МОЛЕКУЛЯРНО-ФИЗИОЛОГИЧЕСКИЕ МЕХАНИЗМЫ ПЛАСТИЧНОСТИ В ПРОСТЫХ СИСТЕМАХ

ИНСТИТУТ ВЫСШЕЙ НЕРВНОЙ ДЕЯТЕЛЬНОСТИ И НЕЙРОФИЗИОЛОГИИ РАН

Балабан П.М.

LOGIC OF SCIENTIFIC SEARCH

John Godfrey Saxe's poem

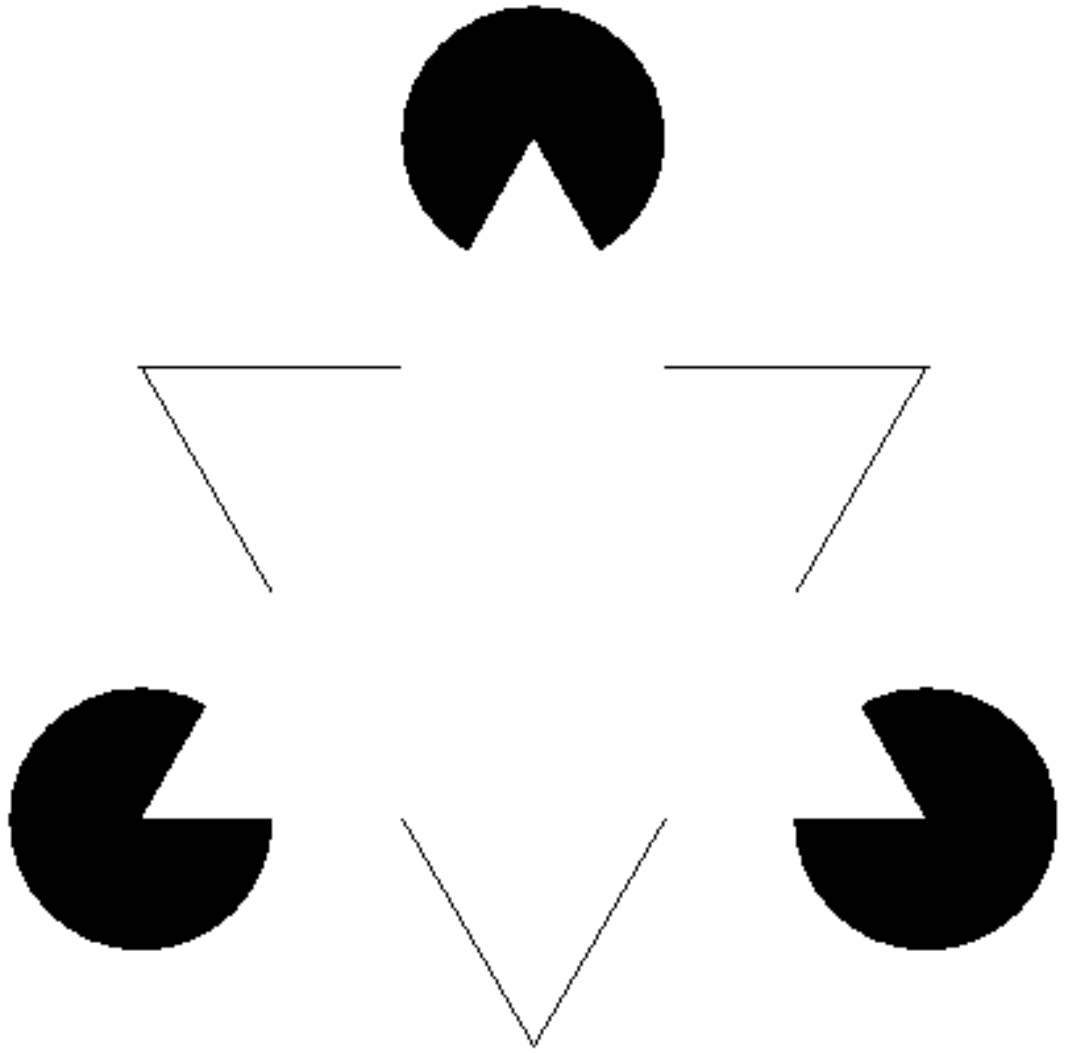
It was six men of Indostan
To *learning* much inclined,
Who went to see the Elephant
(Though all of them were blind),
That each by observation
Might satisfy his mind.

