want to study networks in the brain?

- The function of neuronal networks underlies all animal behavior

How can we study neuronal networks that underlie behaviour?

- **Types of behaviour**
  - Feeding
  - Exploration
  - Perception
  - Learning
  - Memory recall
  - Rule learning

How can we study neuronal networks that underlie behaviour?

- Study neurons that are activated during a specific behavior
  - Action potentials - electrophysiological/ imaging
  - Synaptic activity - electrophysiological
    - markers of activity
  - Correlate their activity with specific aspects of behavior
- Manipulate their activity and study the effect on behavior
  - optogenetics

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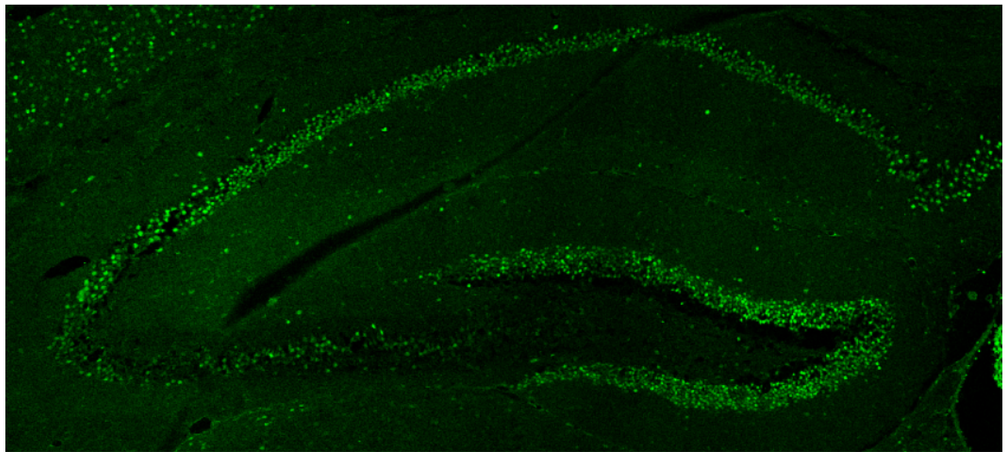
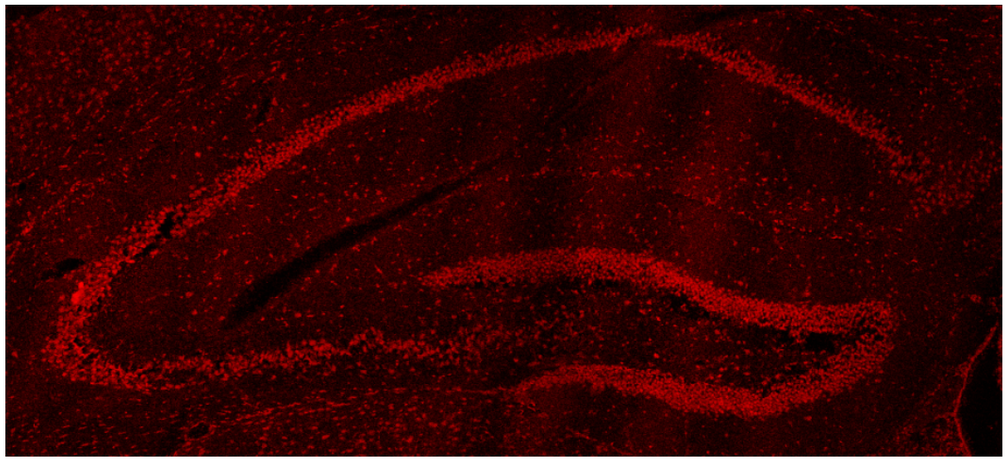
Cellular and molecular techniques

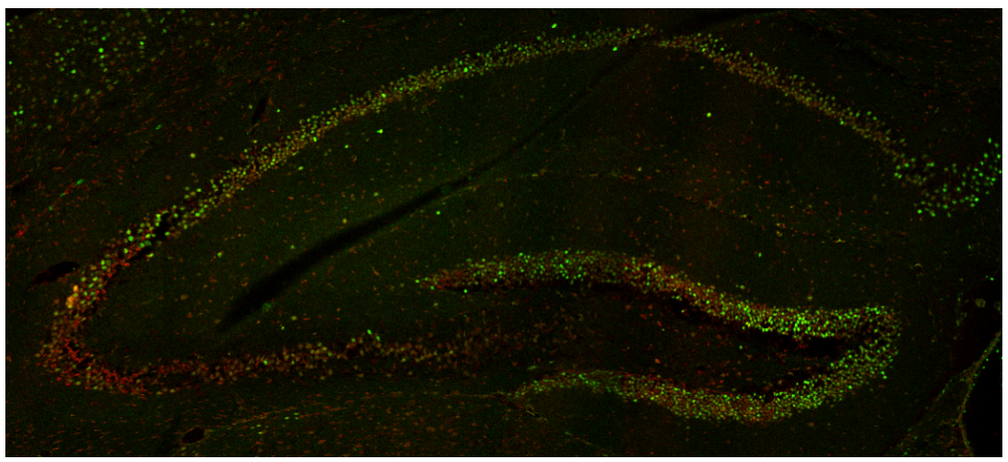
- Immediate early genes - Activity markers
  - Arc
  - c-fos
  - c-jun
- Genes that are transcribed and translated in response to neuronal activity

c-fos activation following epileptic seizures

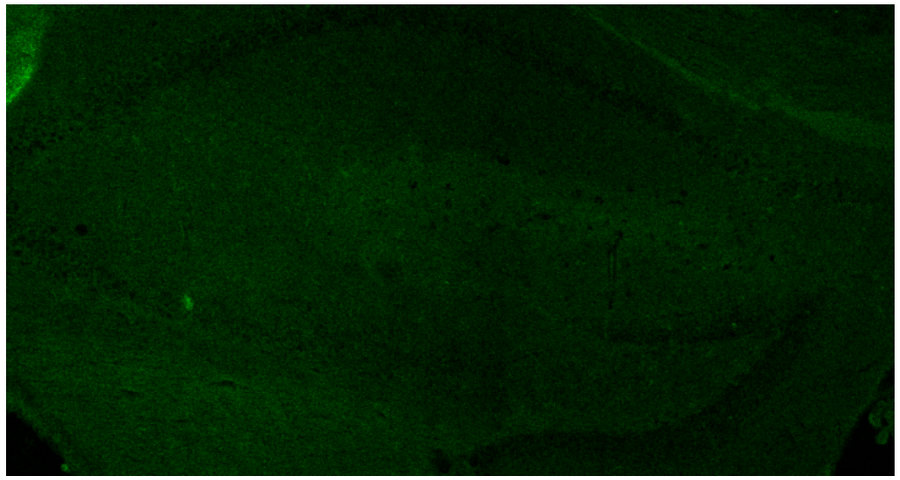
WT mouse, 300mg/kg pilocarpine

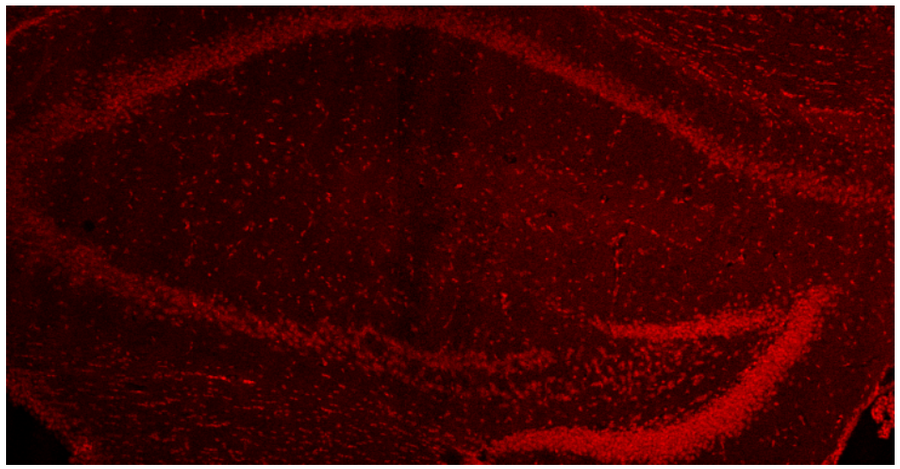


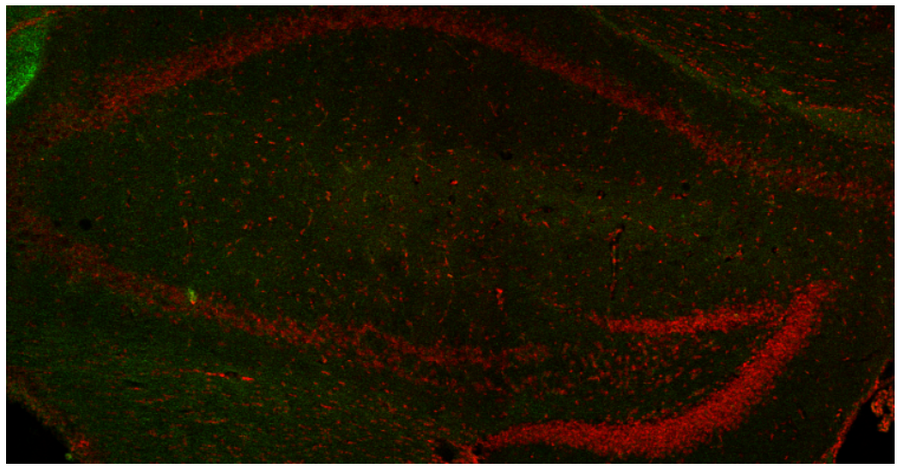




Rac1 conditional KO mouse
100mg/kg pilocarpine

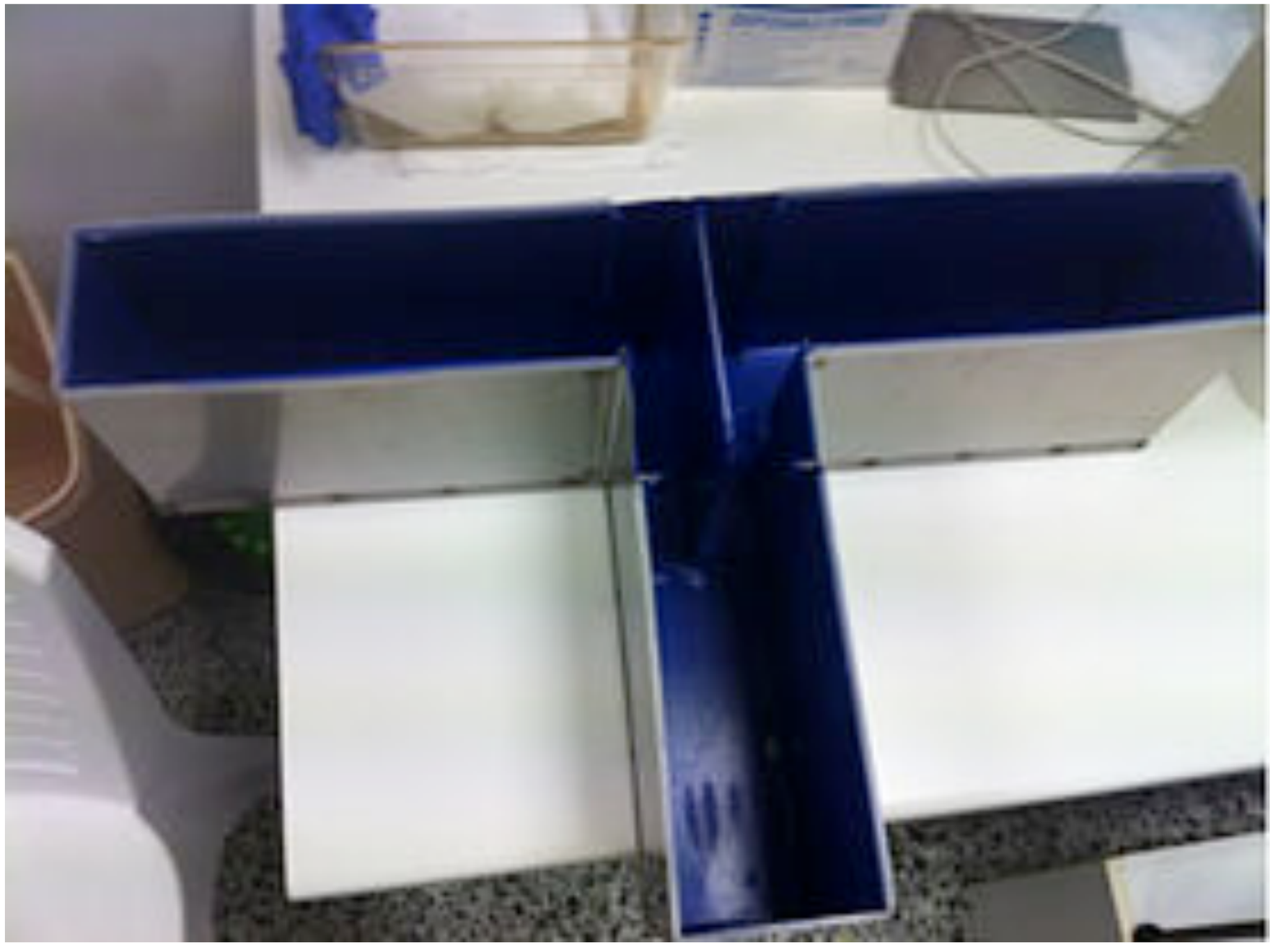






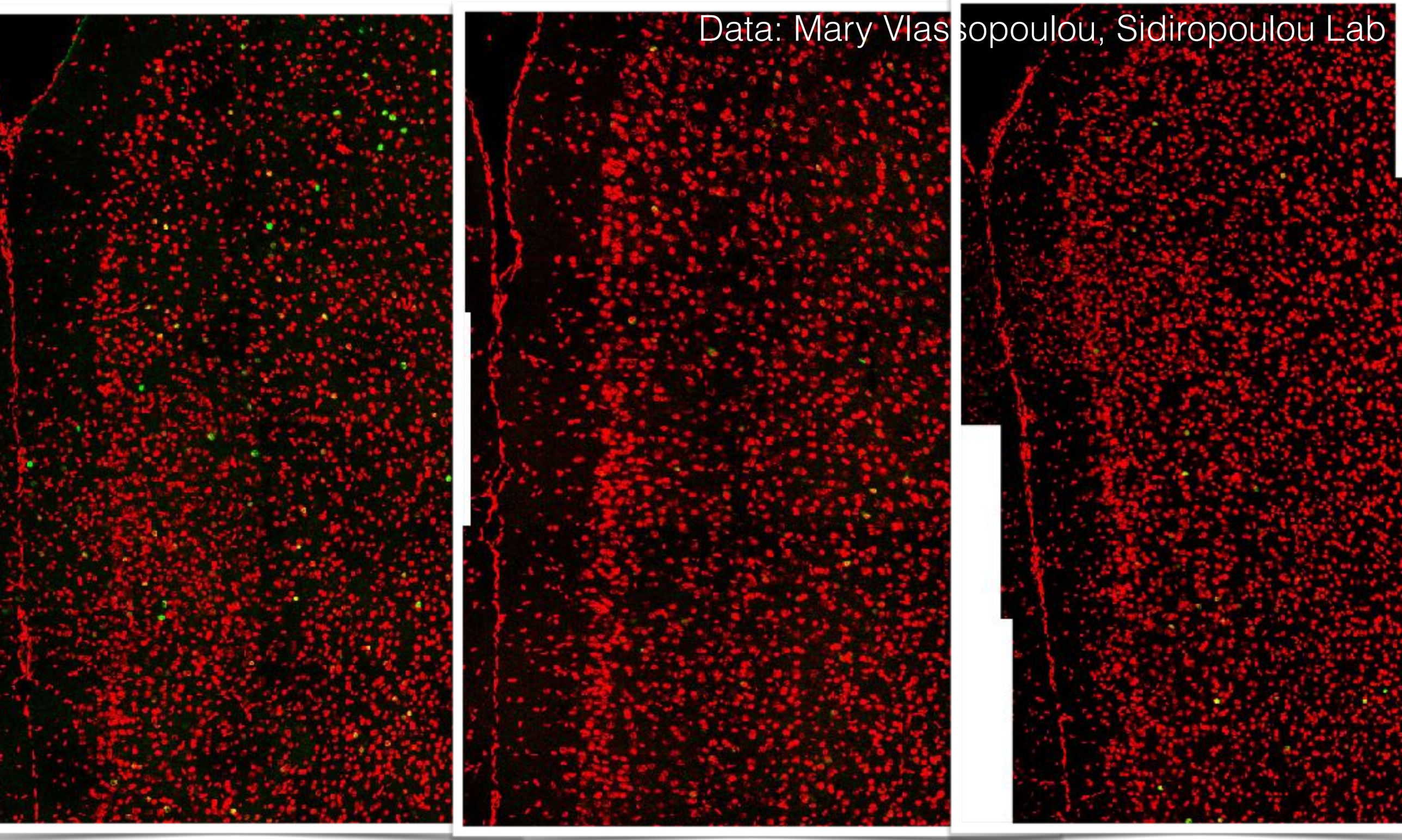
Chalkiadaki, Sidiropoulou, unpublished data

Different types of memory behavior in the T-maze



PREFRONTAL CORTEX

Delayed alternation Left-Right discrimination Open-Field



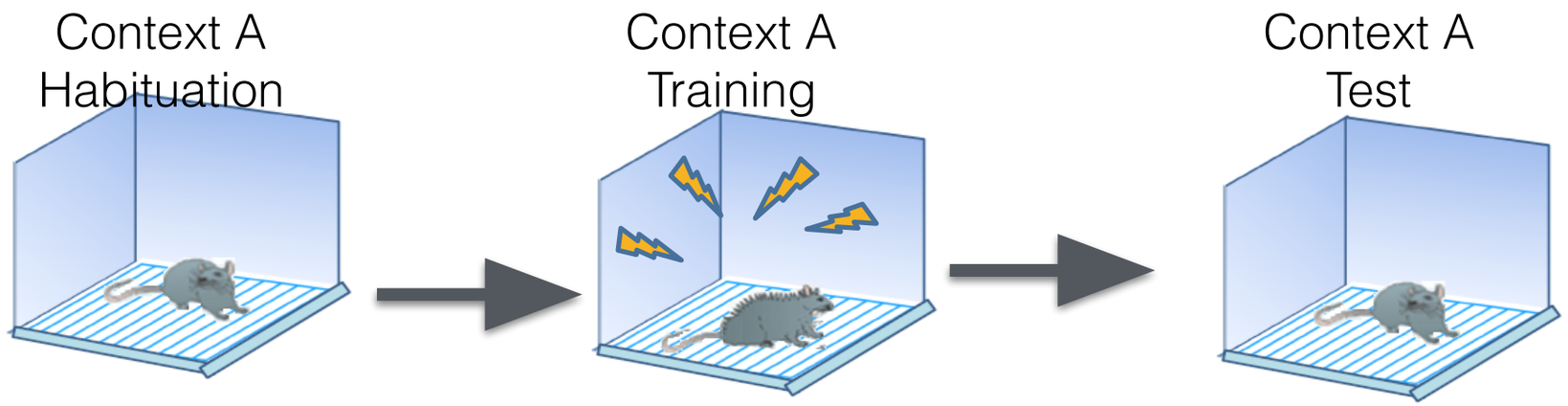
c-fos activation following memory recall (Paul Frankland)

Identification of a Functional Connectome for Long-Term Fear Memory in Mice

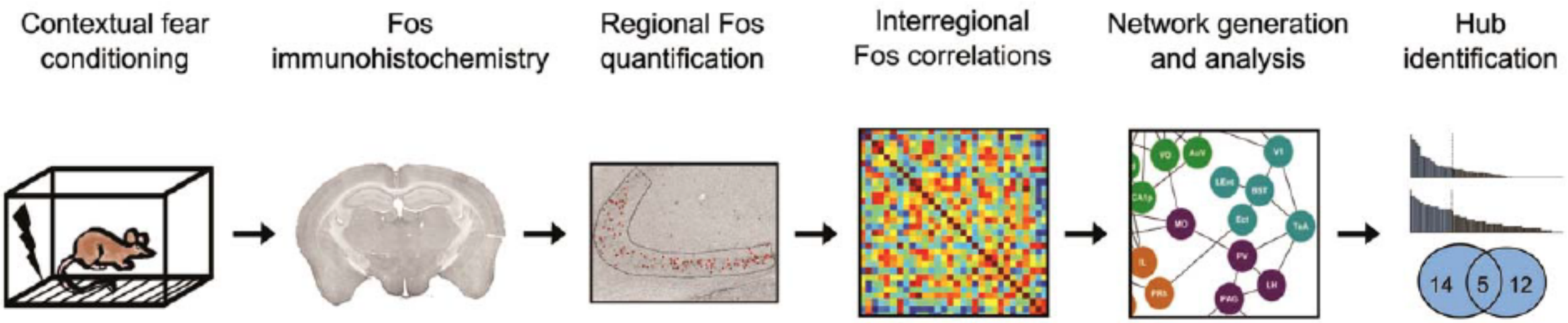
Anne L. Wheeler[1,2], Cátia M. Teixeira[1], Afra H. Wang[1,2], Xuejian Xiong[3], Natasa Kovacevic[4], Jason P. Lerch[1,5], Anthony R. McIntosh[4,6], John Parkinson[3,7], Paul W. Frankland[1,2,8]*

1 Program in Neurosciences and Mental Health, The Hospital for Sick Children, Toronto, Canada, **2** Institute of Medical Science, University of Toronto, Toronto, Canada, **3** Program in Molecular Structure and Function, The Hospital for Sick Children, Toronto, Canada, **4** Rotman Research Institute, Baycrest Centre, Toronto, Canada, **5** Department of Medical Biophysics, University of Toronto, Toronto, Canada, **6** Department of Psychology, University of Toronto, Toronto, Canada, **7** Departments of Biochemistry and Molecular Genetics, University of Toronto, Toronto, Canada, **8** Department of Physiology, University of Toronto, Toronto, Canada

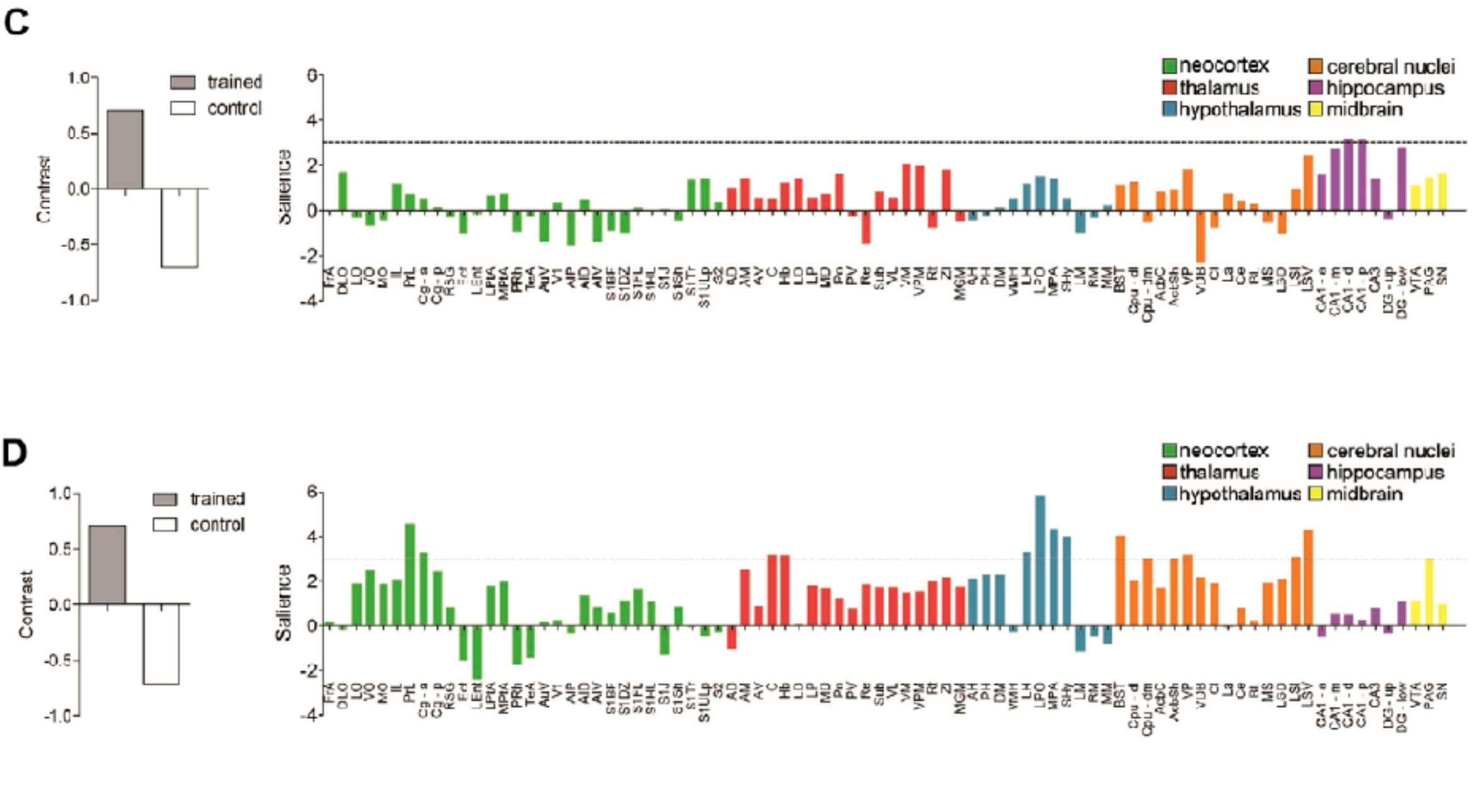
Studying memory in mice



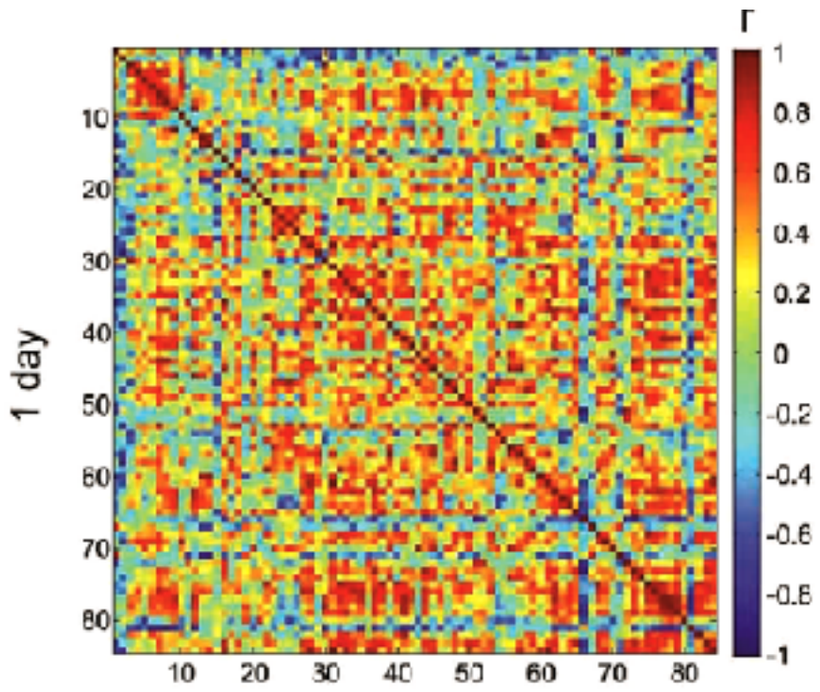
Experimental design

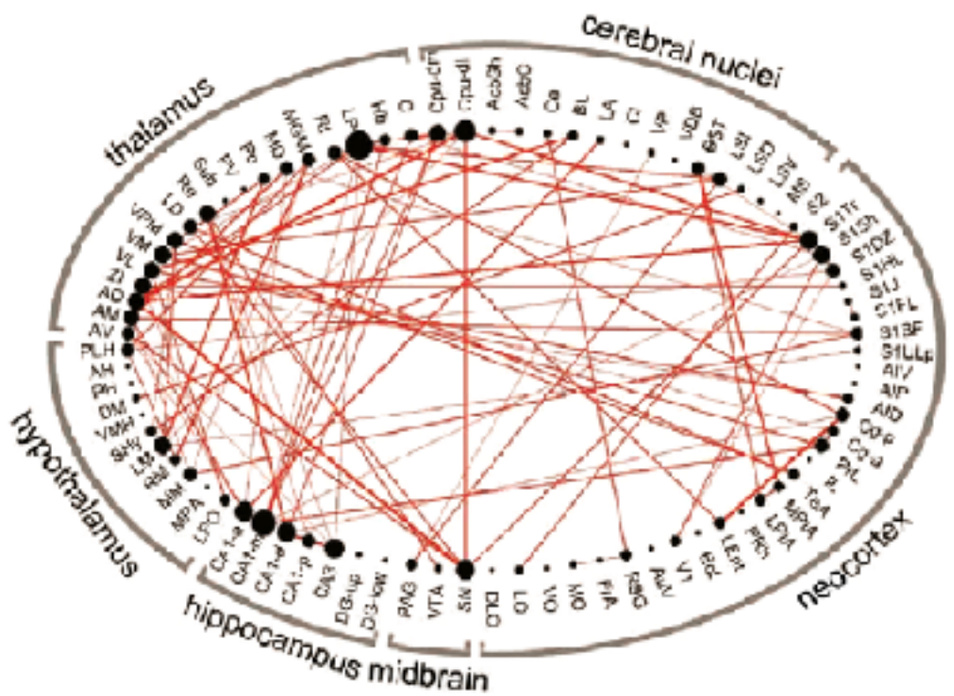


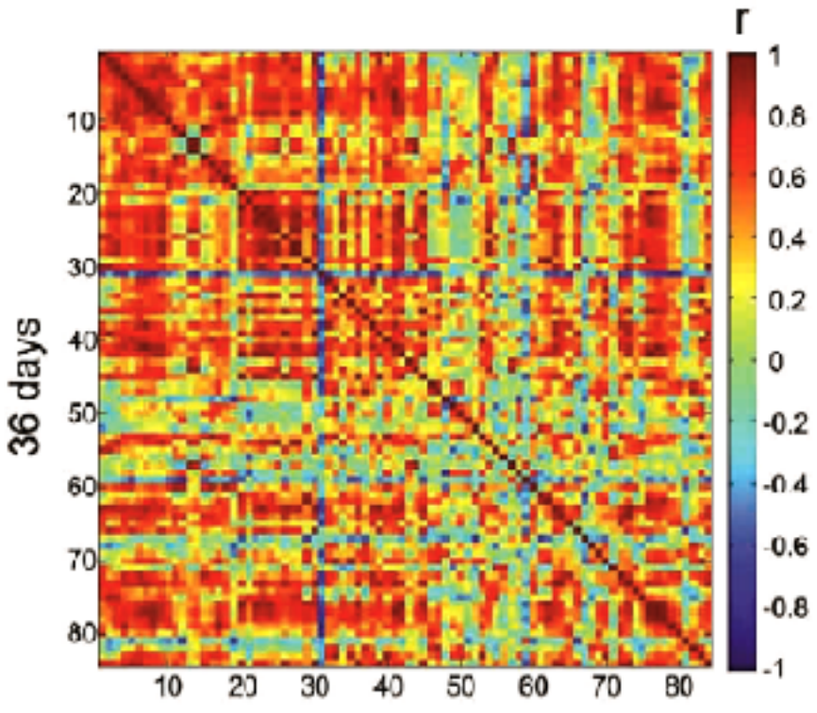
Differential change of c-fos expression between control and trained mice

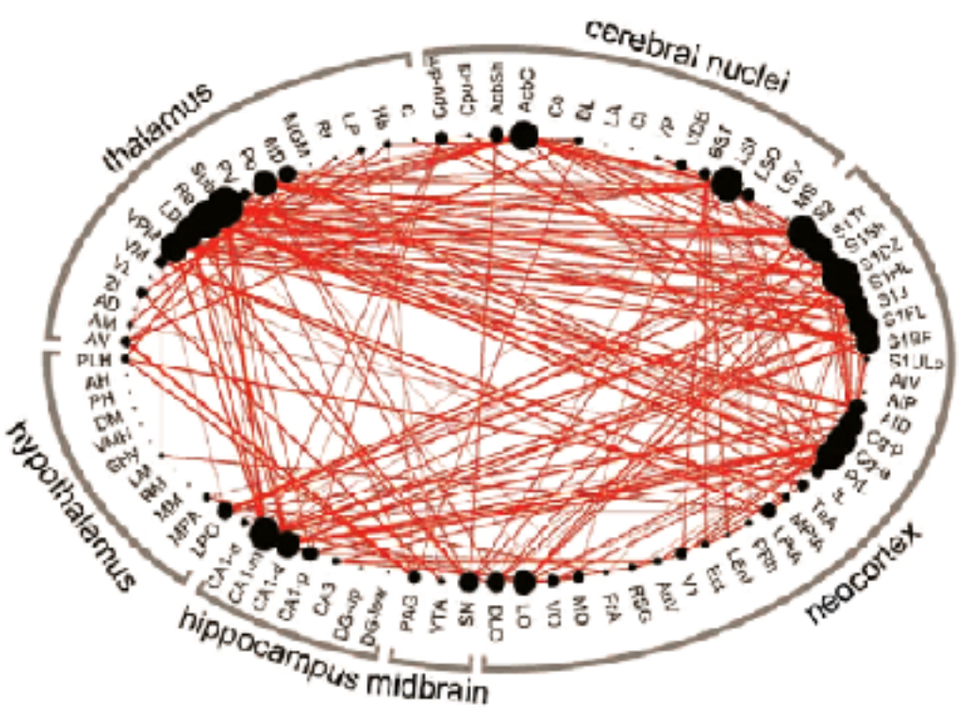


Correlation networks that underlie memory recall









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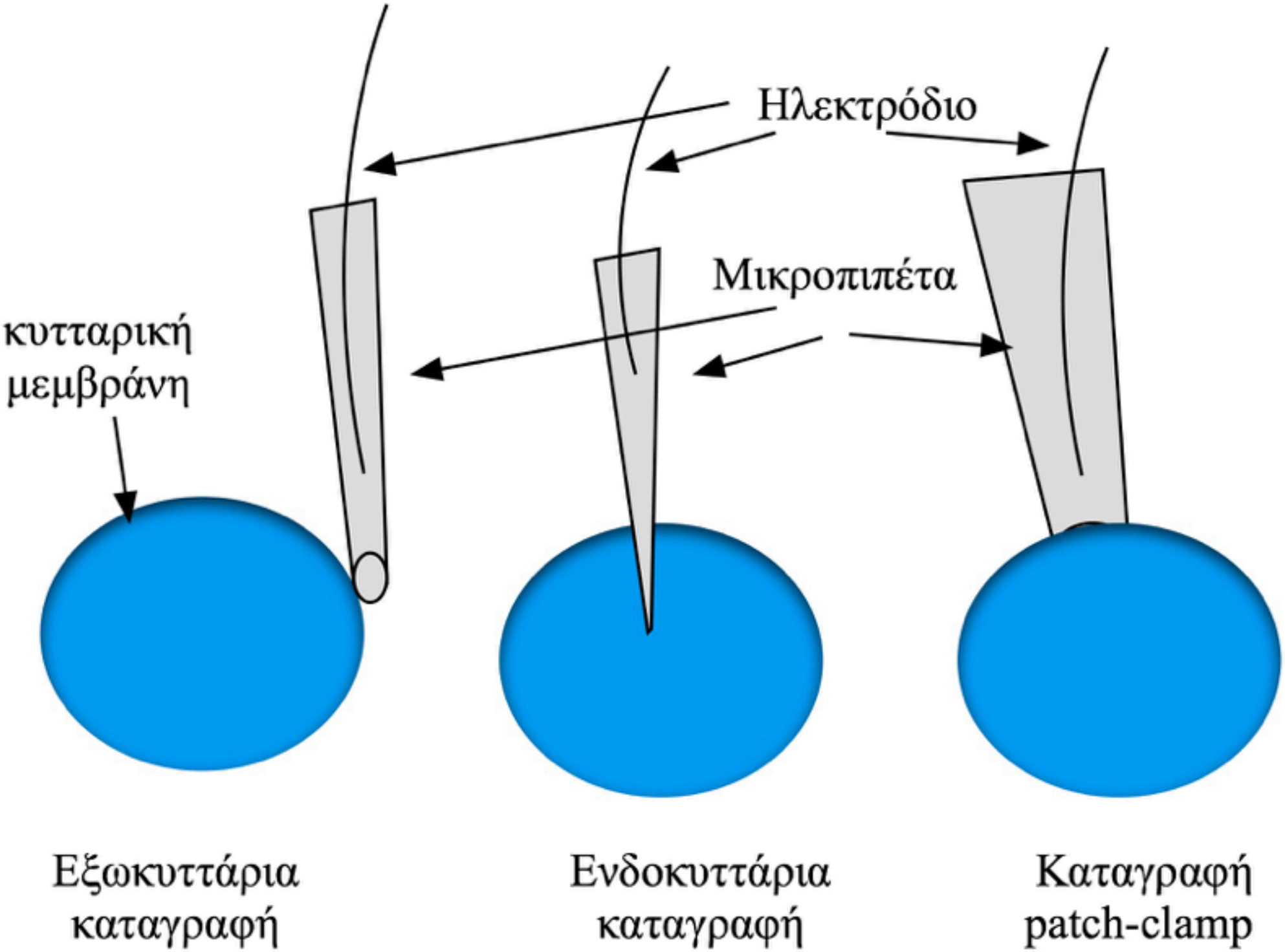
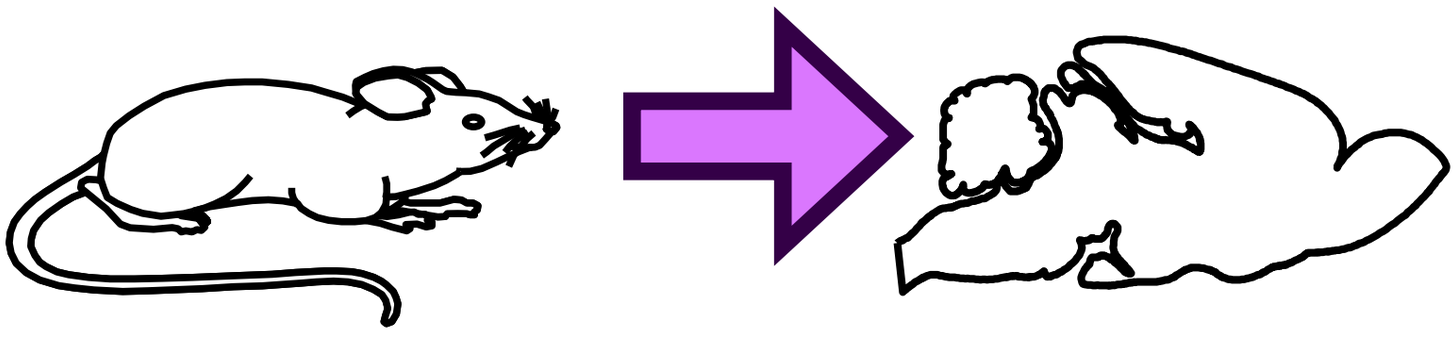
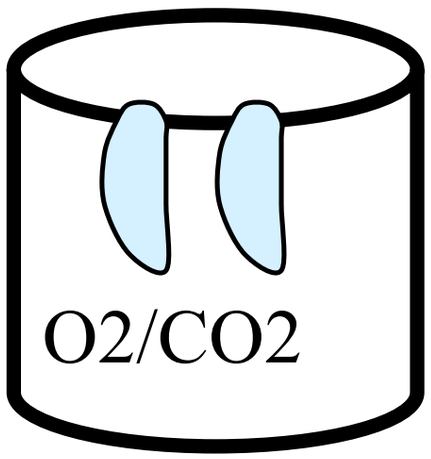
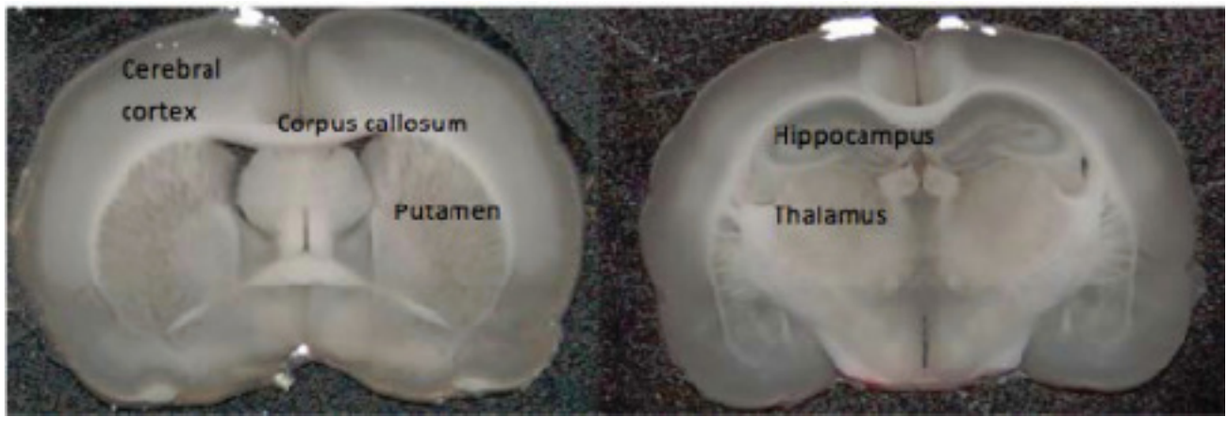
Electrophysiological recordings



Acute brain slice

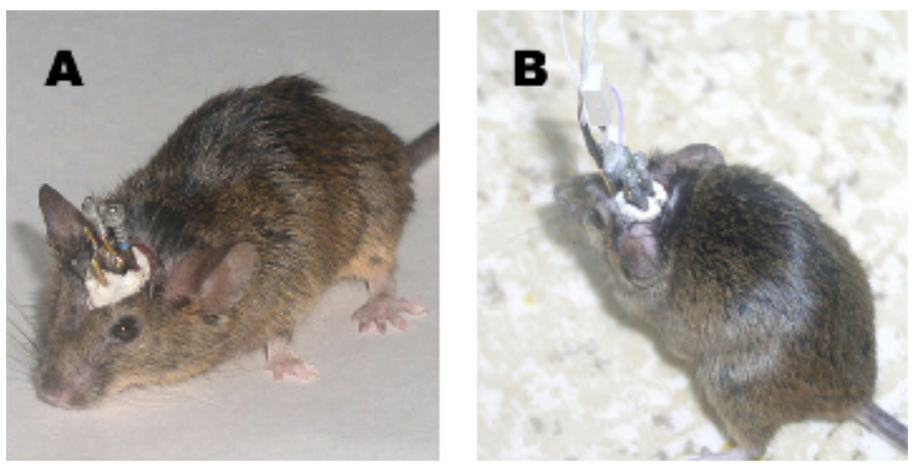






In vivo recordings

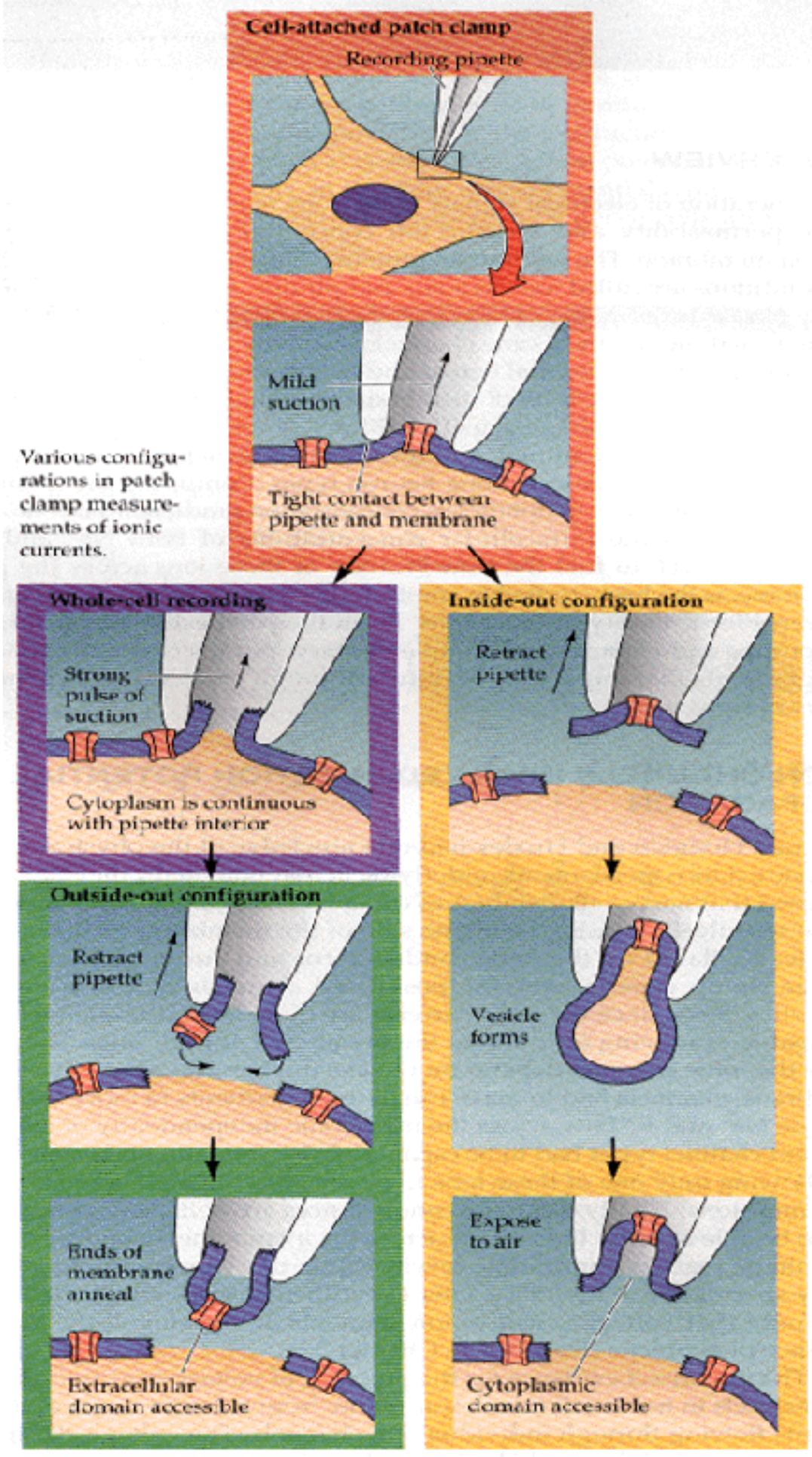
- Anaesthetized
- Head-fixed
- Freely-moving



Intracellular or patch-clamp recordings

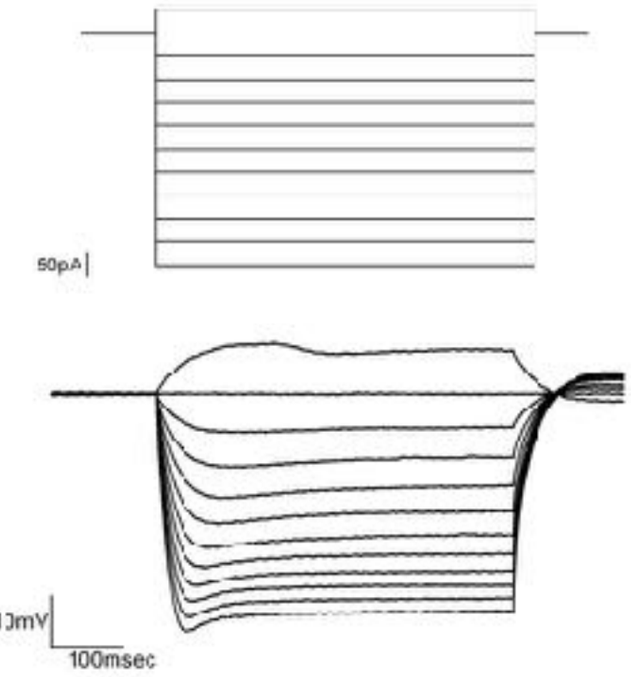
Patch clamp technique Configurations

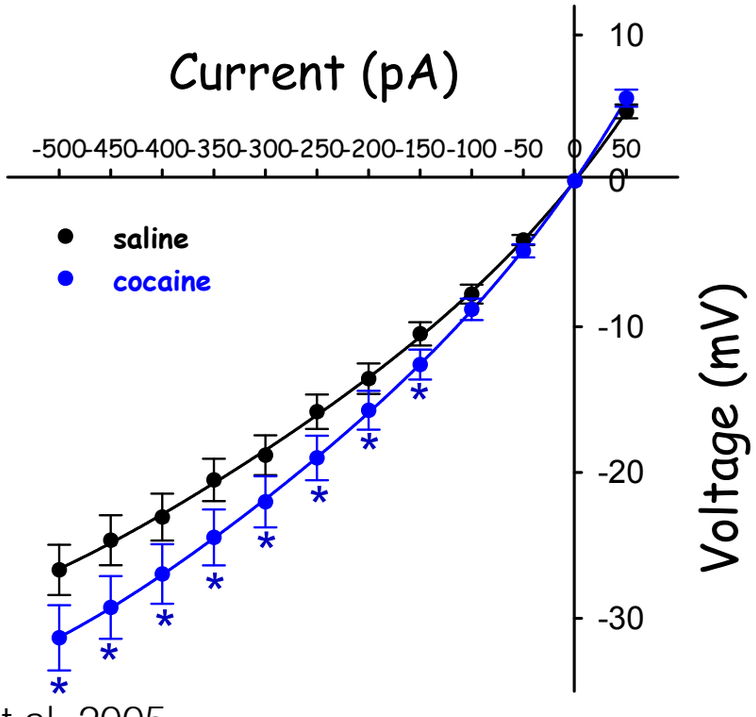
- Current-clamp
  - Give current inputs and record voltage changes
  - Monitor Vm, record Action Potentials
- Voltage-clamp mode
  - "Clamp" voltage and record currents
  - Study different ion channels