In the fable each of the blind men feels a single part of the elephant and comes to a very different conclusion about the nature of the animal. While the man who holds the elephant's trunk concludes that the elephant is "very like a snake," another, who feels only the elephant's ear, claims that the beast resembles a fan. Yet a third blind man, who touches one of the tusks, argues that the animal is like a spear.

As the poem ends,

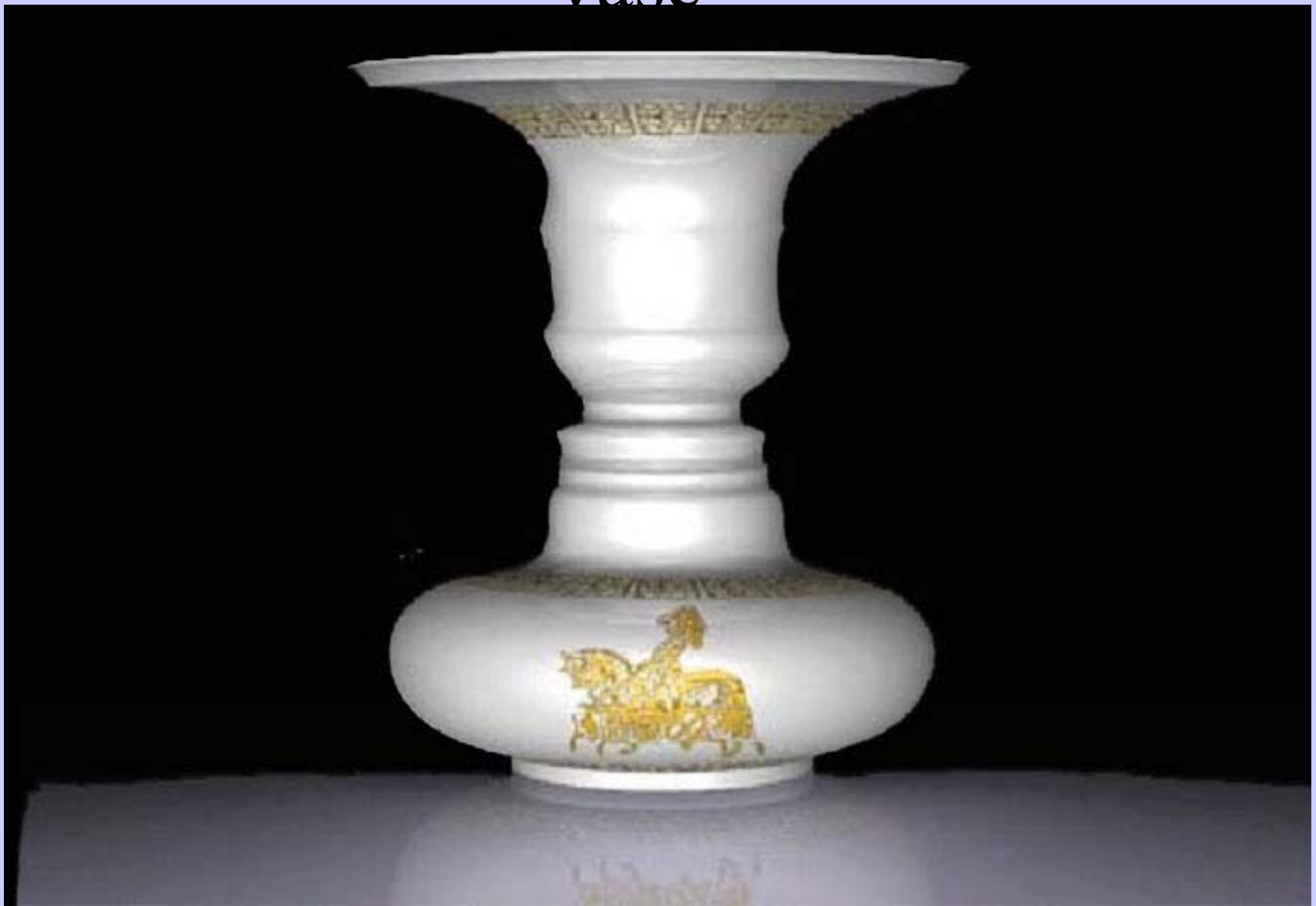
And so these men of Indostan
Disputed loud and long,
Each in his own opinion
Exceeding stiff and strong,
Though each was partly in the right,
And all were in the wrong!
Moral:
So oft in theologic wars,
The disputants, I ween,
Rail on in utter ignorance
Of what each other mean,
And prate about an Elephant

Not one of them has *seen*!