Current-clamp

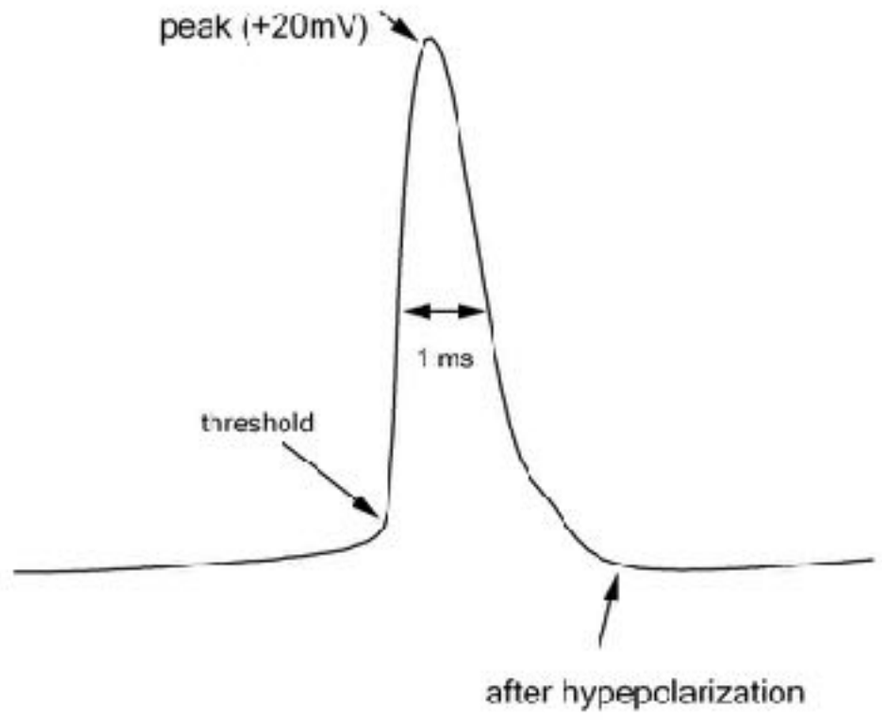
- I-V curve
- Membrane properties





Nasif et al, 2005

- Action potential properties



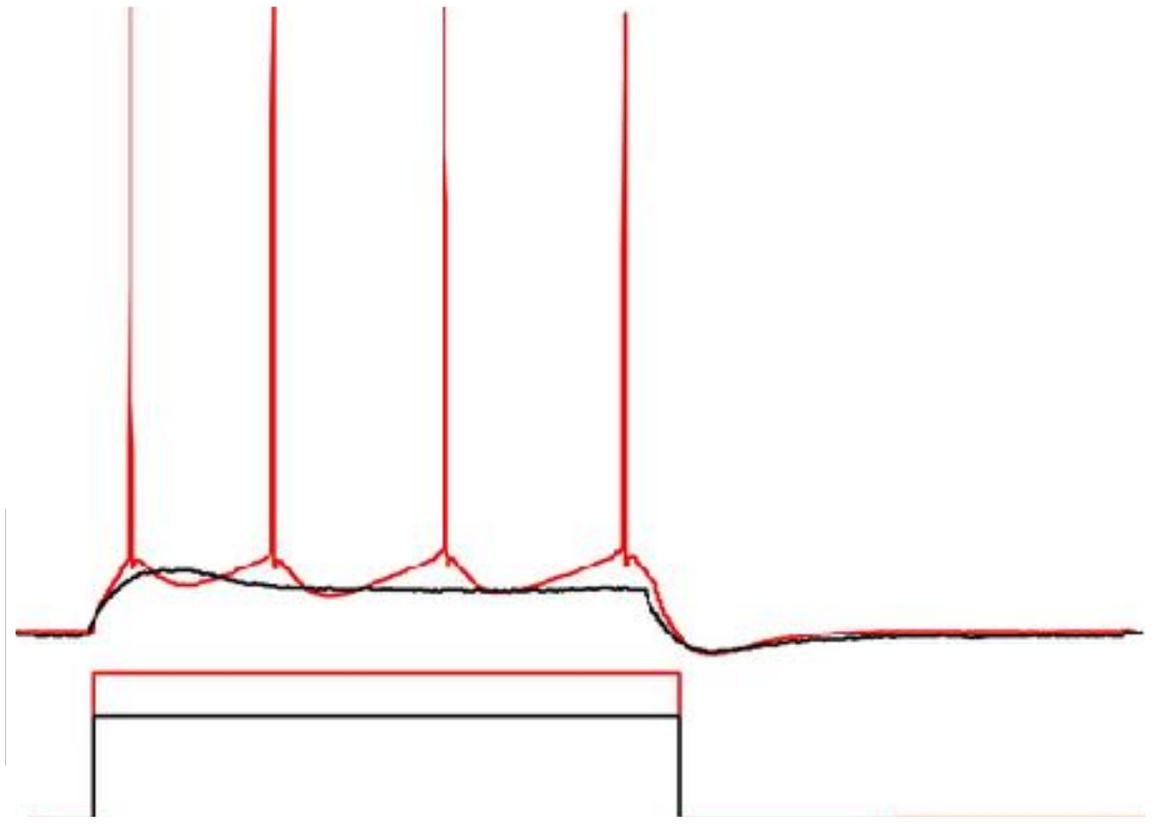
Whole-cell configuration
Current-clamp

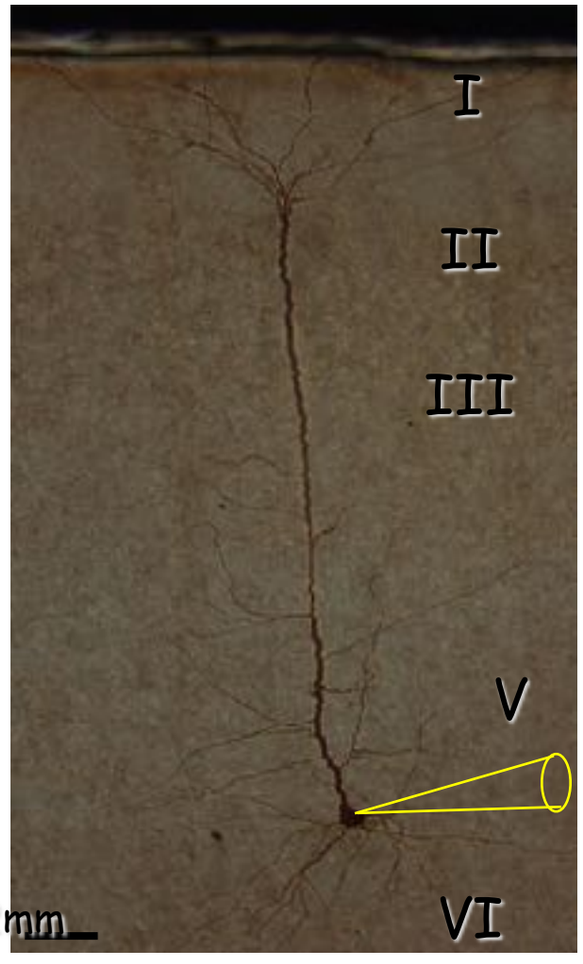
Διαλύματα που χρησιμοποιούνται

Εξωκυττάρια: τεχνητό εγκεφαλονωτιαίο υγρό (artificial cerebrospinal fluid, aCSF), pH,
Ενδοκυττάρια (στο ηλεκτρόδιο): παρόμοια με το ενδοκυττάριο περιβάλλον

Voltage response

Current input





Voltage-clamp

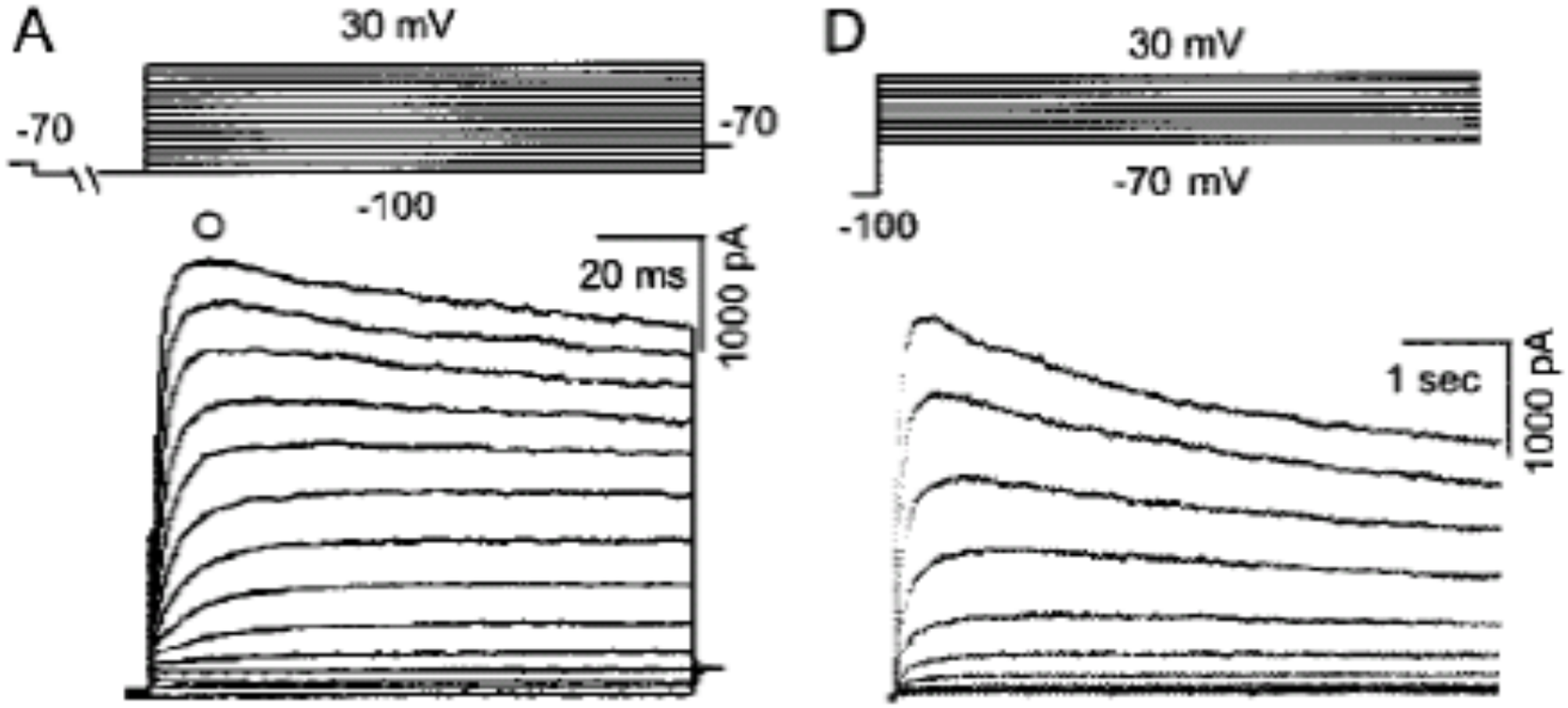
- Διαλύματα (ανάλογα με το ρεύμα που είναι να καταγραφεί)
- Τα περιεχόμενα των διαλυμάτων μπορούν να επηρεάσουν κατά πολύ τα αποτελέσματα των καταγραφών.

Current recordings

- Ρεύμα νατρίου (μπλοκάρουμε τα ρεύματα καλίου και ασβεστίου)
- Ρεύμα καλίου (μπλοκάρουμε τα ρεύματα νατρίου και ασβεστίου)
- Ρεύμα ασβεστίου (μπλοκάρουμε τα ρεύματα νατρίου και καλίου)

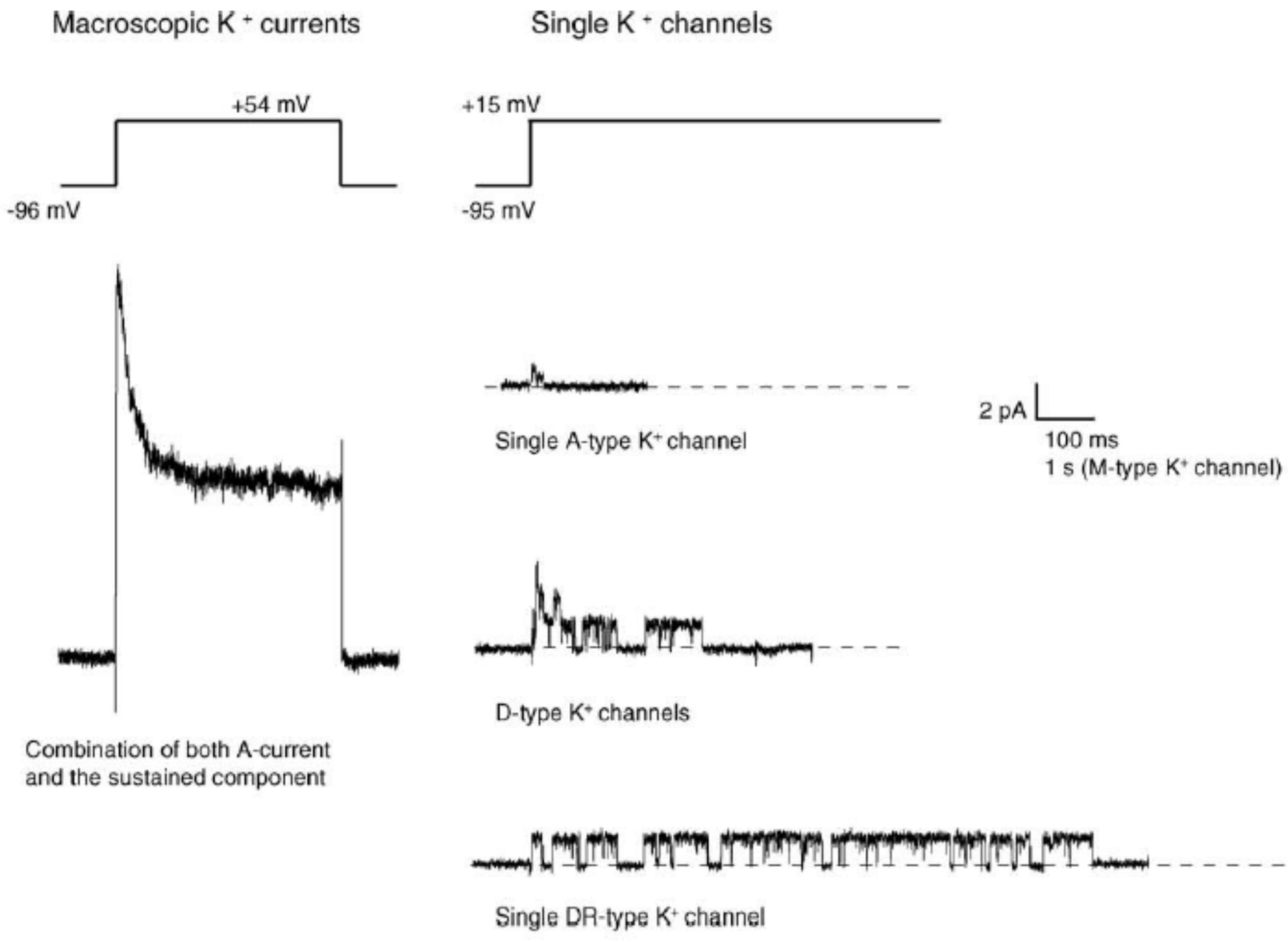
Tetradotoxin, tetra-ethyl-ammonium (TEA), cadmium

Potassium currents voltage-dependent (whole-cell configuration)



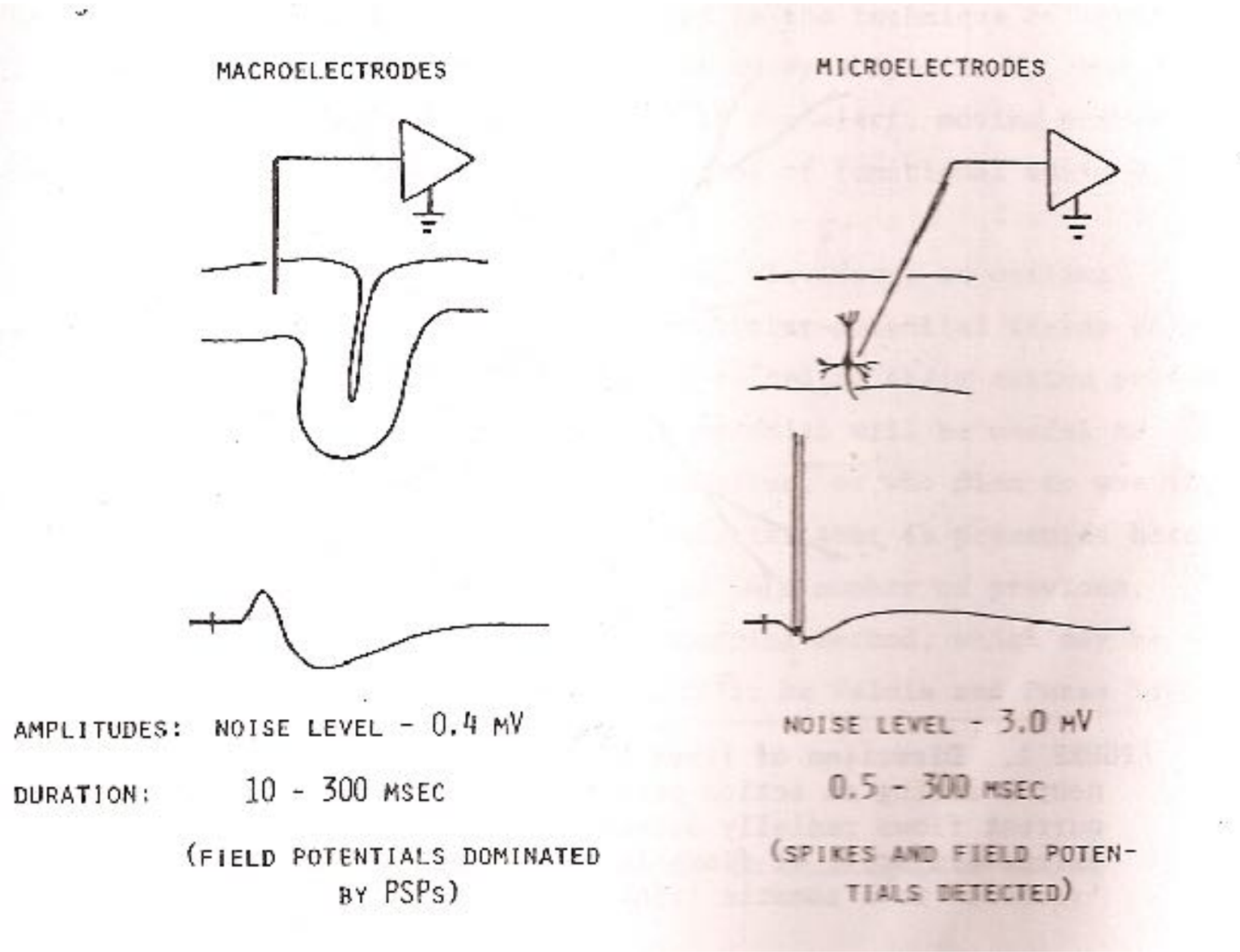
Dong and White, J Neuroscience, 2003

Whole-cell vs single-channel currents



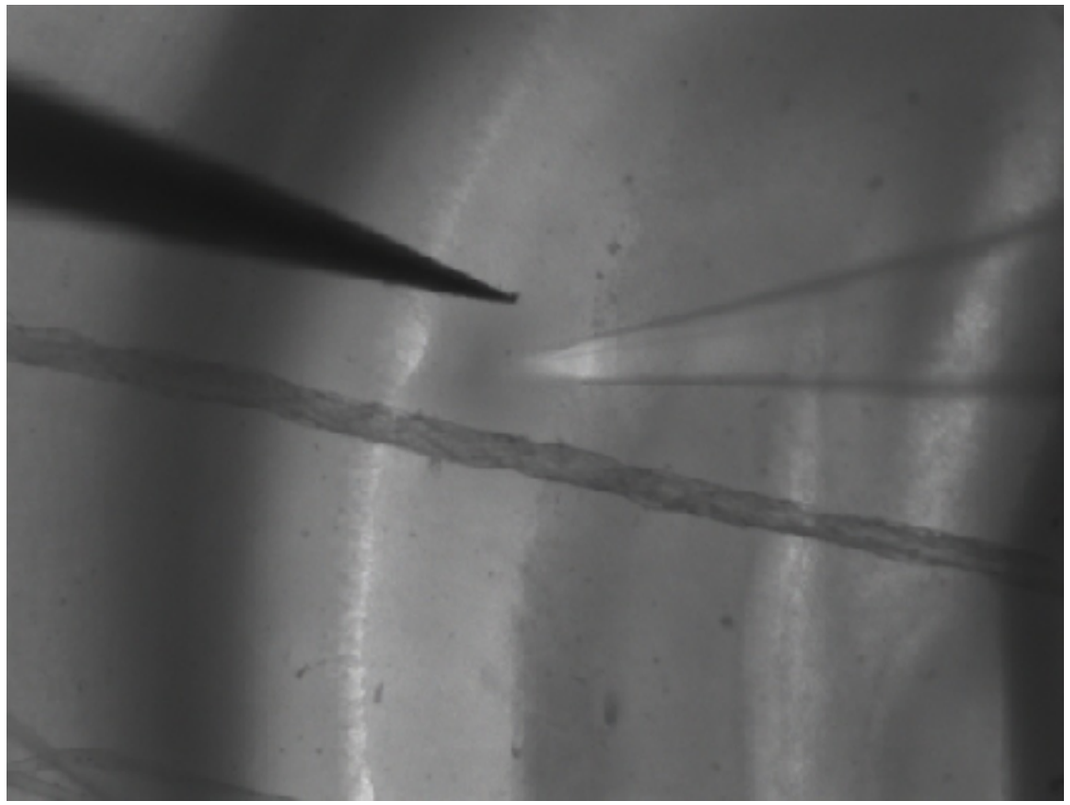
What is the connection to networks and behavior?

Extracellular recordings

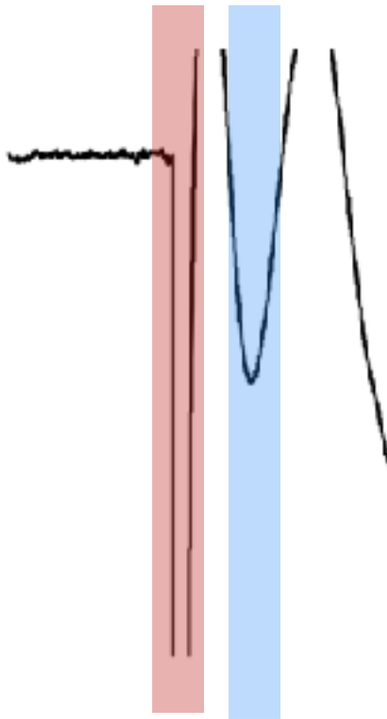


Mountcastle, 1957

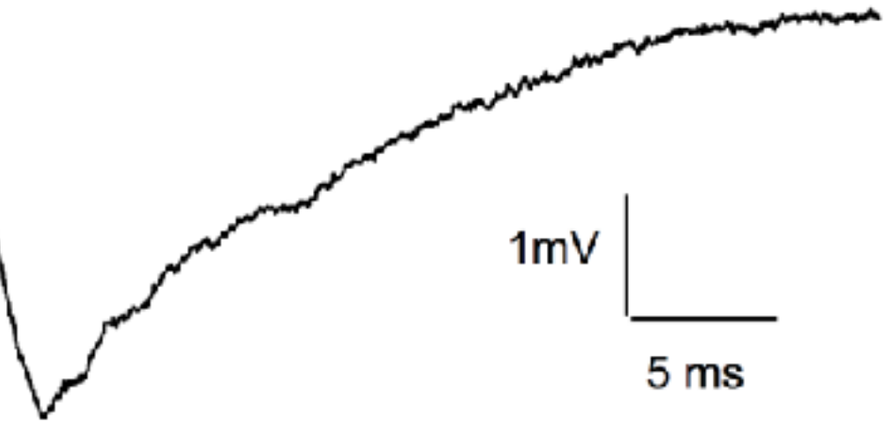
Extracellular Recordings - Local Field Potentials



stimulation artifact



field excitatory post-synaptic potential

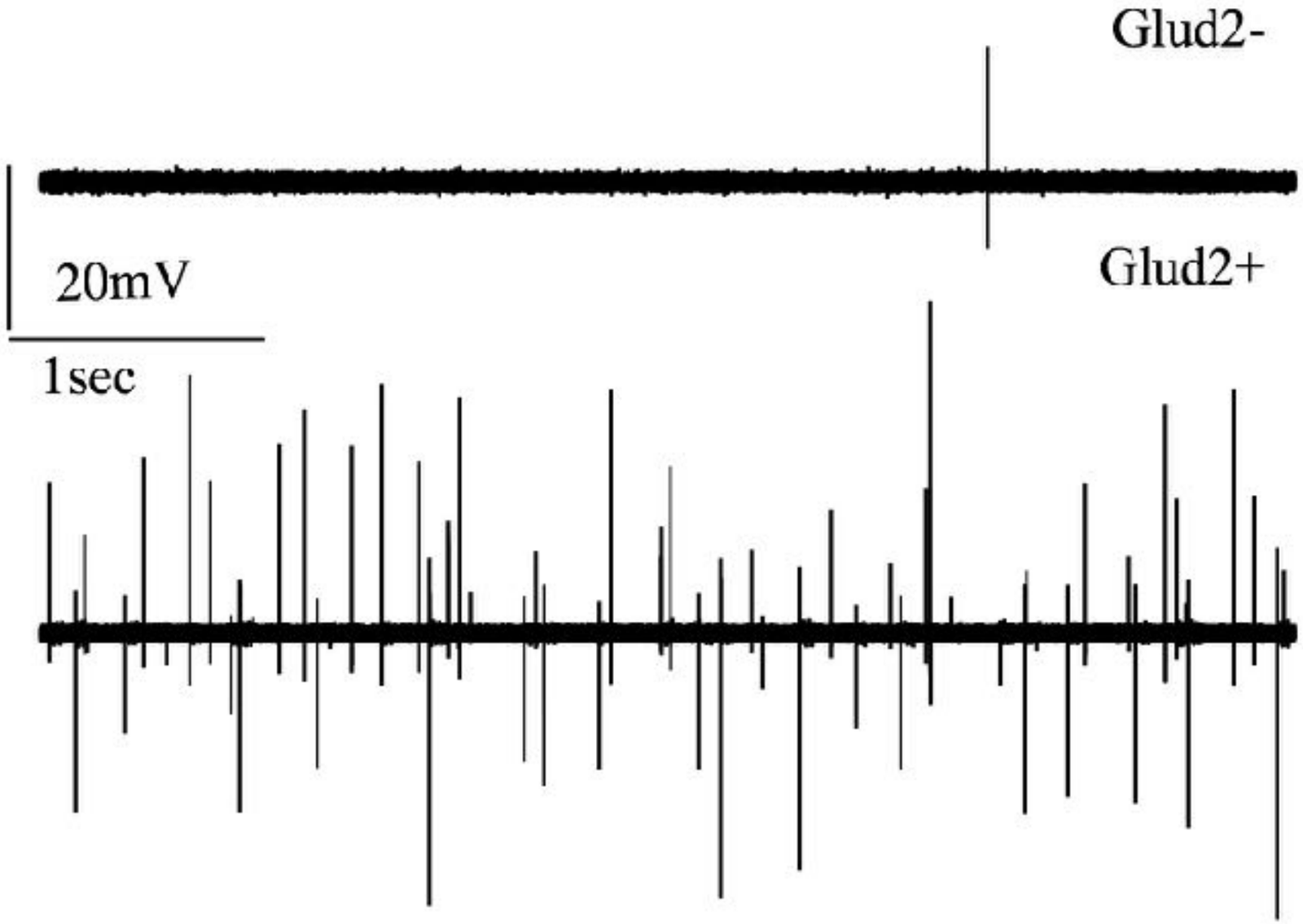


fiber volley

Brain slice - LFPs

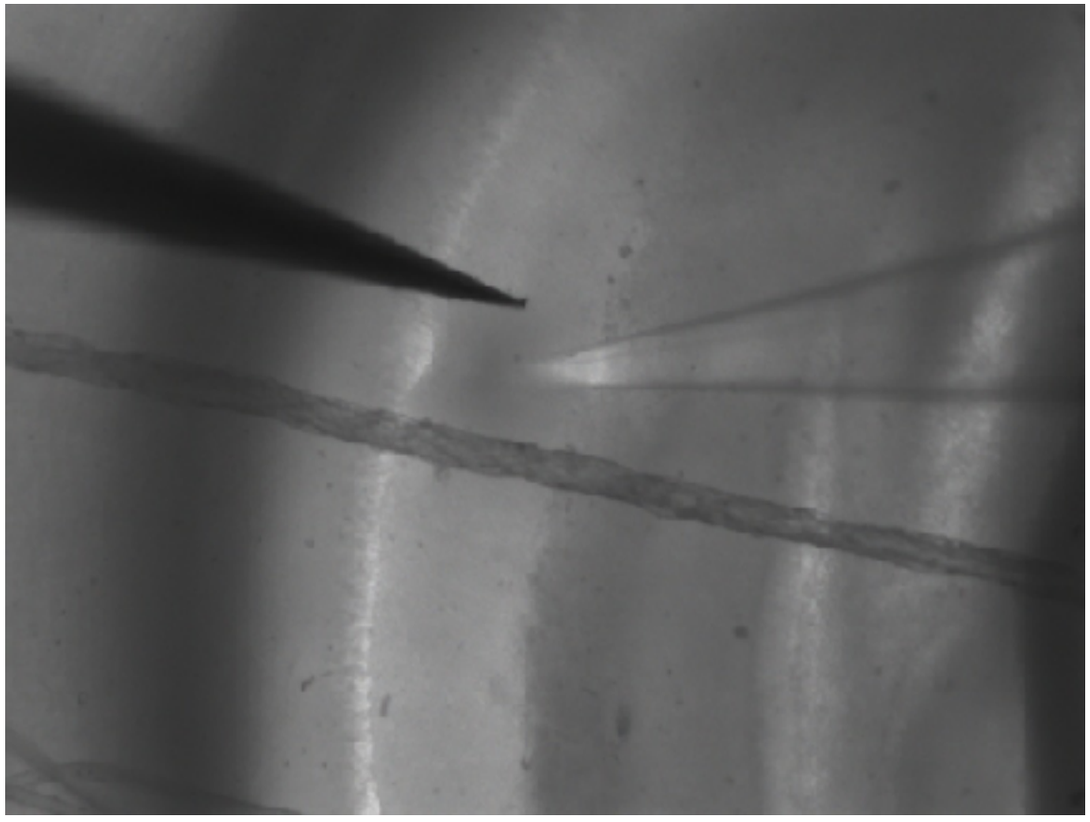
- Record brain areas that are stratified
  - hippocampus
  - Cortex
- Spontaneous/Epileptiform activity
- Evoked field excitatory postsynaptic potentials (fEPSP)
- Long-term potentiation

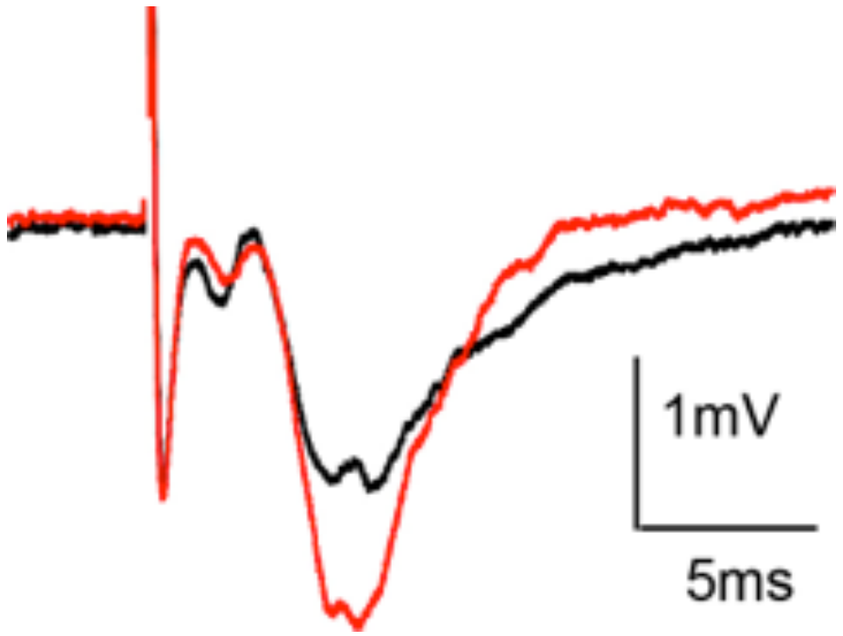
Basal spontaneous activity



Spanaki, Sidiropoulou et al, in preparation

Evoked LFPs - Long-term potentiation





0.1mA

10-20min

Tetanus
100Hz - 1sec
3x - 20sec

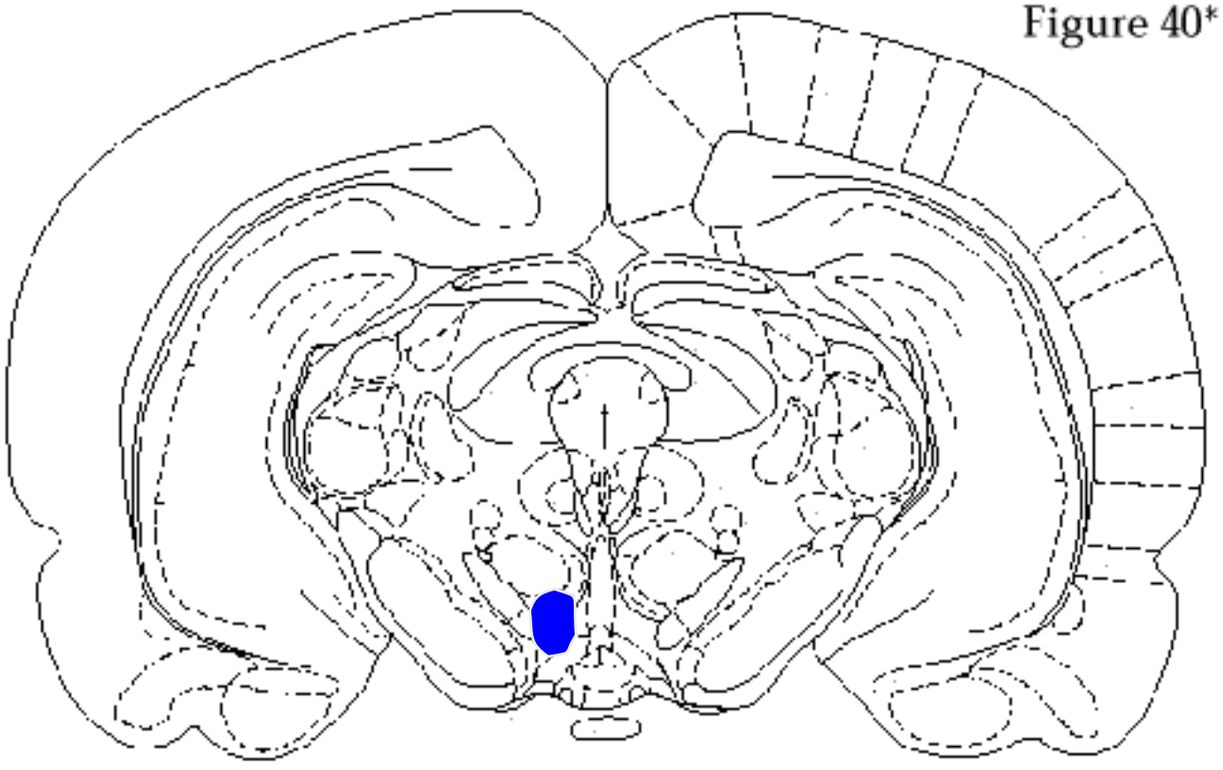
50min

Extracellular Recordings - Single unit recordings
Dopamine neurons

Ventral Tegmental Area
Substantia Nigra
A8, A9, A10

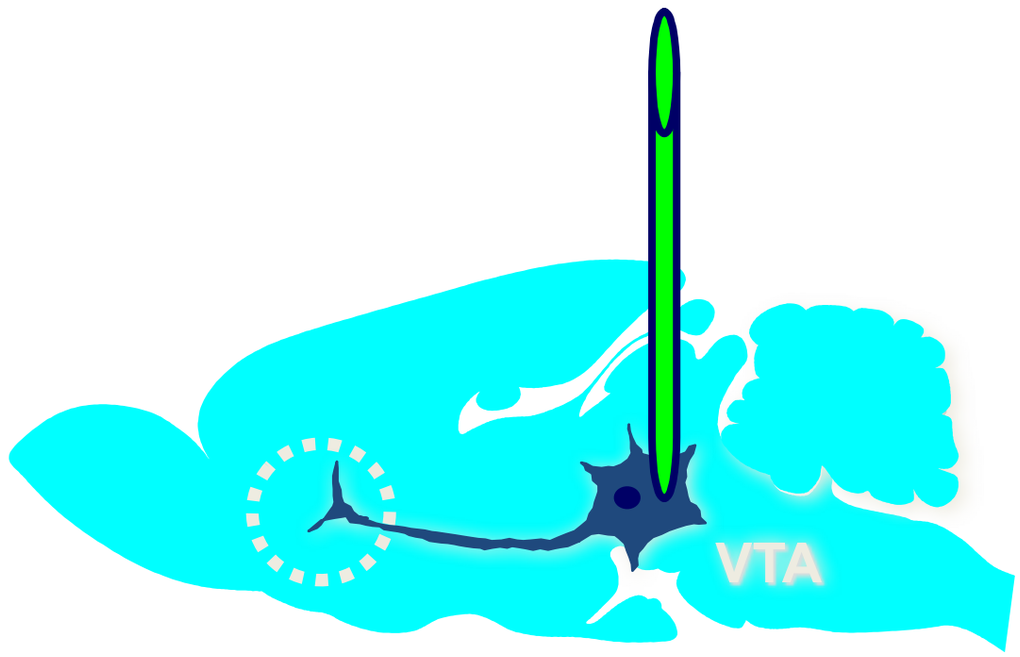
- Διαλύματα
- Ελεκτρόδιο
  - NaCl
  - Fast green

Figure 40*

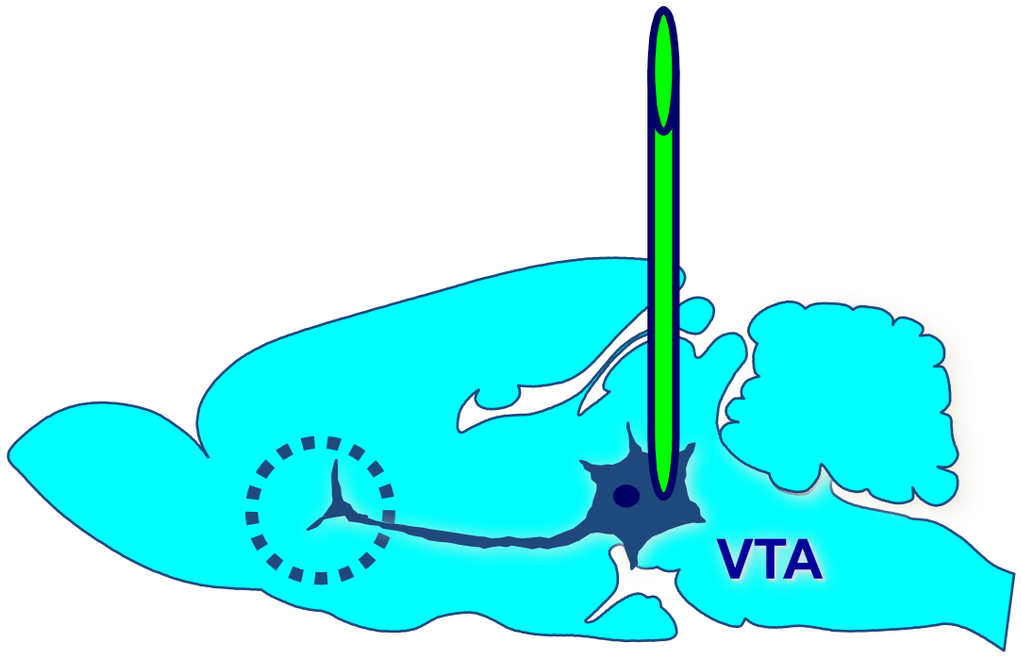


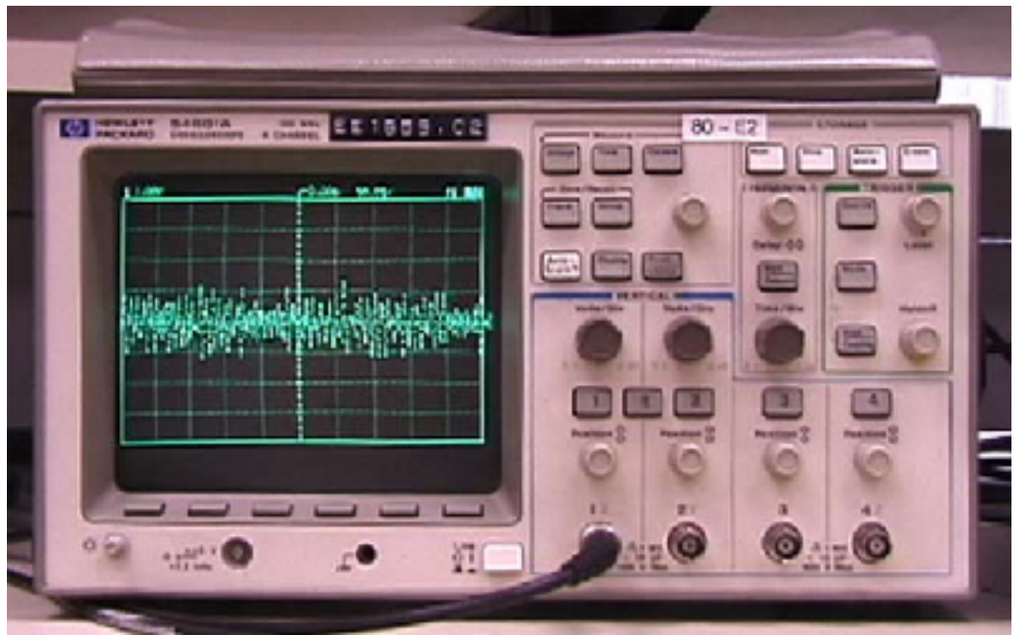
Interaural 3.40 mm*

Bregma -5.60 mm

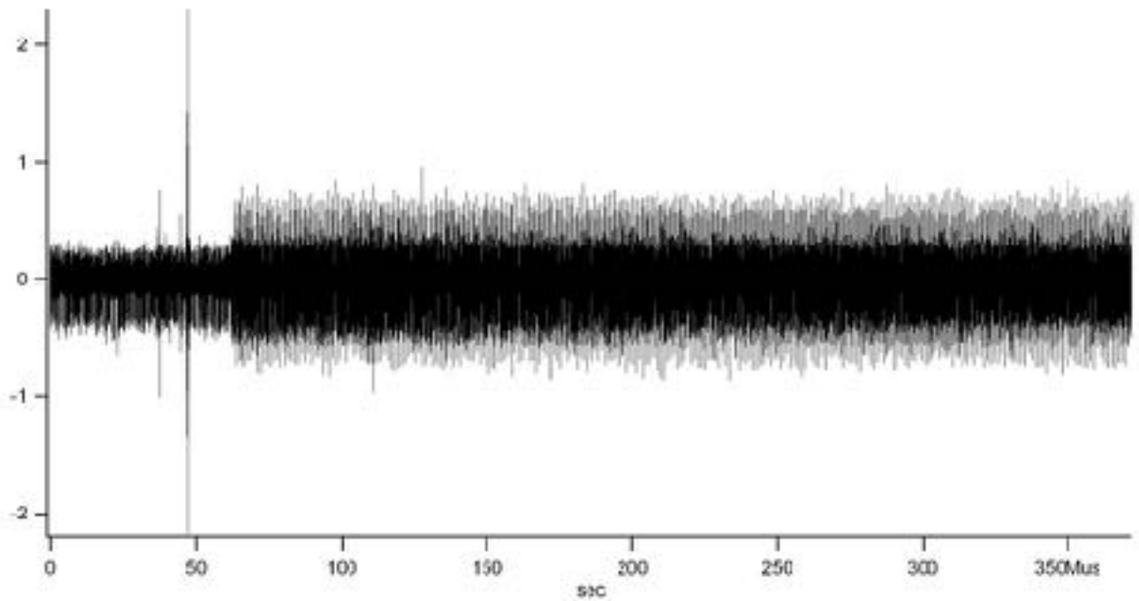


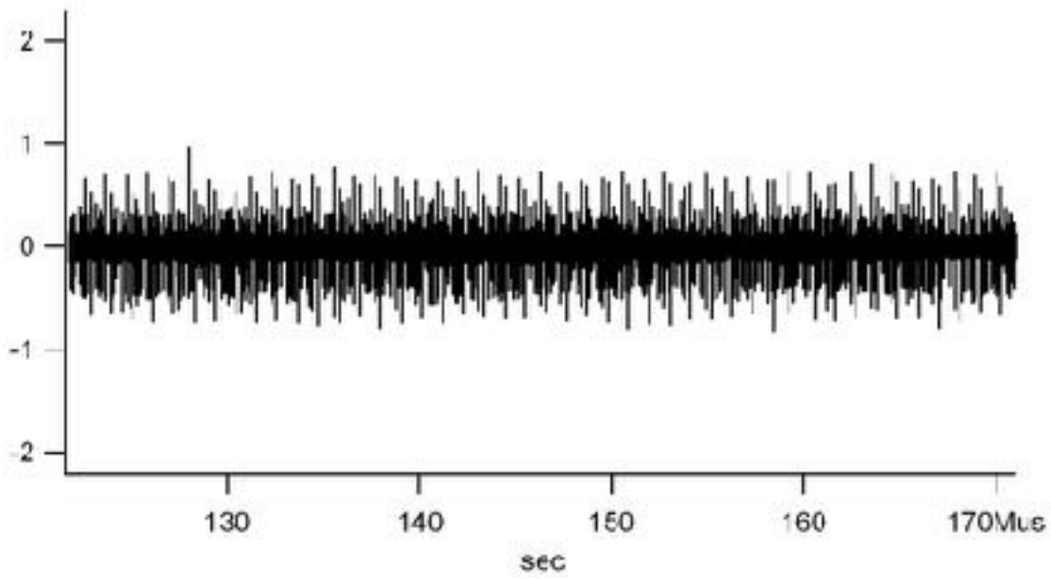
Extracellular Recordings



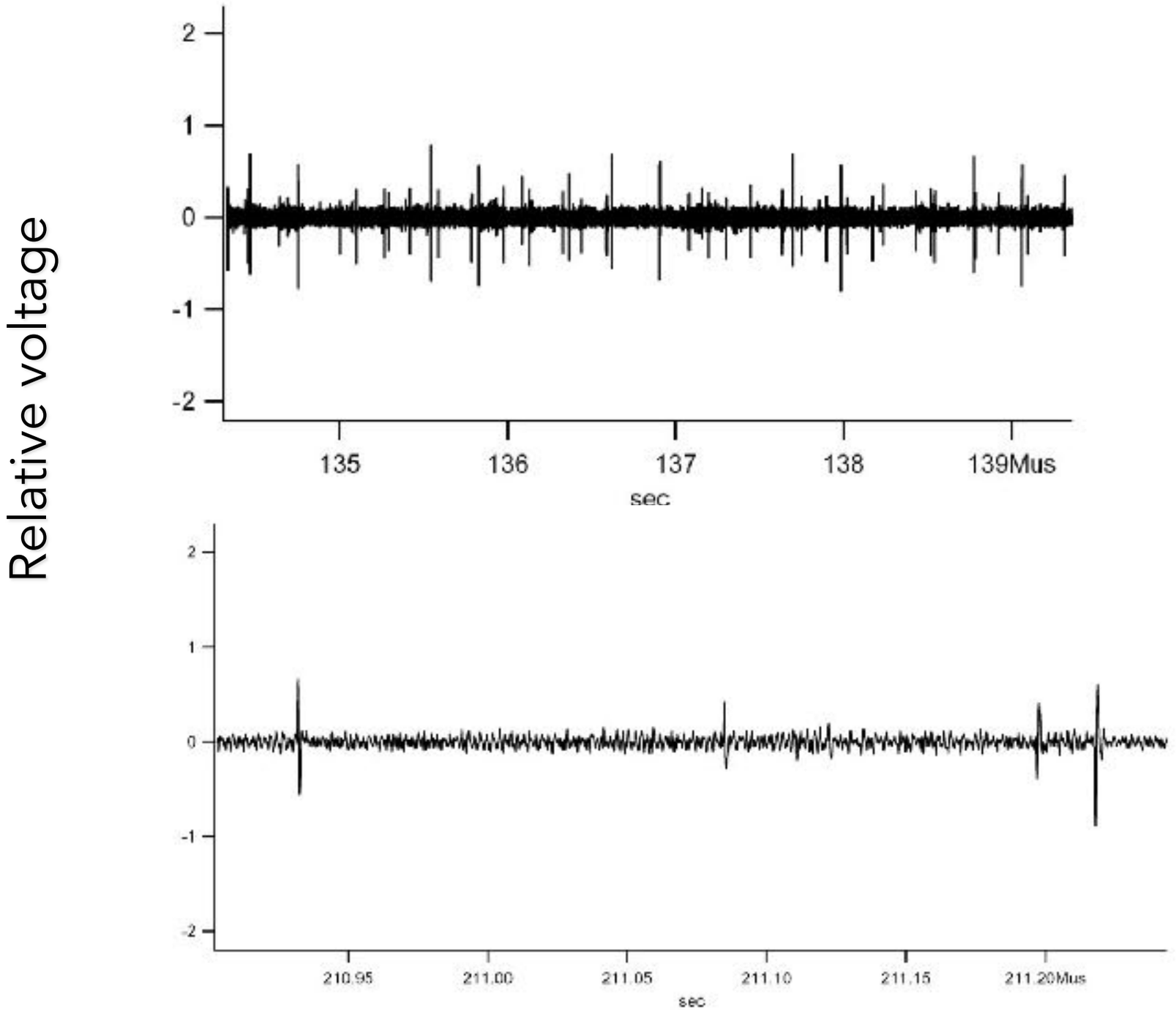


Relative voltage

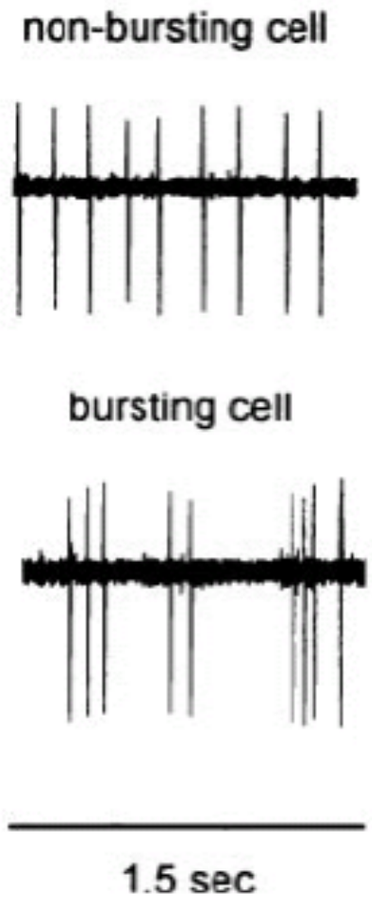


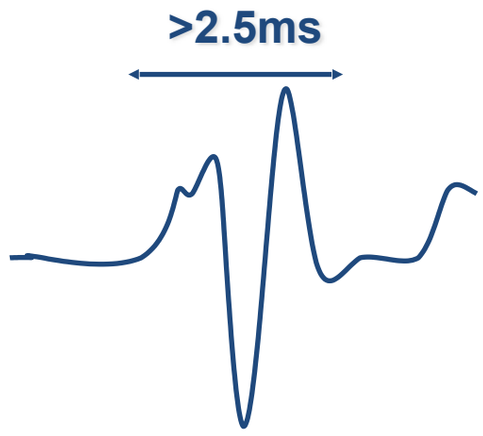


Extracellular Recordings
Dopamine neurons



Dopamine neuron waveforms





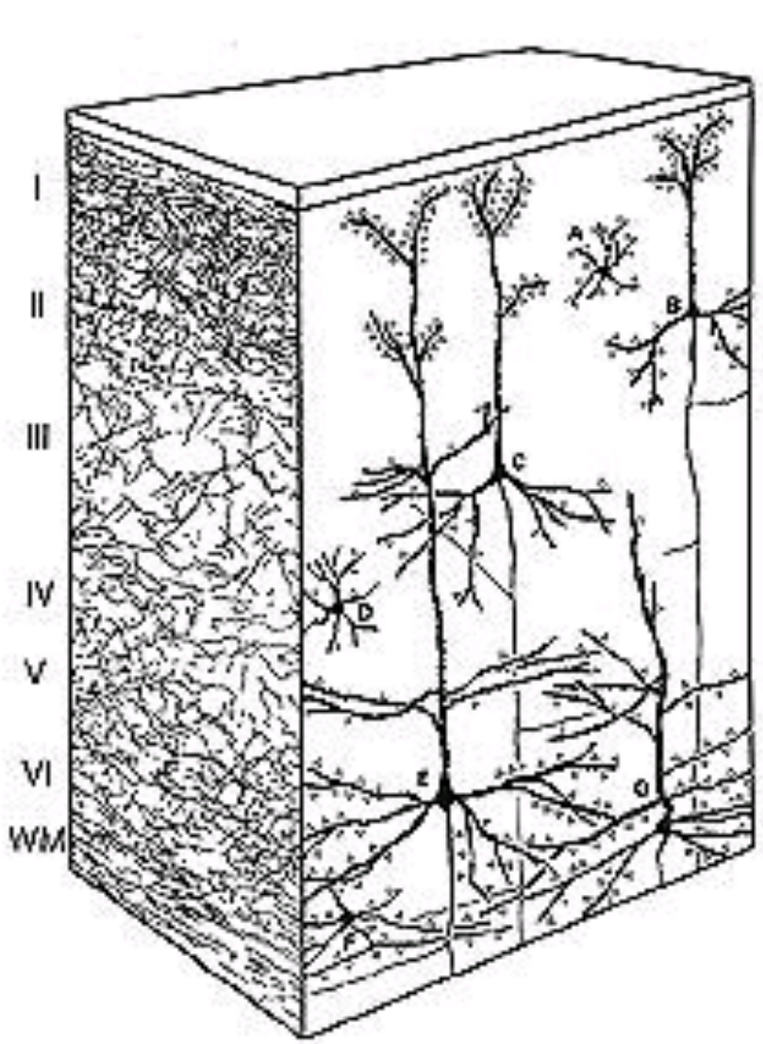
- Spontaneously active, and fire at low irregular frequencies (1-10Hz)
- Triphasic waveform of long duration
- Tonic or burst-firing