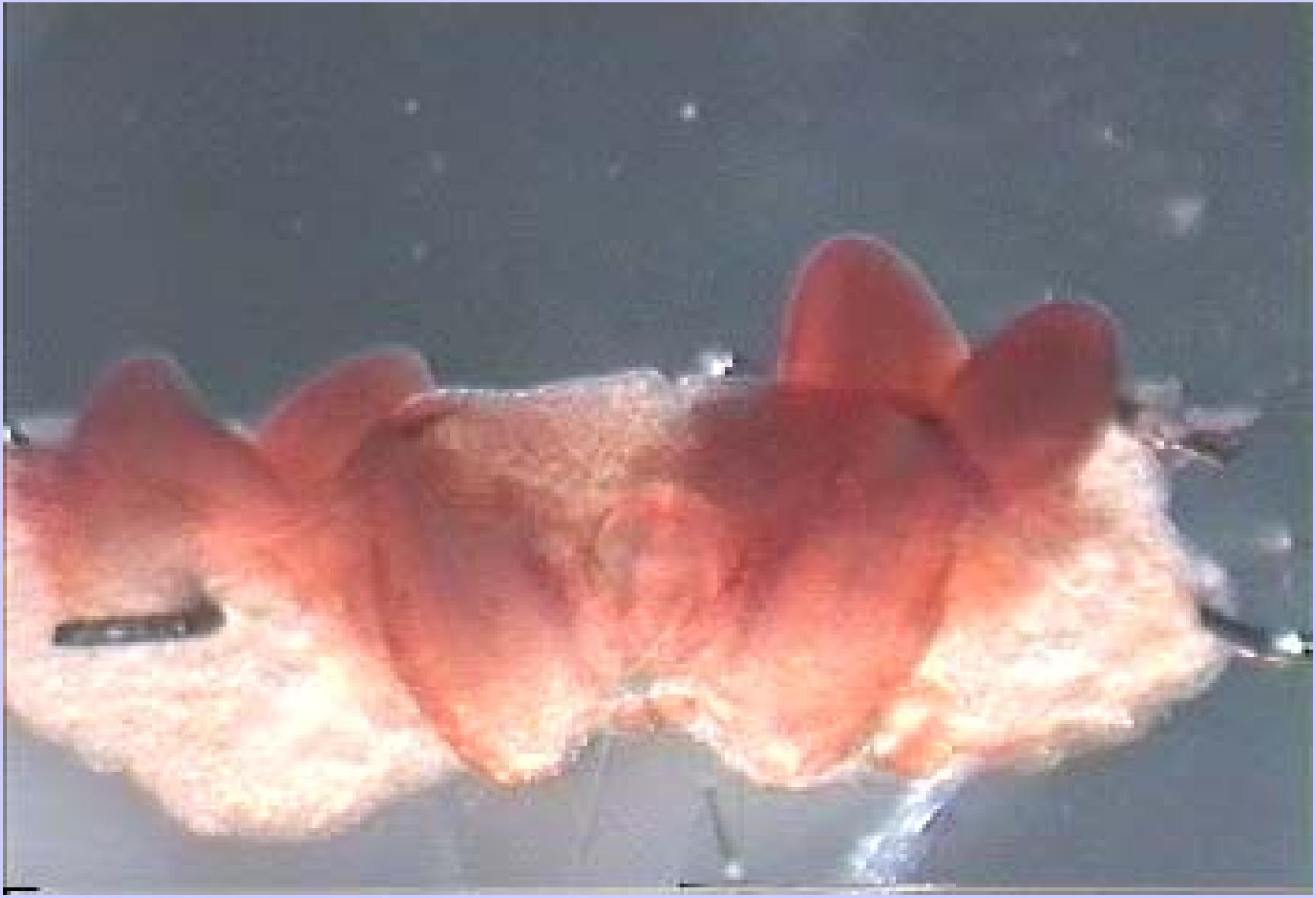


SEEING THE INVISIBLE