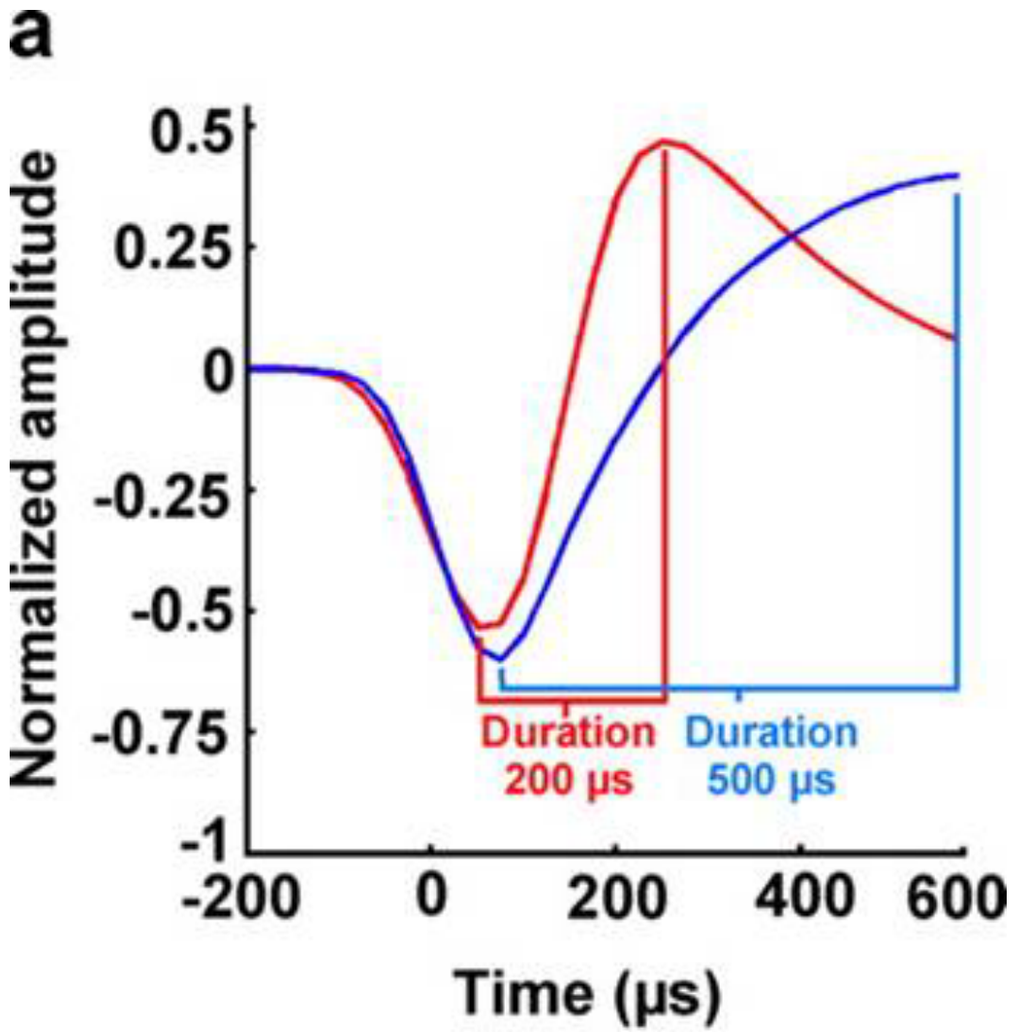
Αναφορά: Marinelli and White, 2000, J. Neuroscience

Correlation between dopamine neuron firing rate and exploratory activity in a novel environment

(Marinelli and White, 2000)

Differentiating the neuronal type based on the waveform properties



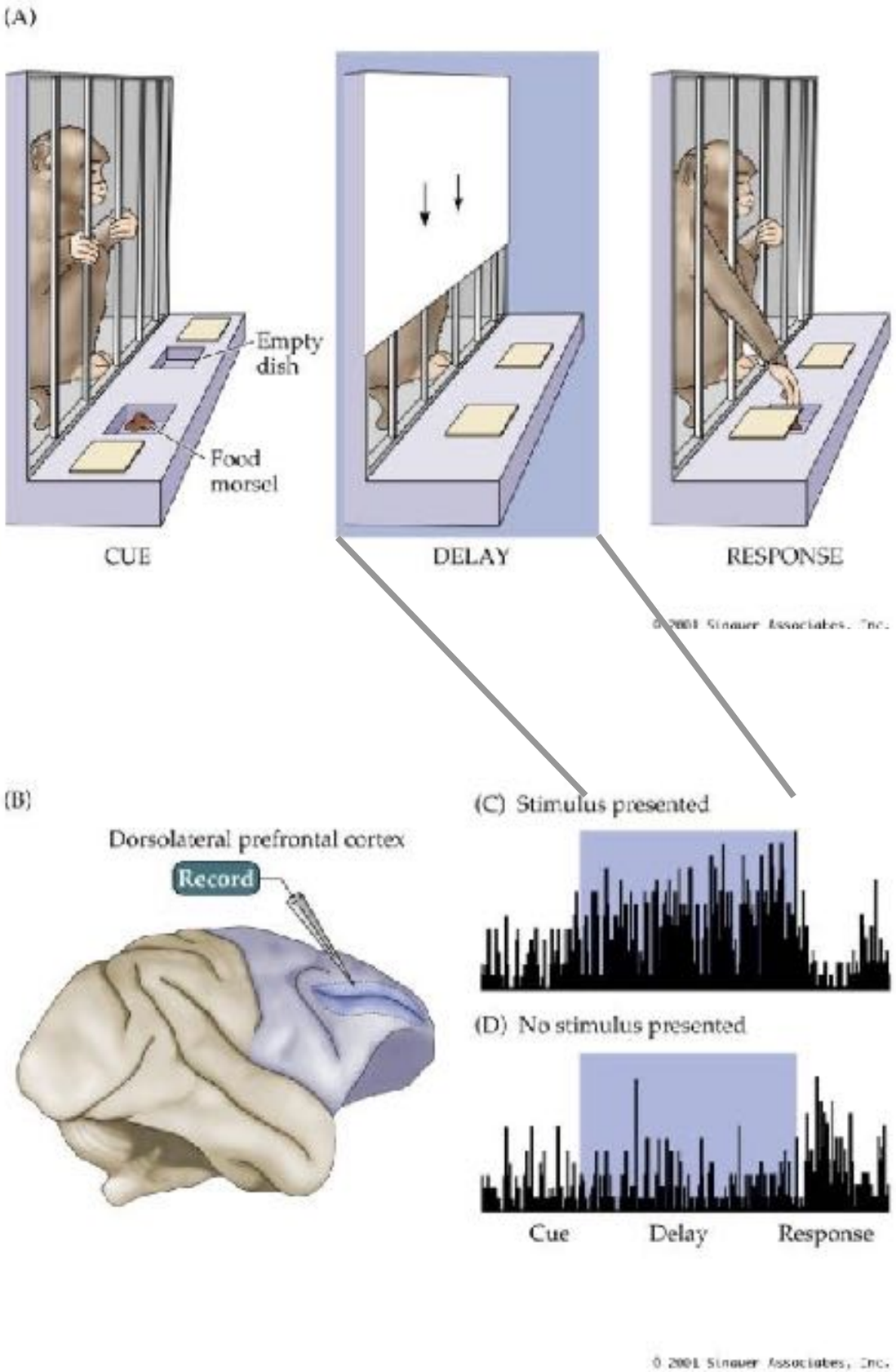


Cortex

Interneurons

Pyramidal neurons

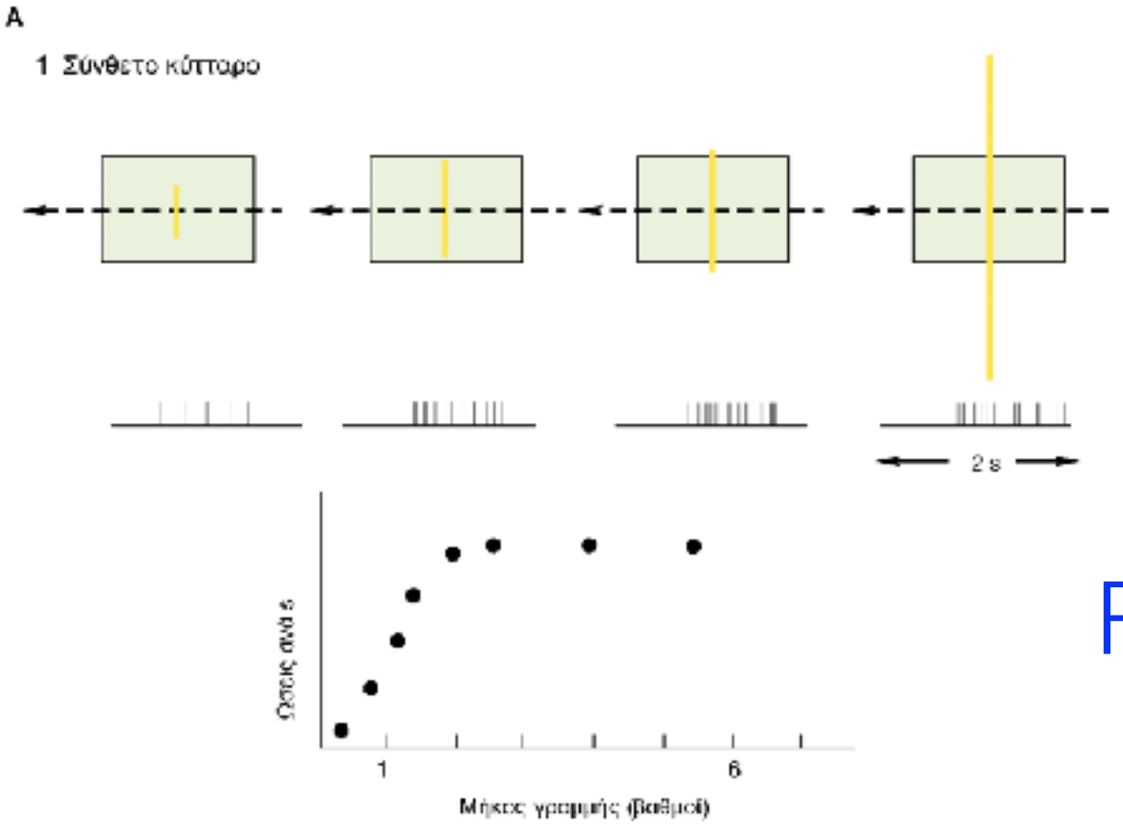
Electrophysiology and behavior



- Working memory
- Persistent activity

Goldman-Rakic, 1995, Neuron

Receptive fields in the visual cortex

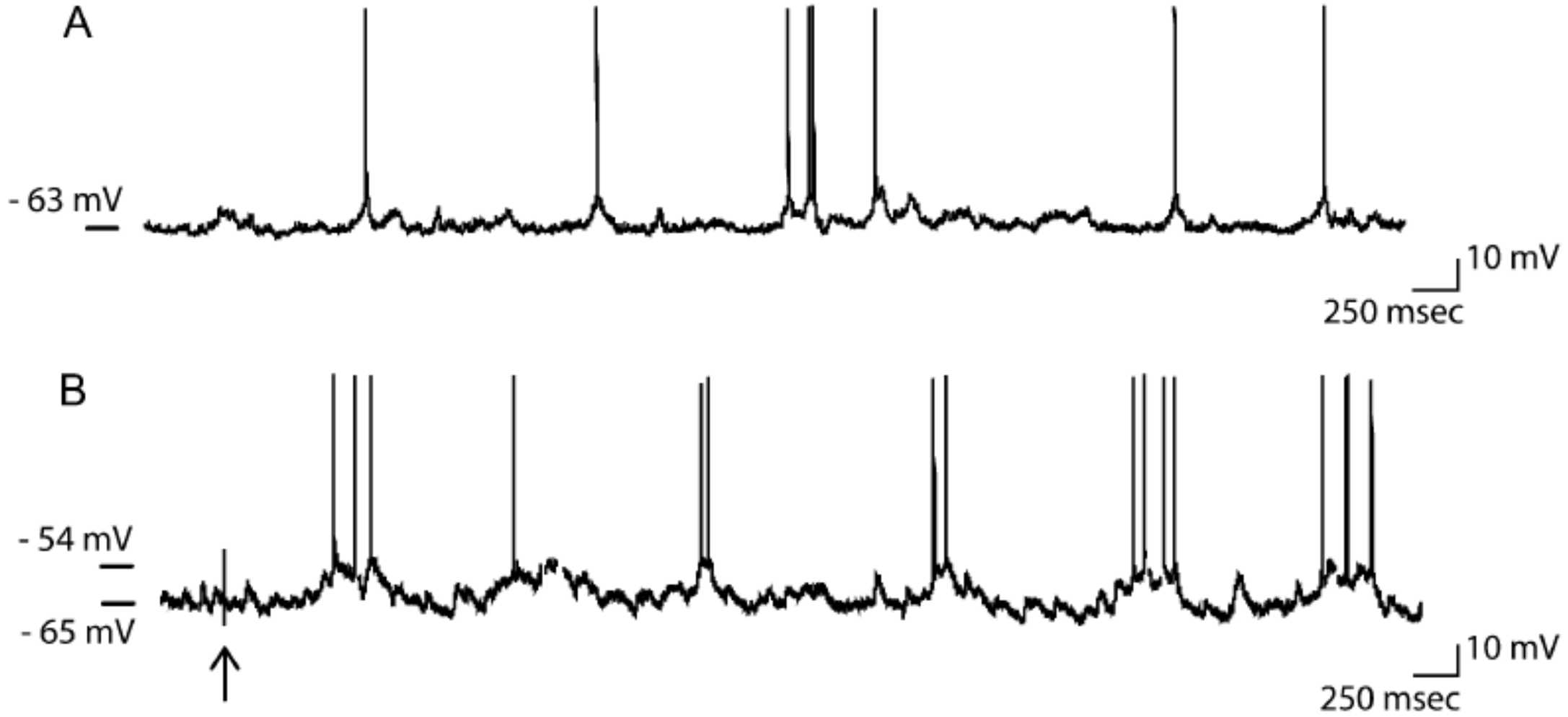


Εικόνα 23-12 Τα ακρο-αναστελλόμενα κύτταρα είναι ανώτερης τάξης σύνθετα κύτταρα που έχουν σχέση με τα χείλη, τις γωνίες και τις καμπύλες. (Από Hubel και Wiesel, 1965, τροποποιημένη.)

Α. Τα κύτταρα του οπτικού φλοιού αποκρίνονται επιλεκτικά σε μια συγκεκριμένη περιοχή μηκών μιας κατακόρυφης φωτεινής γραμμής. **1.** Η απόκριση αυτού του σύνθετου κυττάρου αυξάνεται καθώς το μήκος της γραμμής αυξάνεται μέχρι 2° περίπου, ενώ έπειτα δεν υπάρχει μεταβολή. **2.** Η απόκριση αυτού του ακρο-αναστελλόμενου κυττάρου βελτιώνεται, καθώς η γραμμή αυξάνεται μέχρι 2°, αλλά κατόπιν μειώνεται, έτσι ώστε μια γραμμή 6° ή μεγαλύτερη δεν προκαλεί απόκριση.

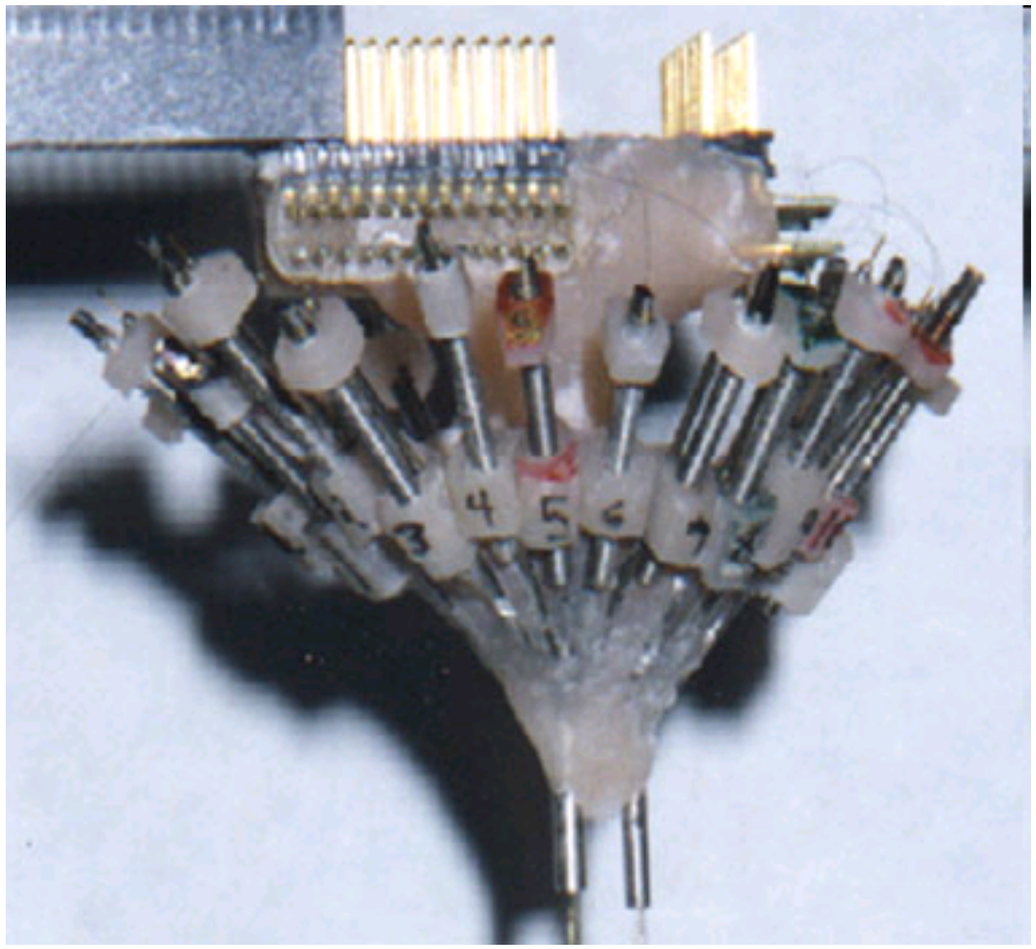
(συνεχίζεται)

In vivo intracellular recordings



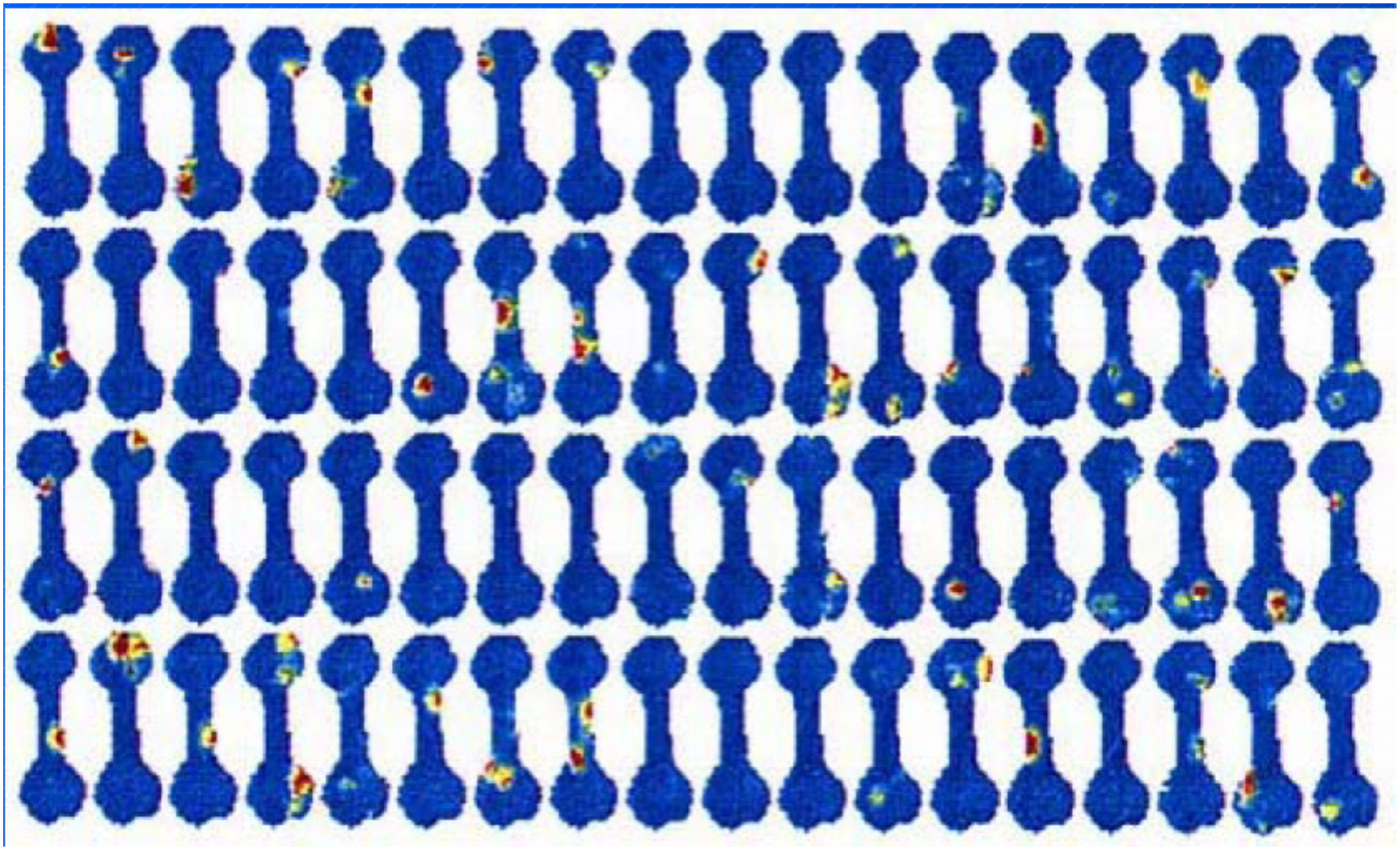
Tetrode-array technology

- Bruce McNaughton (Arizona)
- Matt Wilson, MIT (open course)



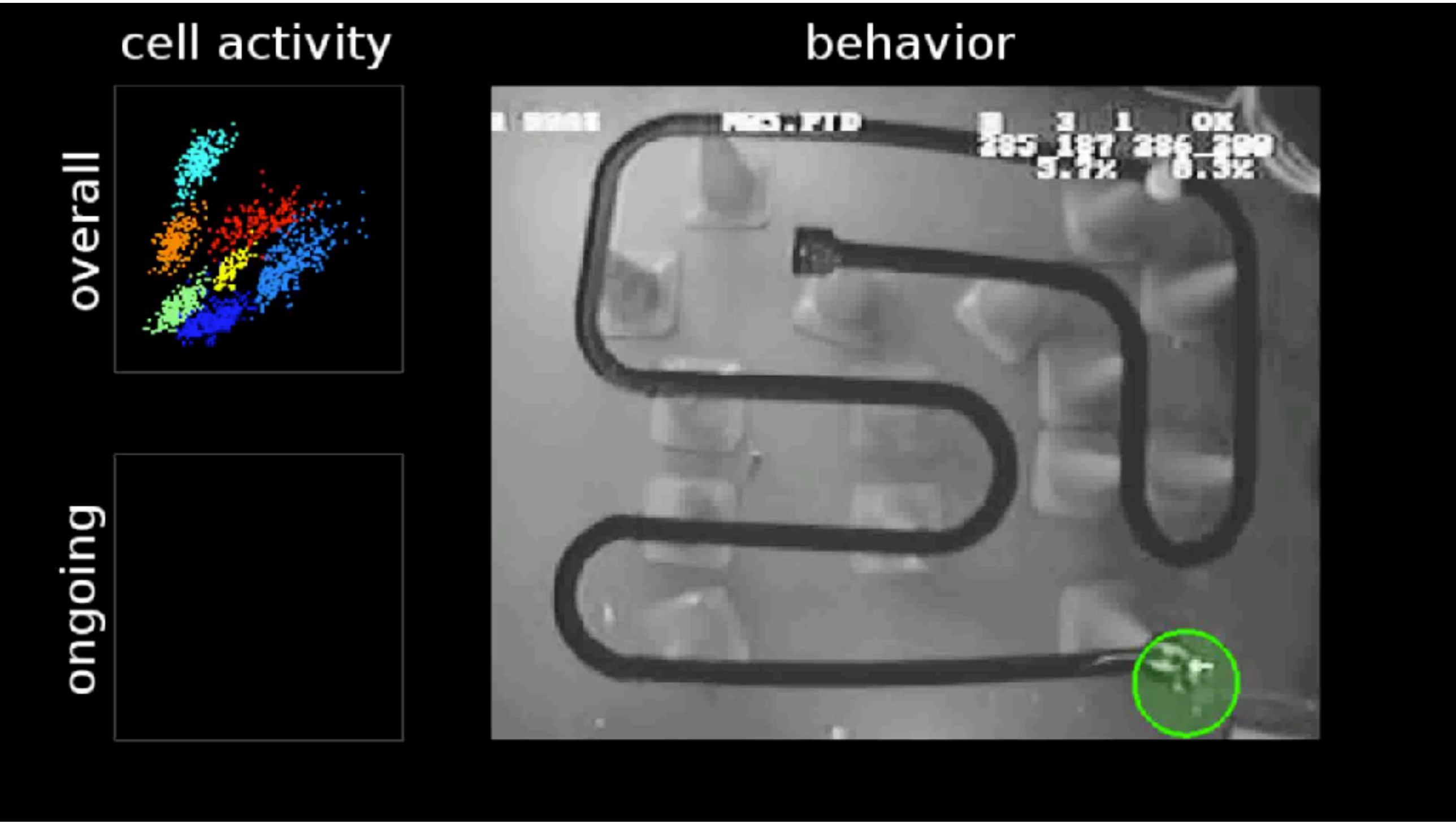
http://ocw.mit.edu/OcwWeb/Brain-and-Cognitive-Sciences/9-96Experimental-Methods-of-Adjustable-Tetrode-Array-NeurophysiologyJanuary--IAP-2001/CourseHome/index.htm

Place cells (Hippocampus)

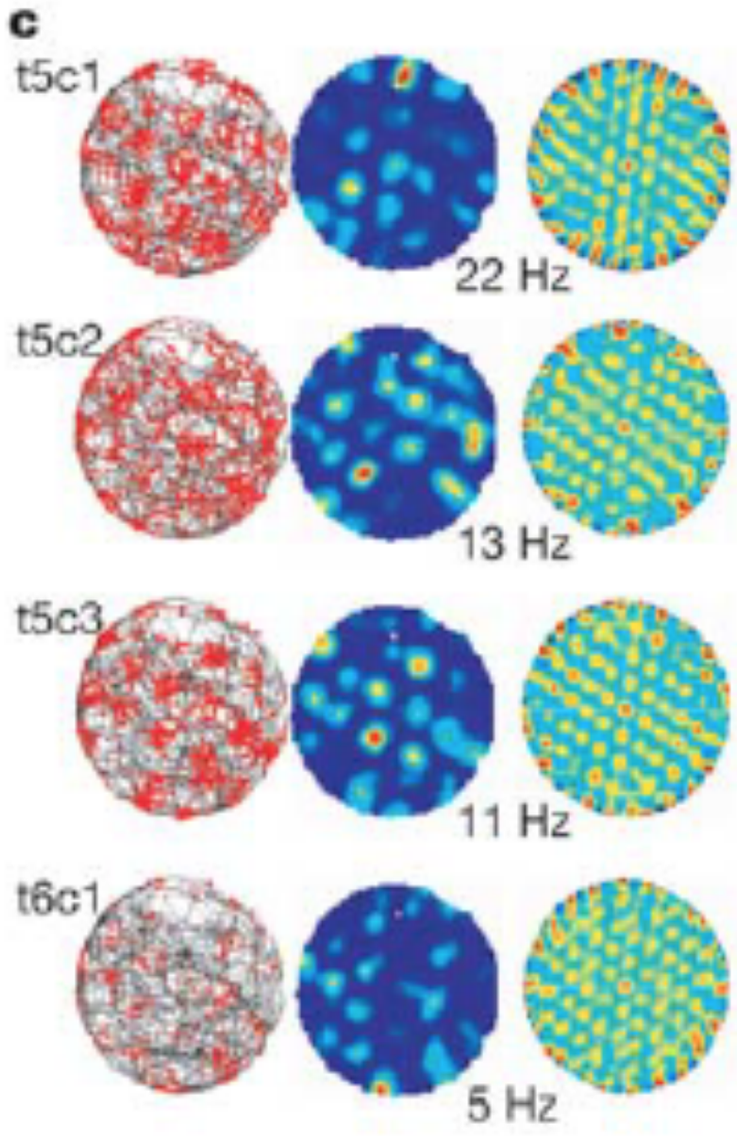


Matt Wilson, MIT

Place cells movie



Grid cells in enthorhinal cortex



Moser group

2014 Nobel prize in Medicine

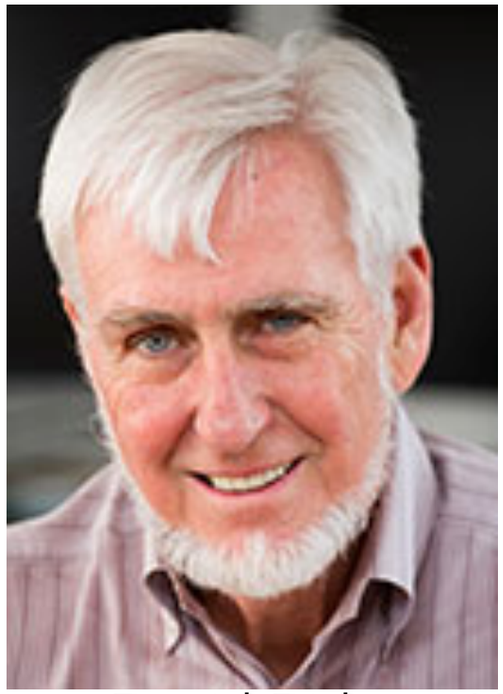


Photo: David Bishop, UCL

John O'Keefe

Prize share: 1/2

May-Britt Moser

Photo: G. Mogen/NTNU

May-Britt Moser

Prize share: 1/4

Edvard I. Moser

Photo: G. Mogen/NTNU

Edvard I. Moser

Prize share: 1/4

Functional imaging techniques

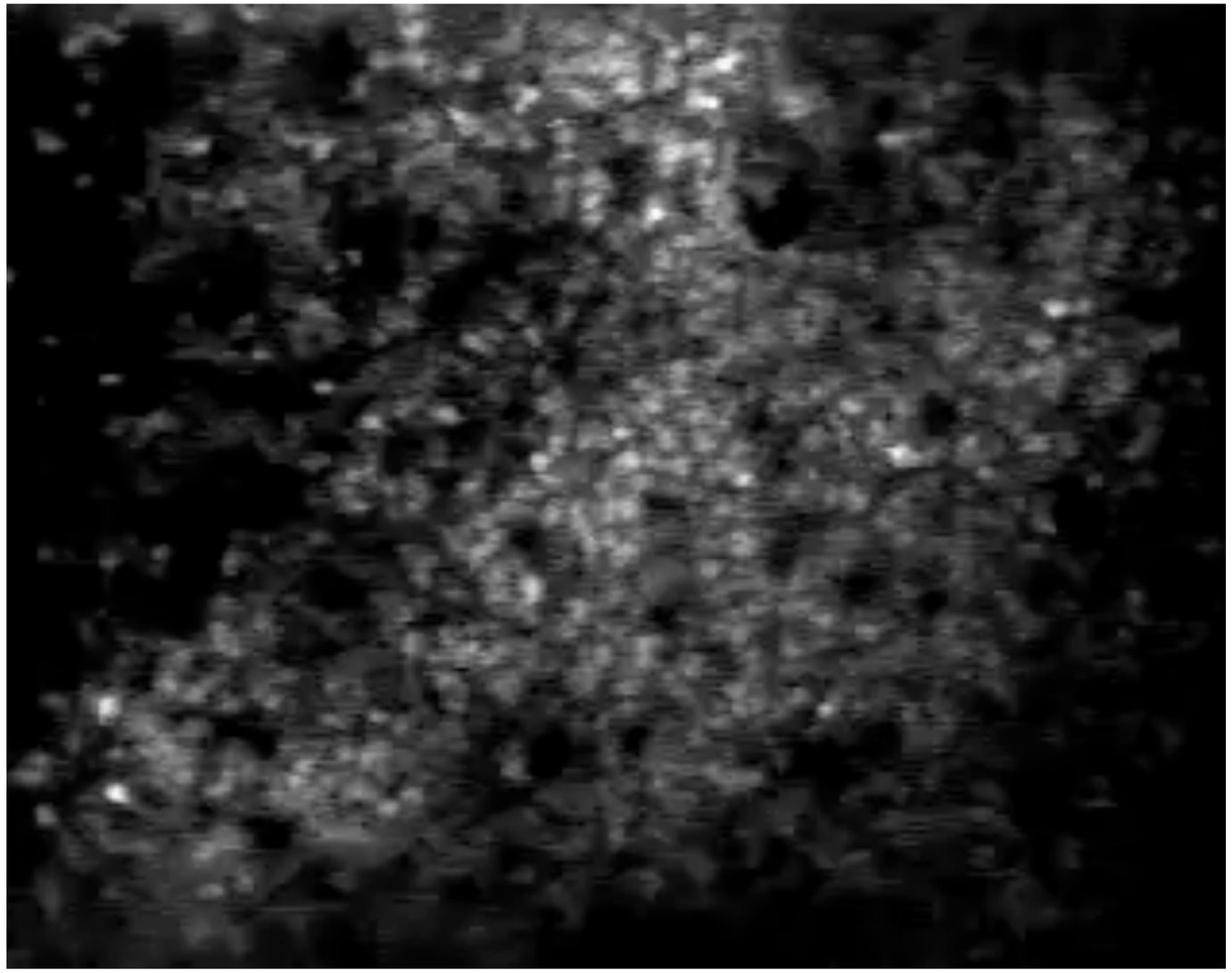
- **Calcium imaging**
- Voltage imaging
- PET
- MRI, fMRI
- Real-time imaging

Calcium imaging

- Calcium cannot be measured directly
- Calcium indicators used
  - Calcium-binding molecules are used that change their fluorescence properties
  - calcium changes can be measures in multiple milliseconds (compared to microseconds in electrophysiology)
- Requirements for successful calcium imaging
  - fast calcium indicator
  - high signal-to-noise ratio
  - proper instrumentation (images without photobleaching, fast scanning)

Advantages compared to recording techniques

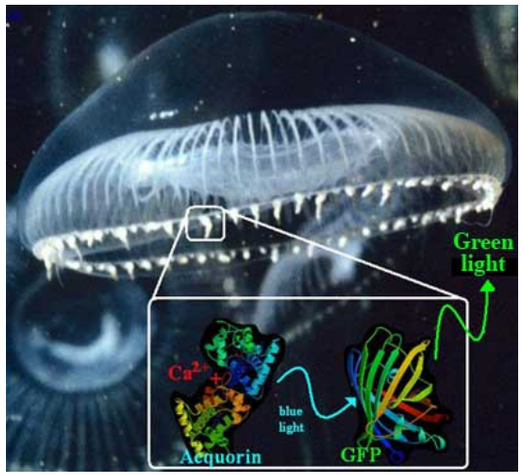
- Multiple cells
- Cell-type identification

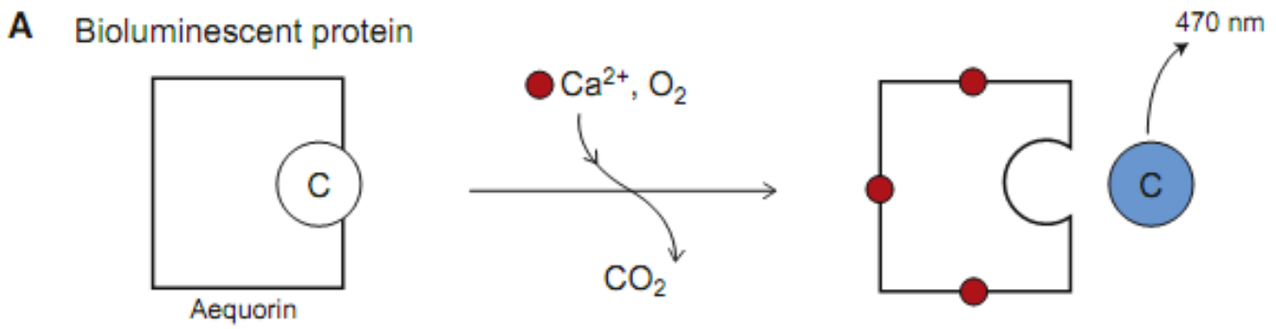


Calcium -imaging video of spontaneous neuronal activity, Golshani Lab, UCLA, http://golshanilab.neurology.ucla.edu/techniques

Molecules used for calcium imaging

- Natural calcium-binding proteins with fluorescent properties, aequorin





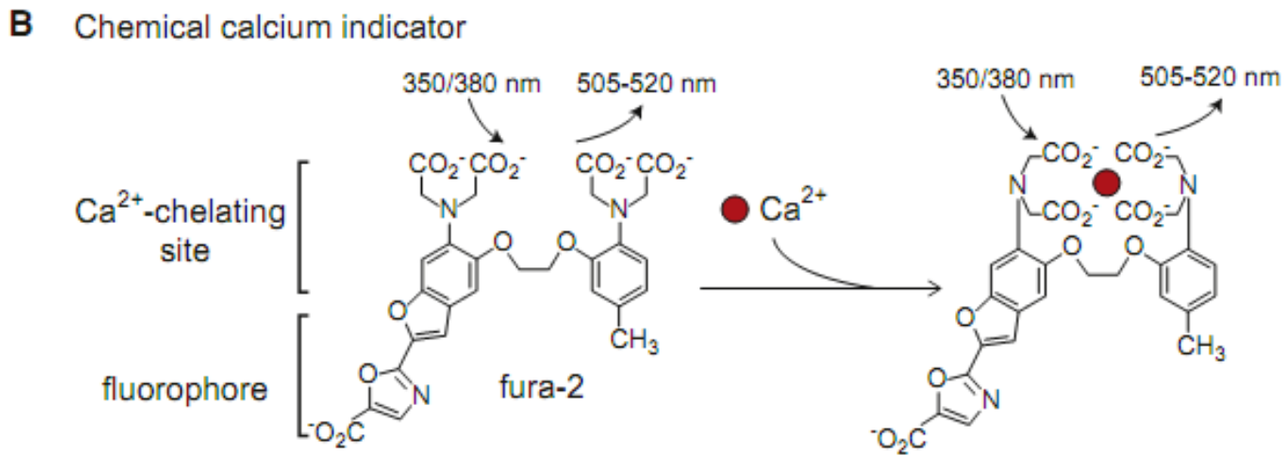
- Synthetic chemicals

- Genetically modified calcium indicators

Synthetic chemical compounds

- Fura-2/Fura-2AM (membrane permeable)
- BAPTA-AM
- Orange green

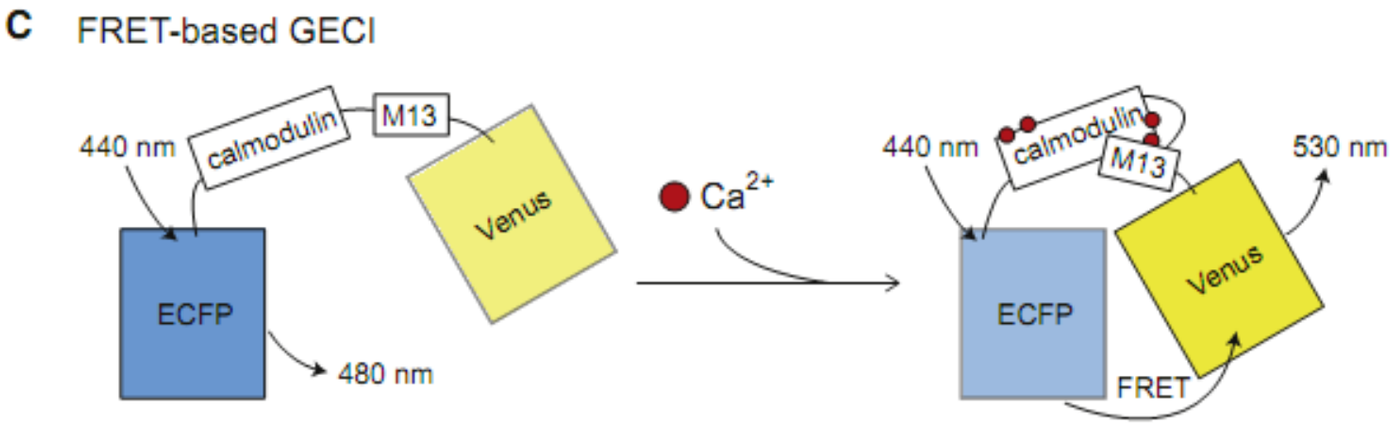
Roger Tsien
Nobel prize in chemistry 2008
Green Fluorescent Protein



Genetic calcium indicators

- Forster resonance energy transfer (FRET)
- Yellow cameleon
- Two fluorescent substances (donor and recipient)

Roger Tsien
Nobel prize in chemistry 2008
Green Fluorescent Protein

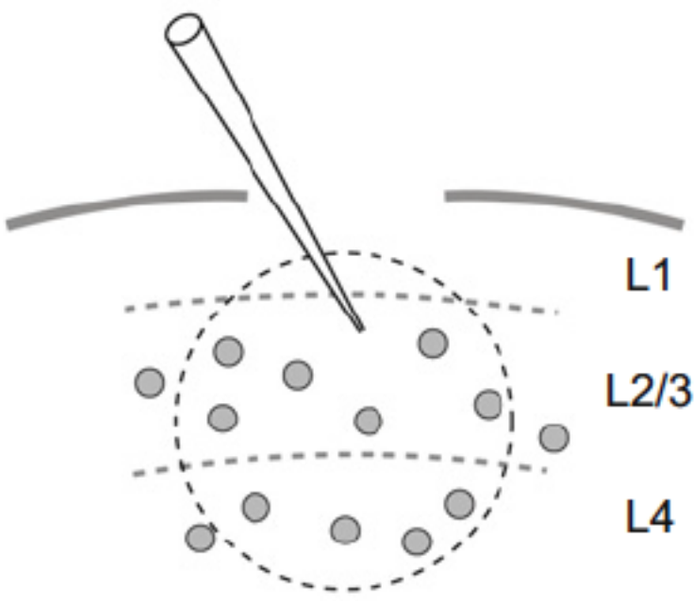


Genetically-encoded calcium indicators (2012)

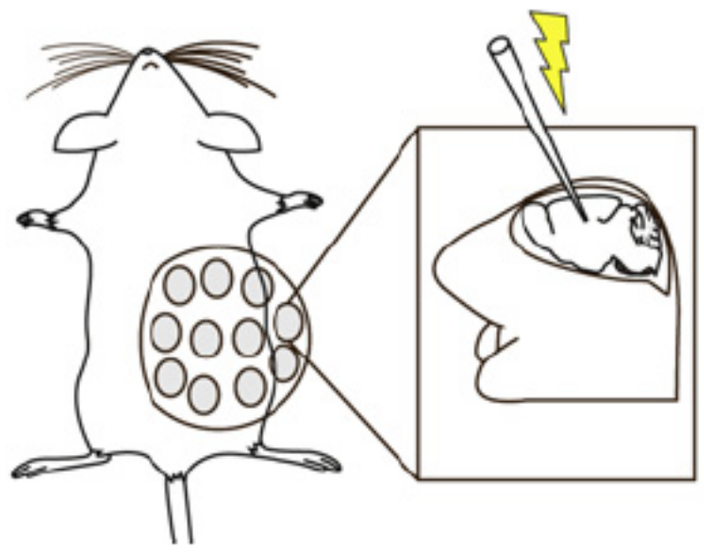
Camgaroo 1
Camgaroo 2
Inverse pericam
GCaMP 2
GCaMP 3
Yellow Cameleon 3.6
Yellow Cameleon Nano
D3cpV
TN-XL
TN-L15
TN-XXL

Genetically encoded calcium indicators

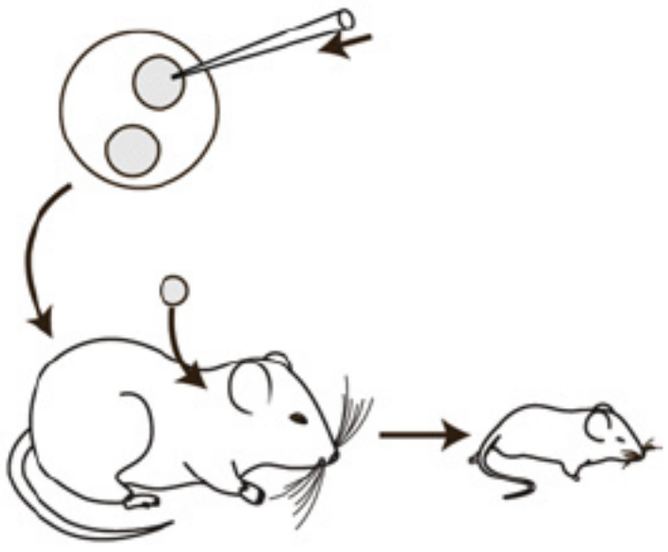
GECI expression



Viral transduction



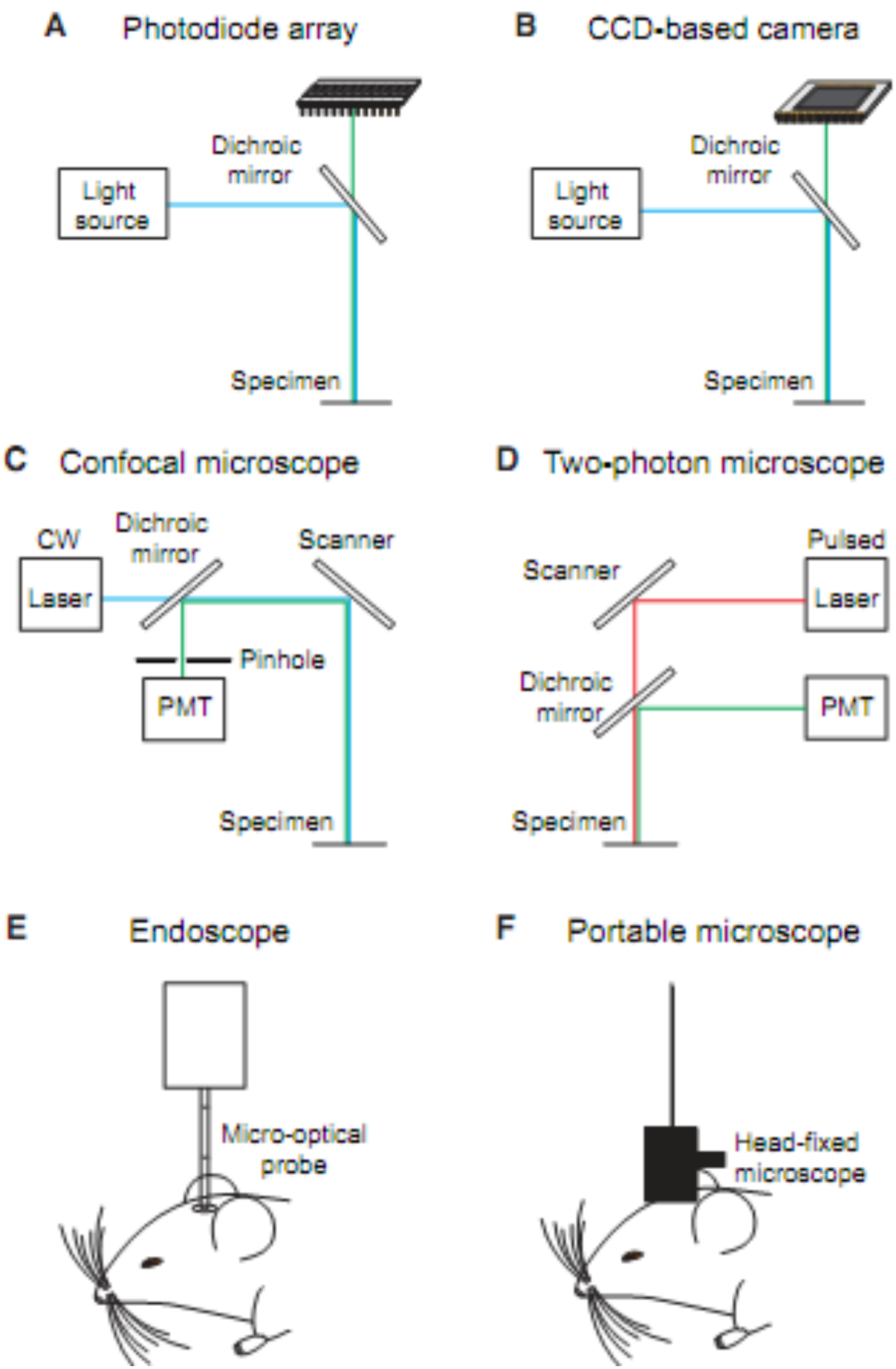
In utero electroporation



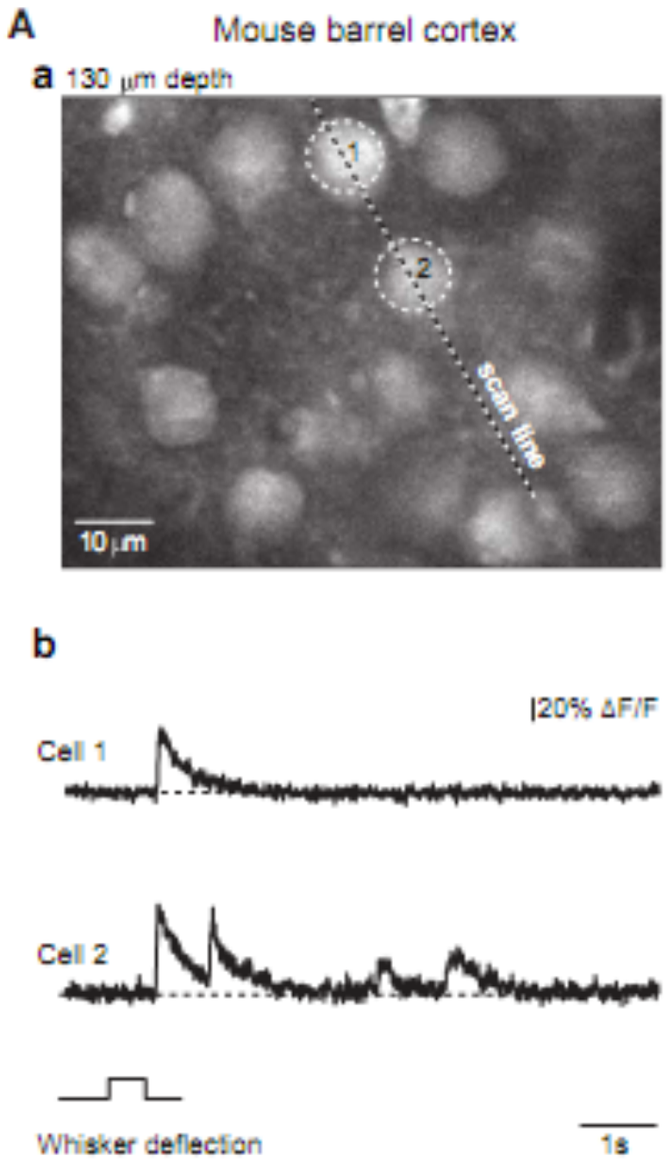
Transgenic mice

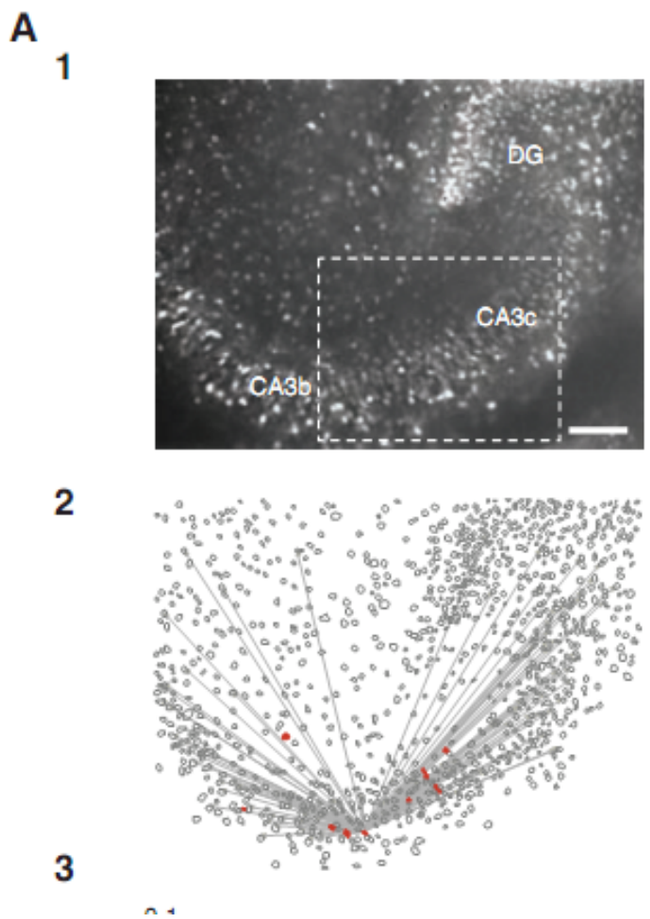
Microscopy to study calcium imaging

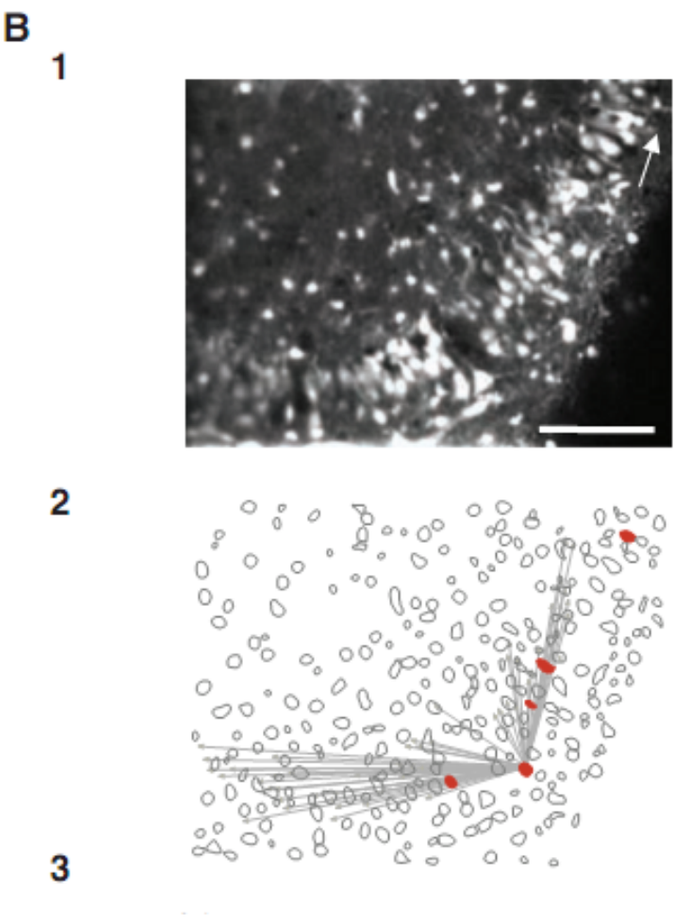
- Fluorescence microscopy
- confocal laser microscopy
- portable microscopy devices



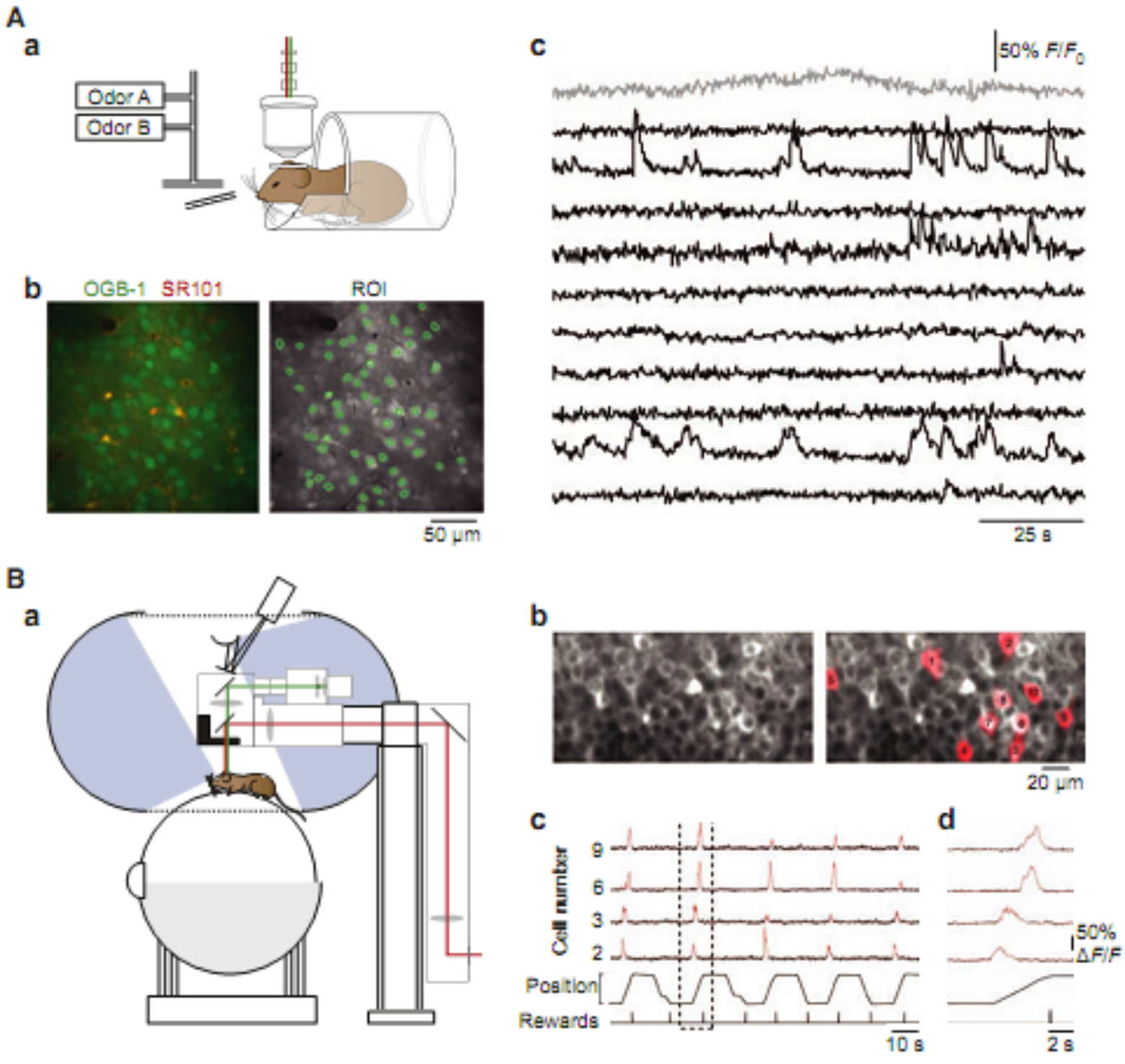
Study activation of neural networks



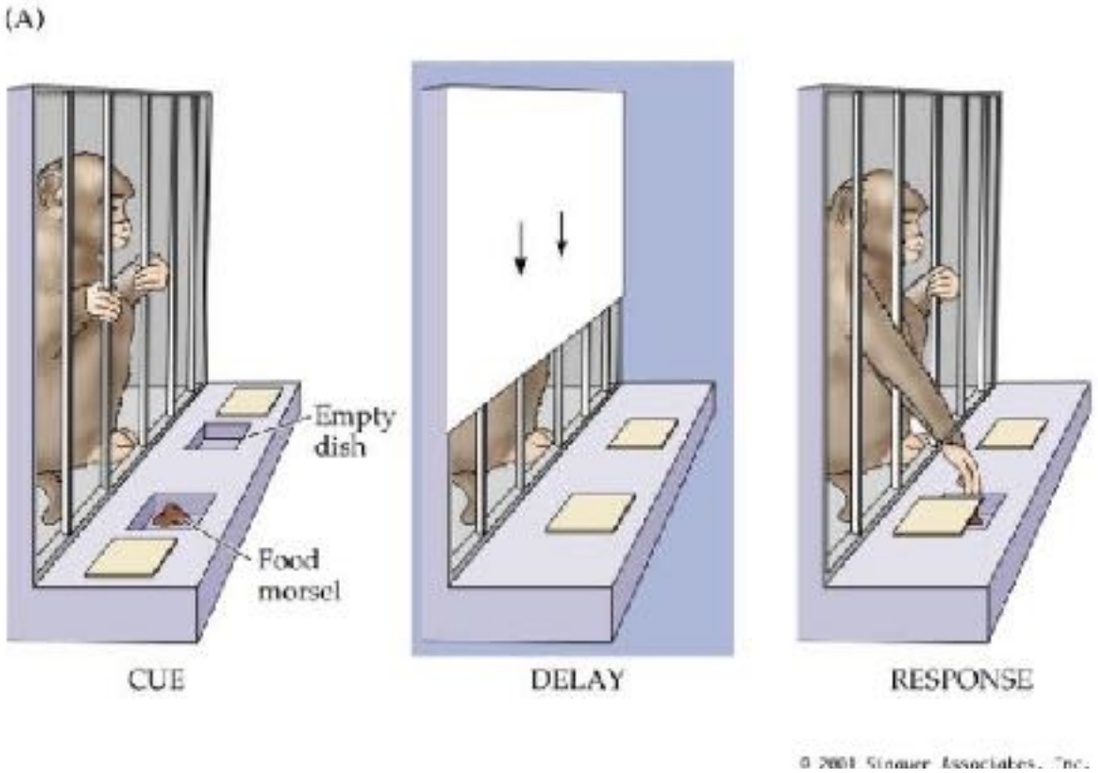




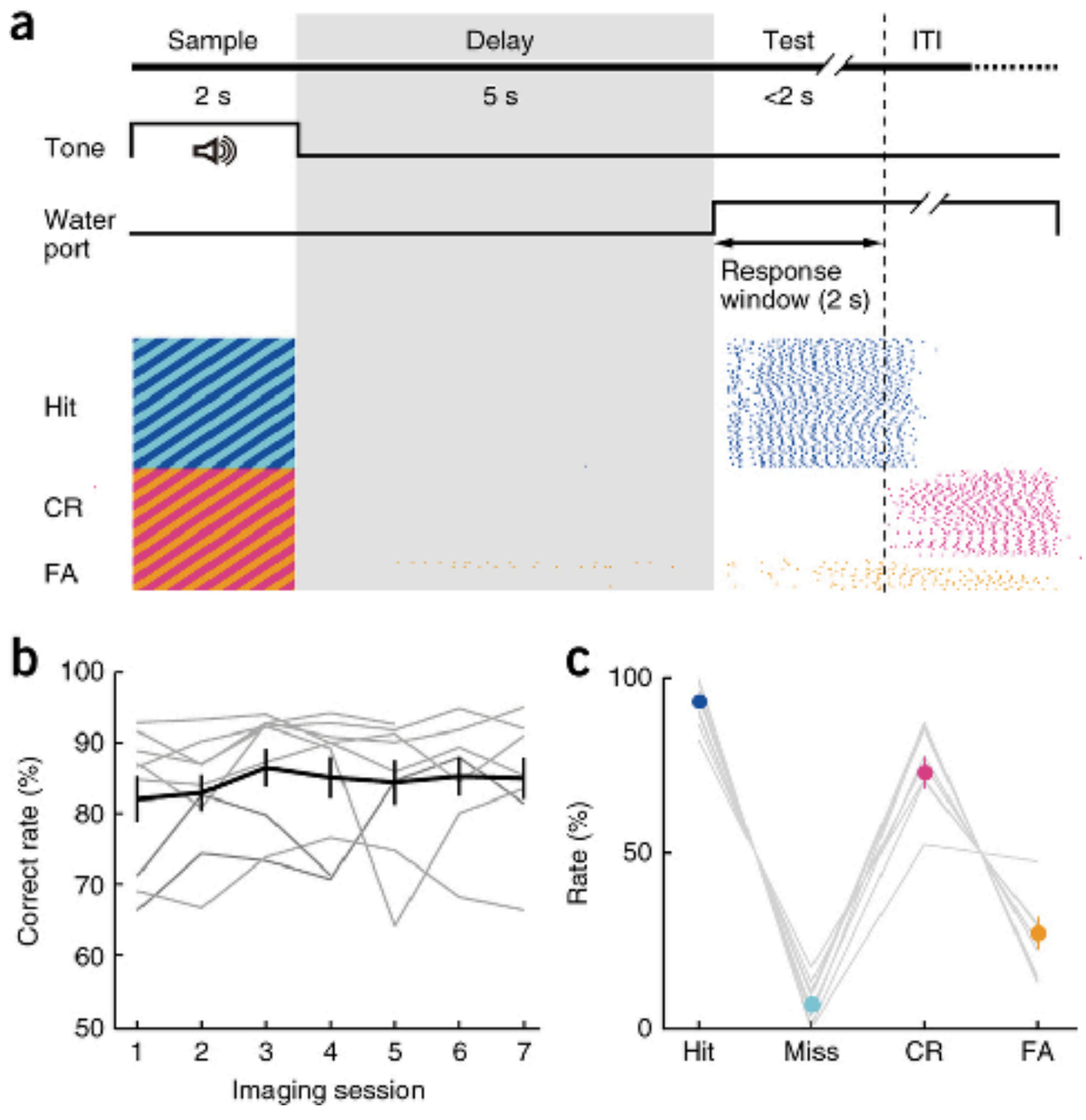
In vivo activation of neural networks



Neuronal activity during working memory tasks

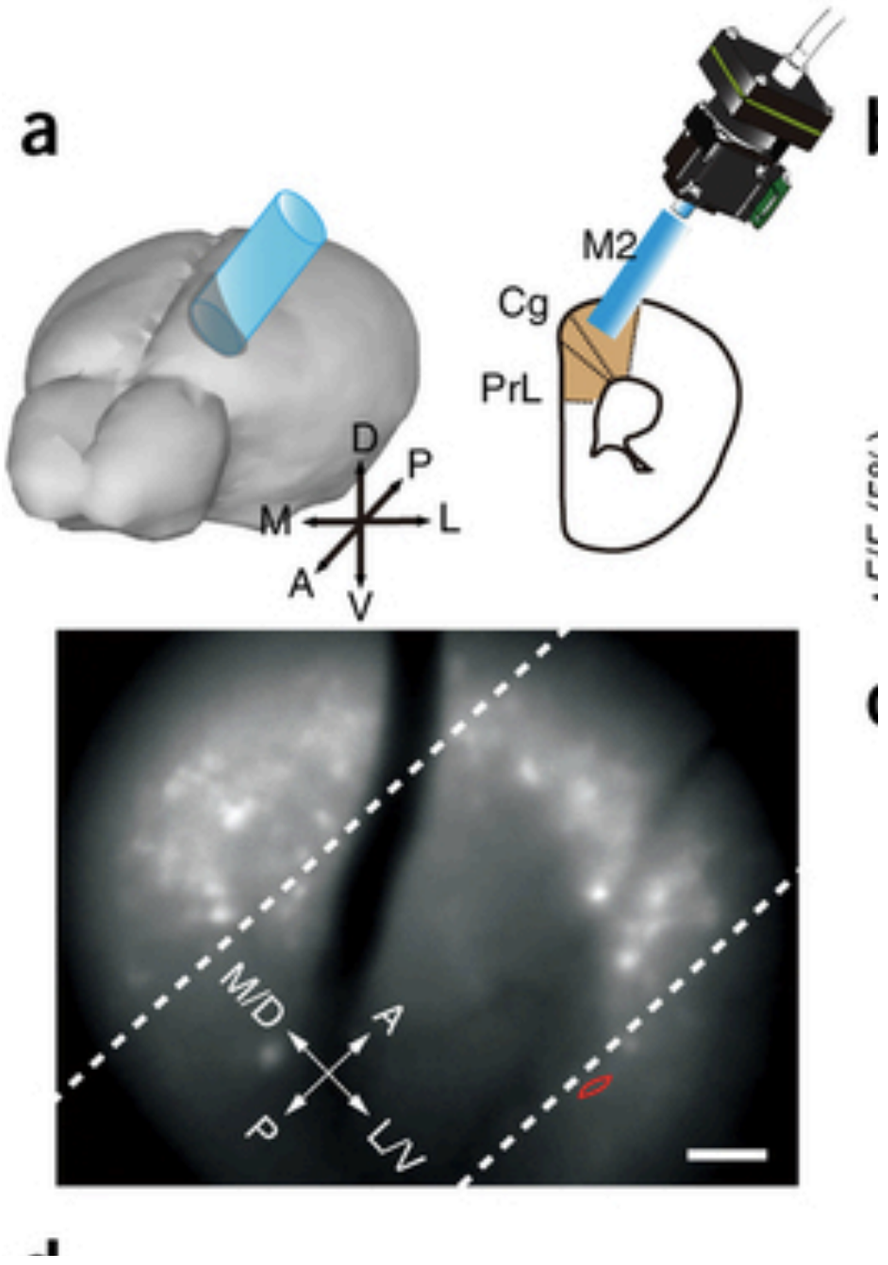


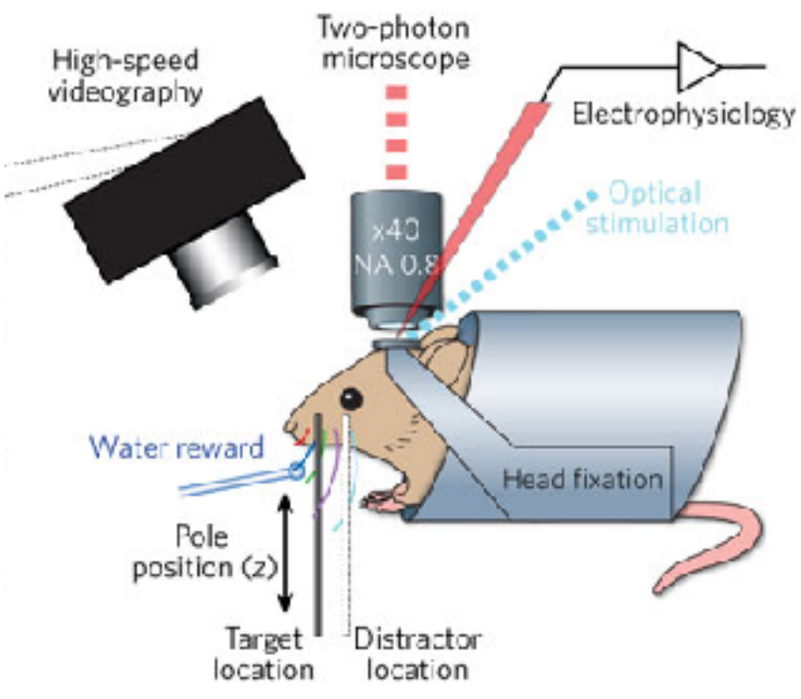
Working memory task in head-fixed animals



To image task-related neuronal activity….

- head-fixed mice
- CaMKIIα-Cre mice
- Cre-inducible adeno-associated virus (AAV) expressing the calcium indicator GCaMP6f30 into the dorsomedial PFC (dmPFC)

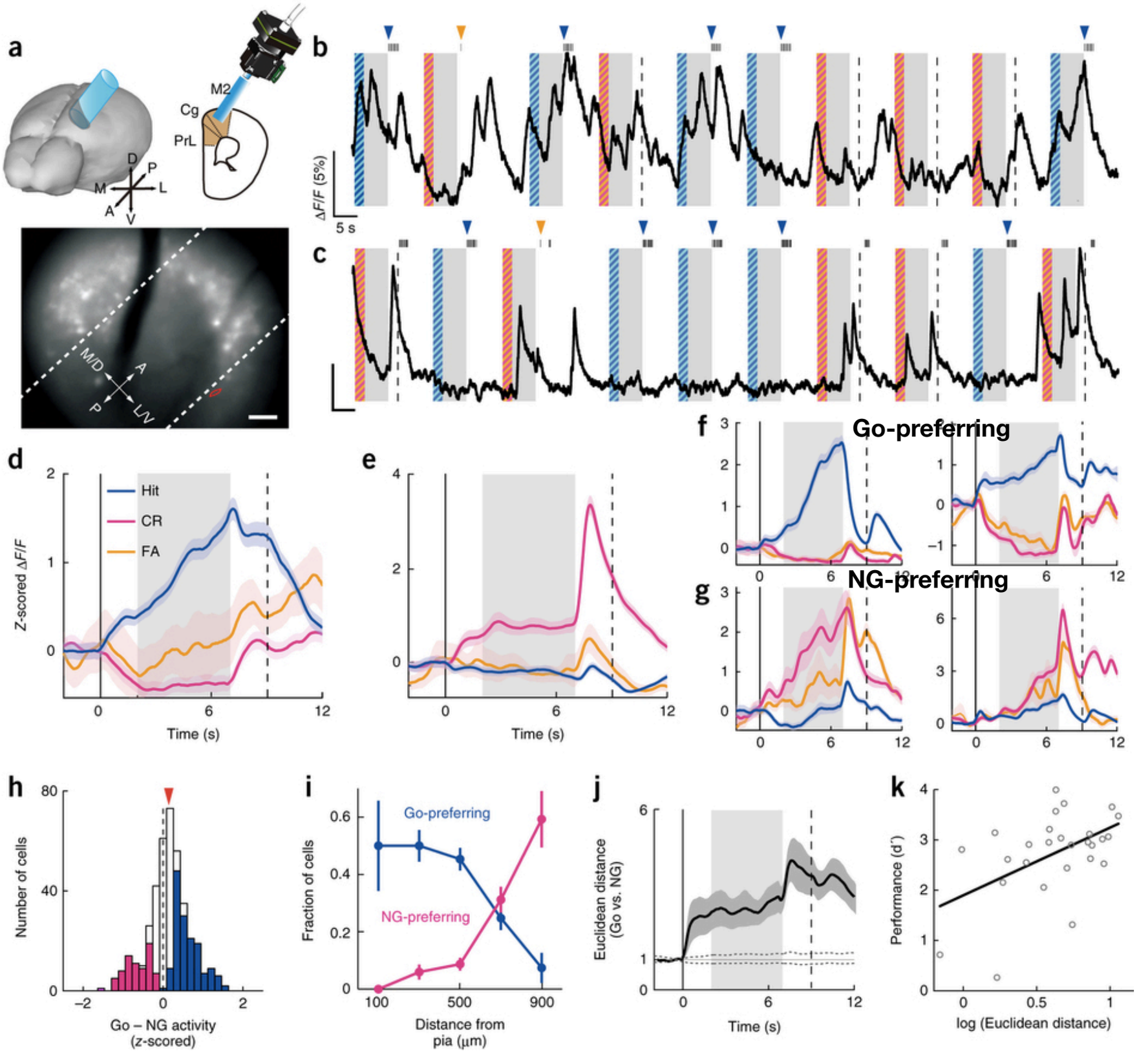




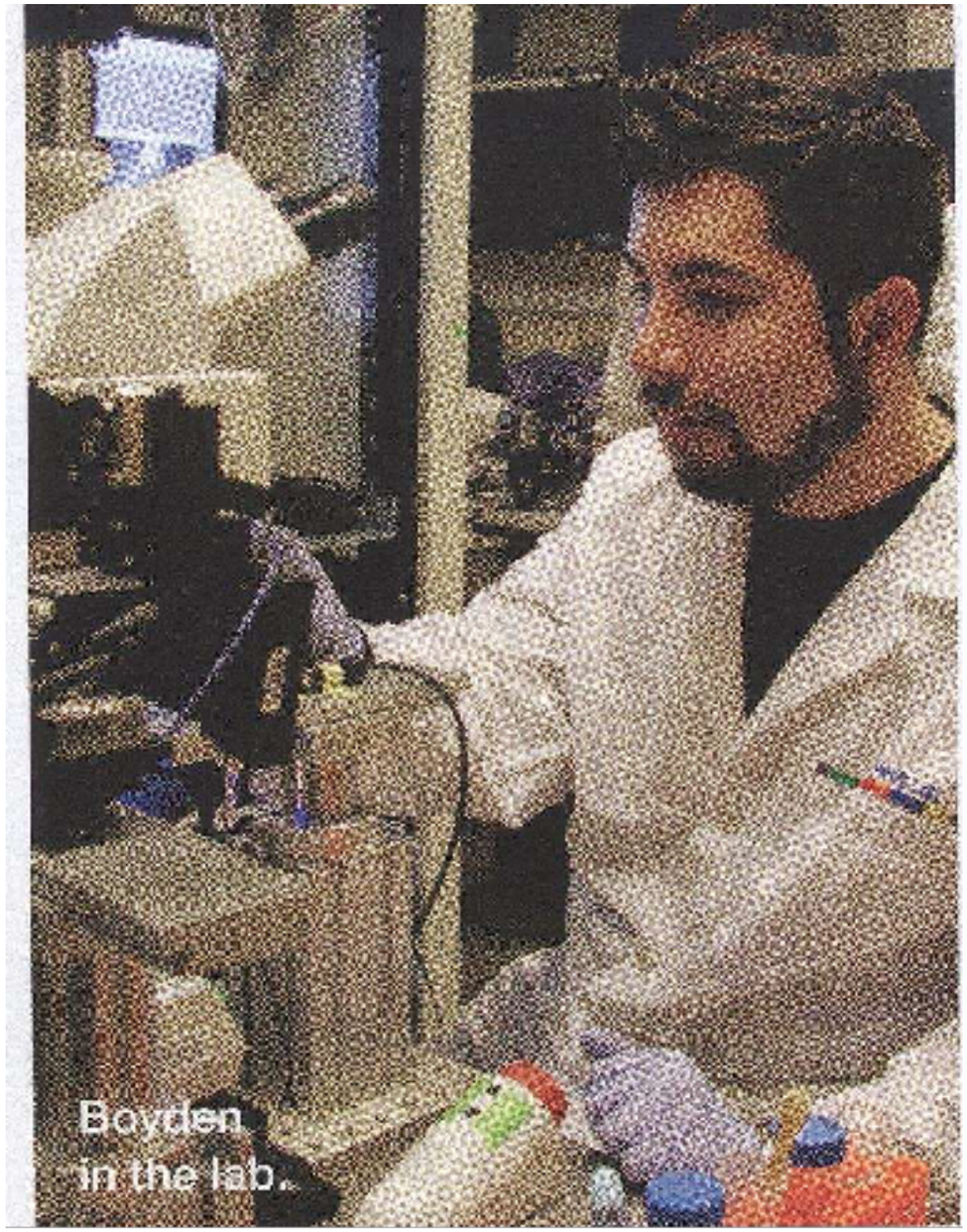
modified from O'Connor DH et al 2009

Pyramidal neuron activity during the delayed Go vs. No-Go task.

Gray shading: delay period
Blue stripes: sample periods with target tone
Orange stripes: sample periods with nontarget tones,
Dashed line: end of response window in CR trials.
Black tick on top: lick response.
Blue arrowheads: delivery of reward
Orange arrowheads: punishment



Optogenetics



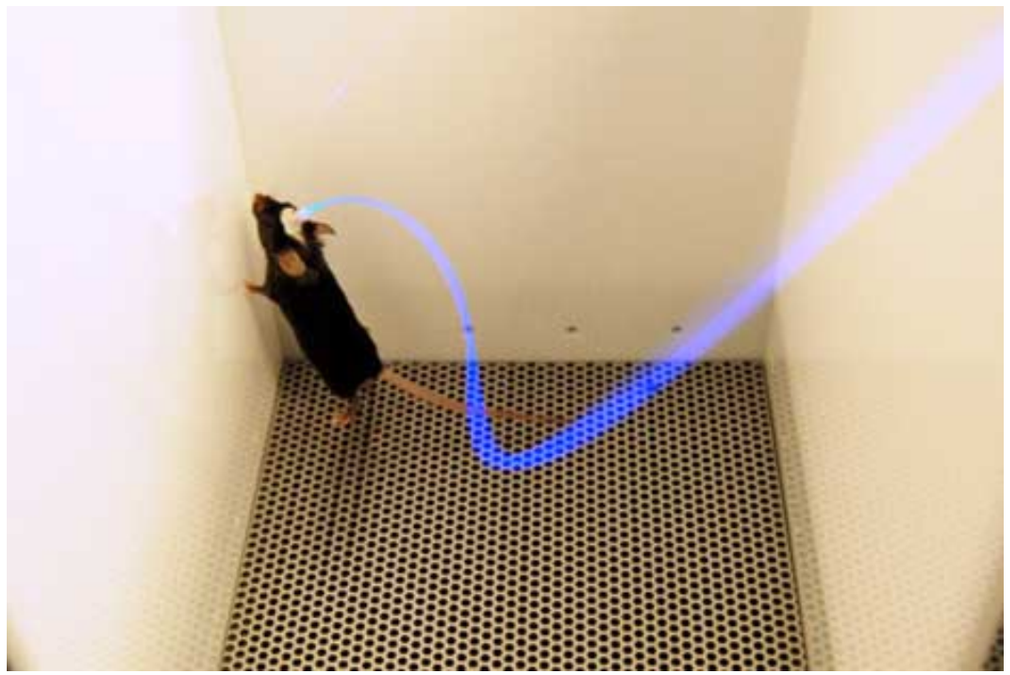
Ed Boyden, MIT

Karl Deisseroth, Stanford

Optogenetics

"The major challenge facing neuroscience was the need to control one type of cell in the brain while leaving others unaltered. Electrical stimuli cannot meet this challenge."

- Francis Crick



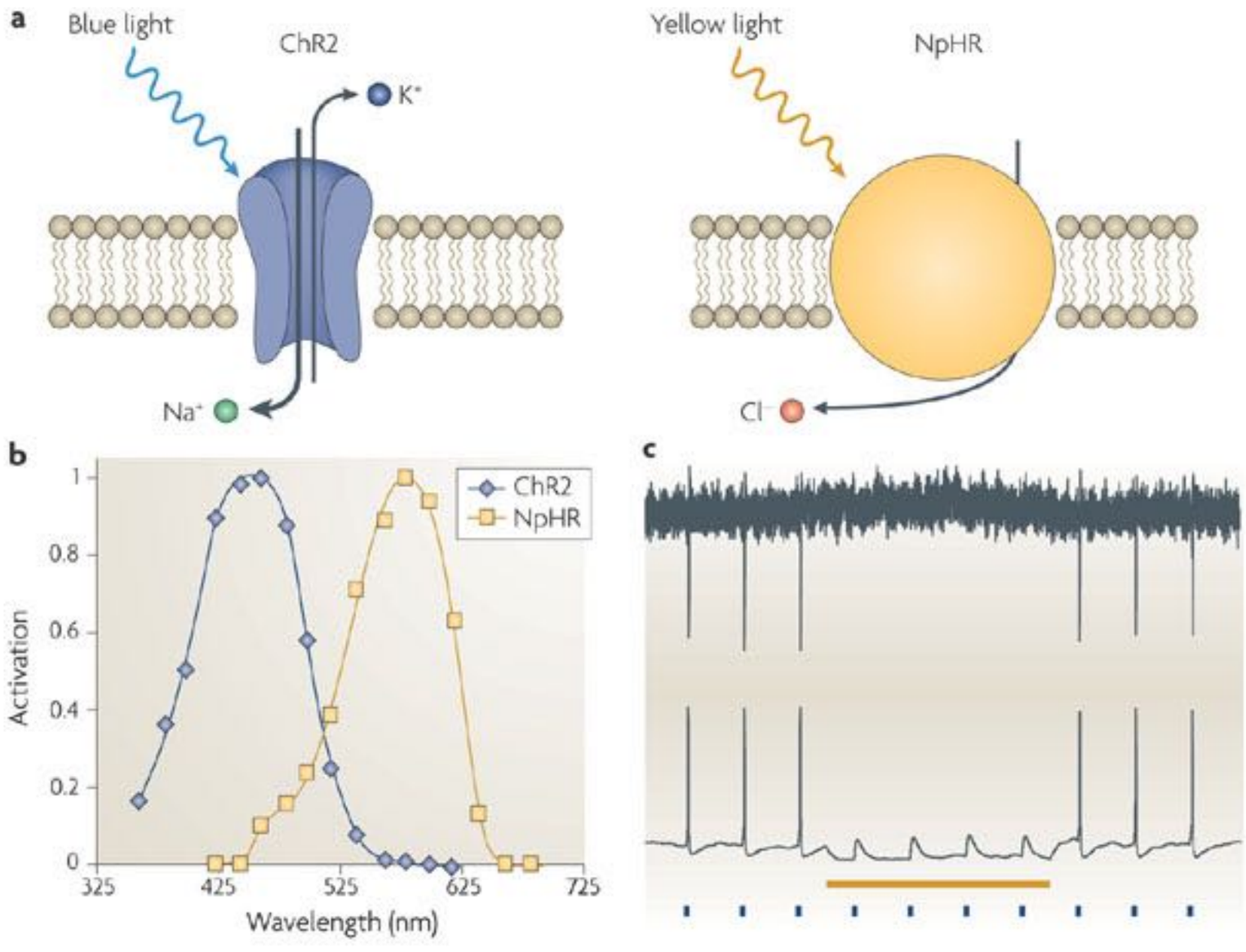
Optogenetics

- Combination of optical and genetic techniques for specifically controlling neuronal subtypes
- Use of ion channels that are activated by light, are expressed in archeobacteria and not expressed in animals

Channels used to control excitability
Opsins

Chlmydomonas reinhardtii
Channelrhodopsin-2

Natronomonas pharaonis
Halorhodopsin



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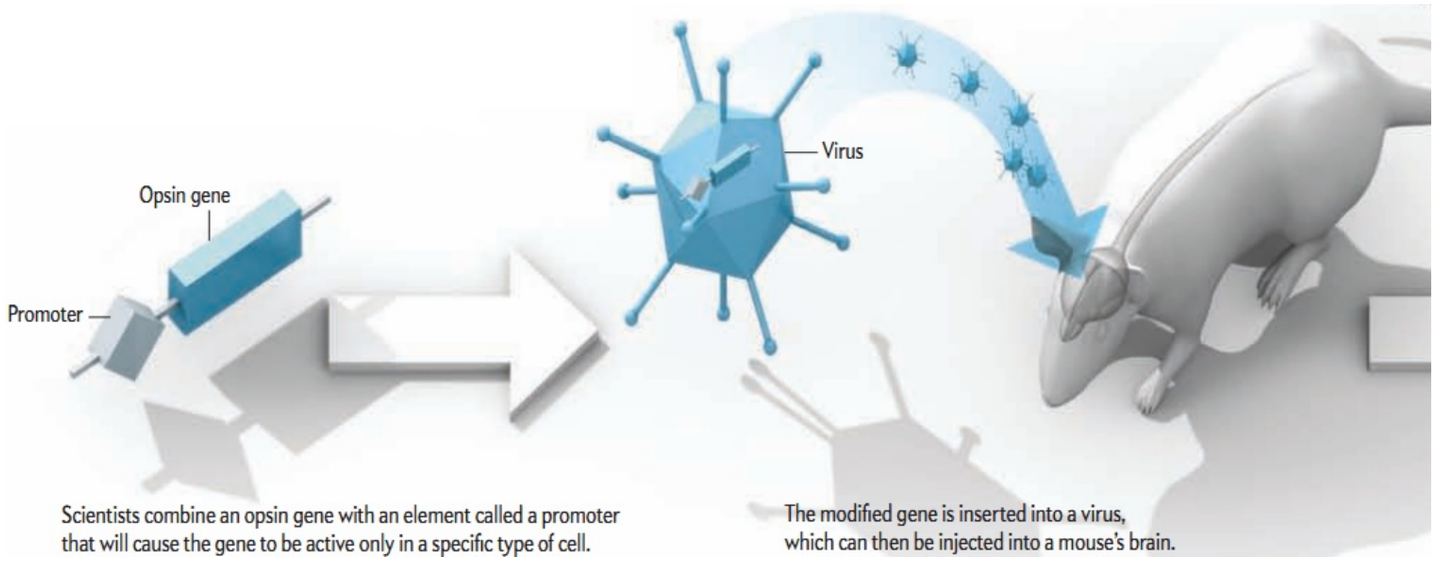
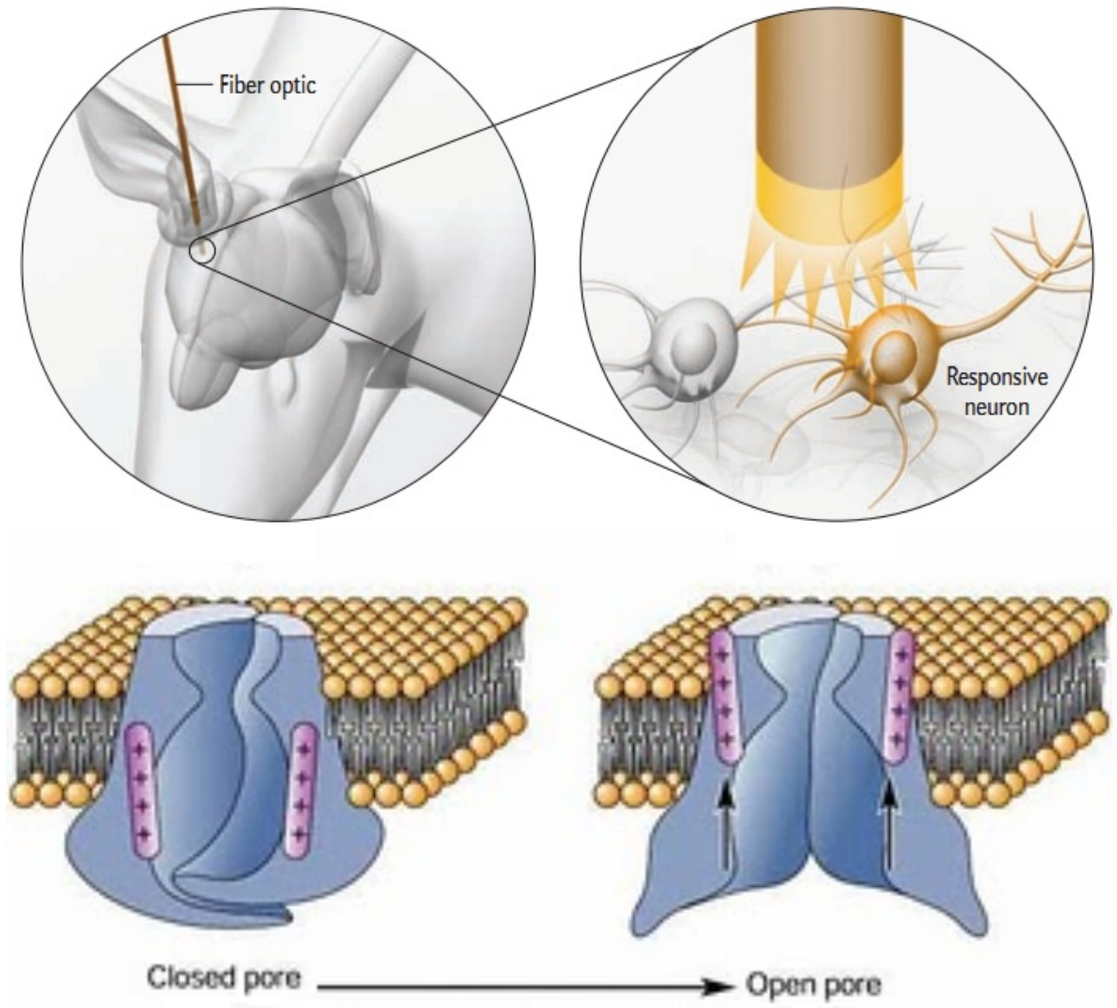
Step 1: Expression of opsin in the desired neuronal population



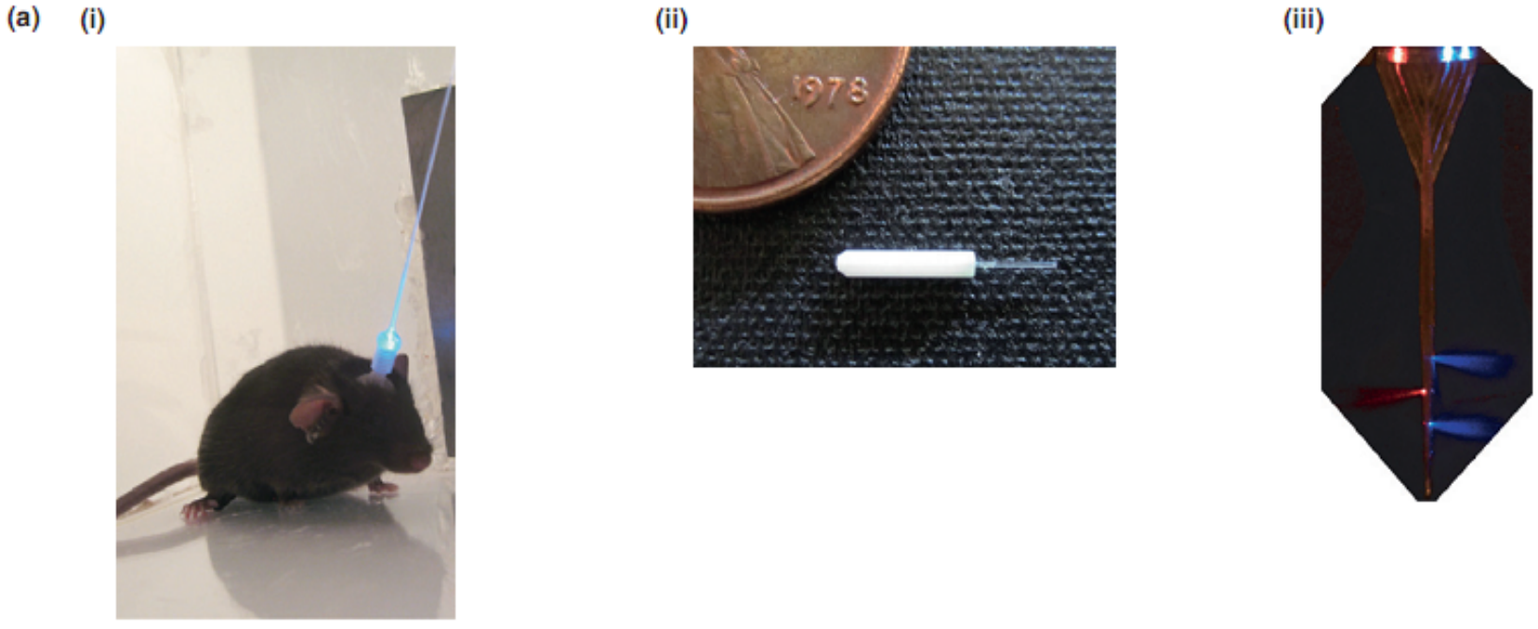
- Viral transfection
- Use of Cre-loxP system in mice
- PV-cre mice
- Som-cre mice
- CamKII-cre mice

Step 2: Activate opsin with light

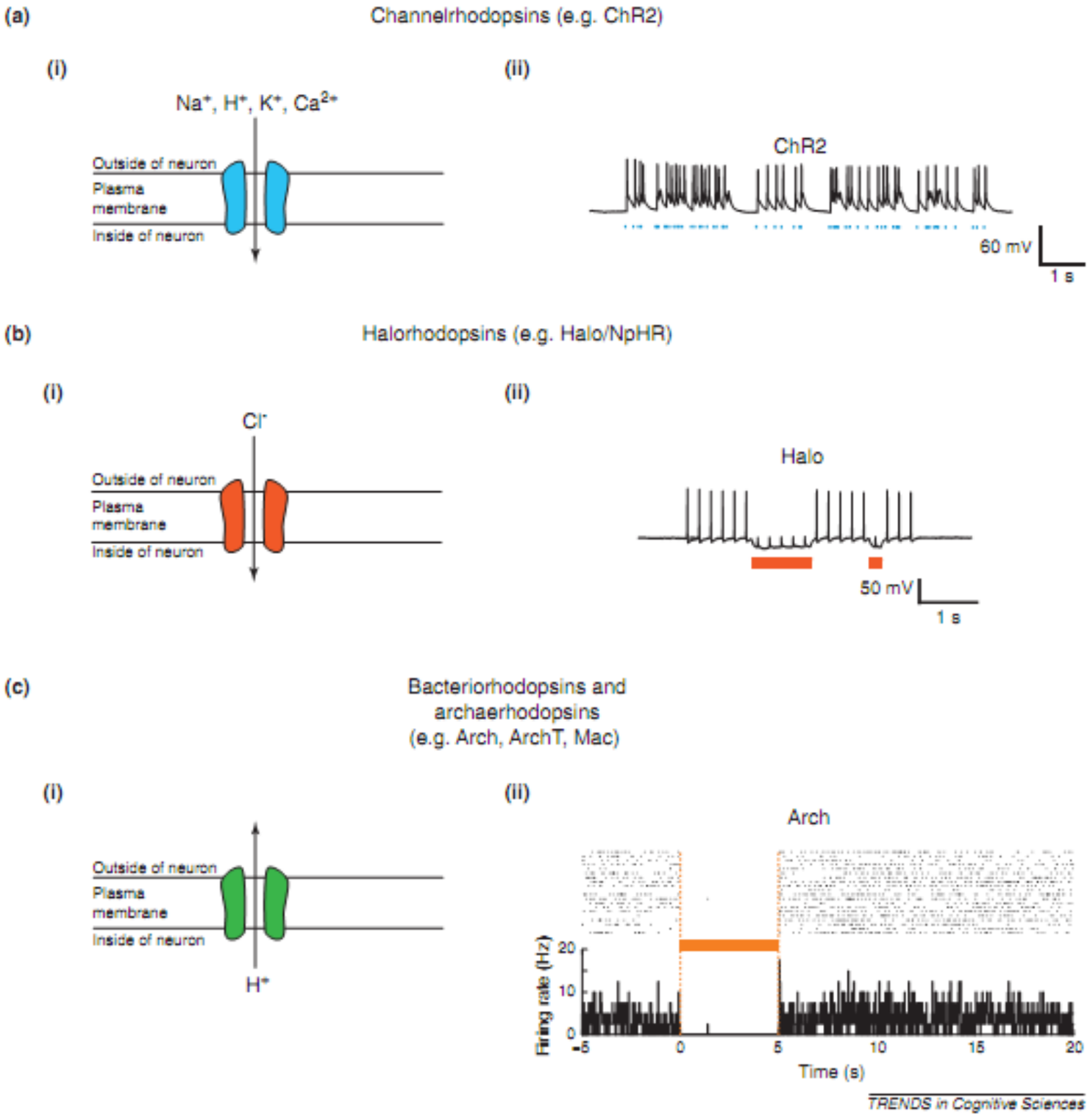


1: *Controlling the Brain with Light* by Karl Deisseroth, Scientific American, November, 2010, pages 49-55

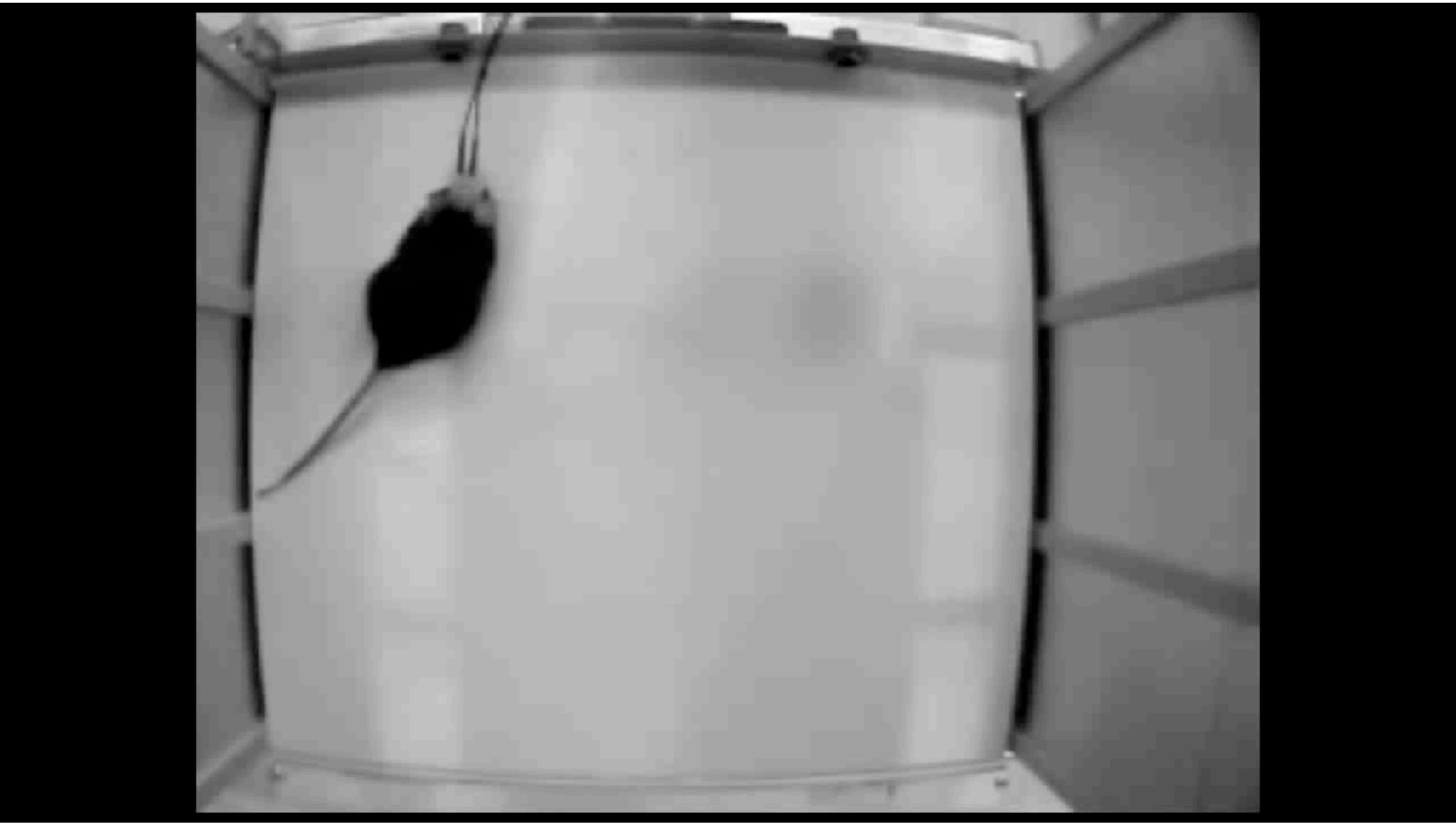
How to direct light into the brain: Optical fibres



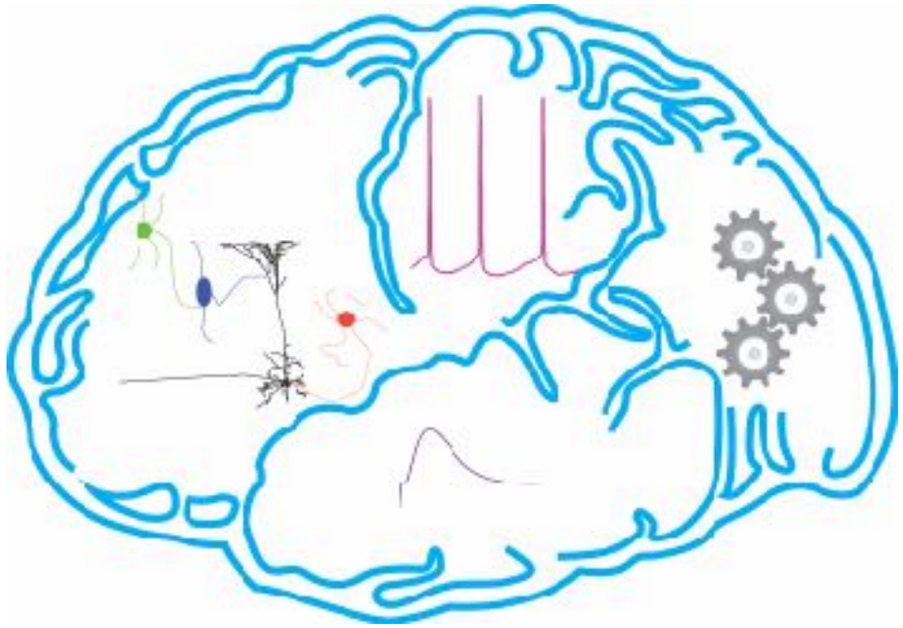
Verifying the effect of opsin expression in neurons



Using optogenetics to study behaviour



Βασικές αρχές λειτουργίας του νευρικού συστήματος, Κυριακή Σιδηροπούλου
http://repository.kallipos.gr/handle/11419/4828



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8th - 10th December 2017
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