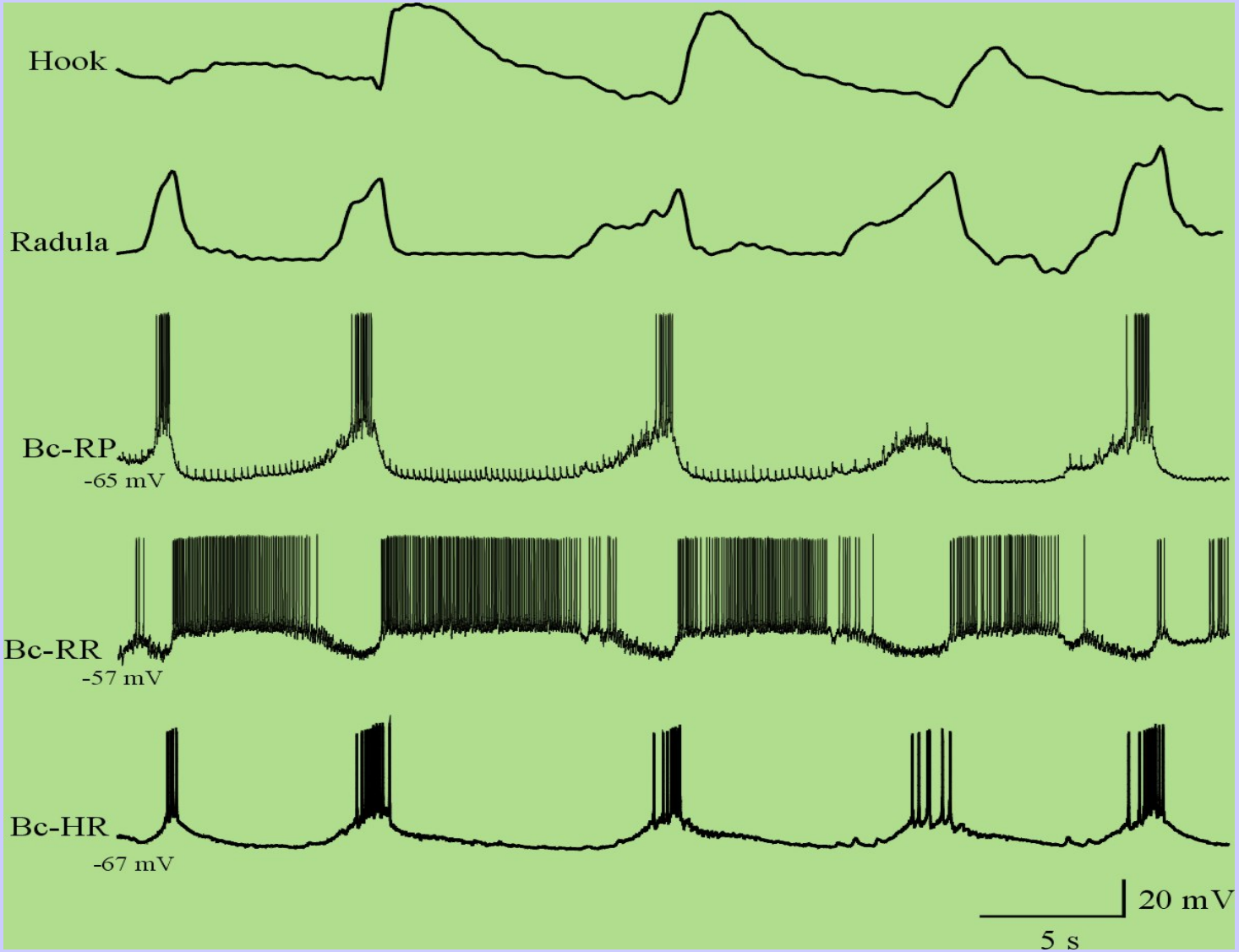
Rubin's vase -face -Illusion with realistic vase



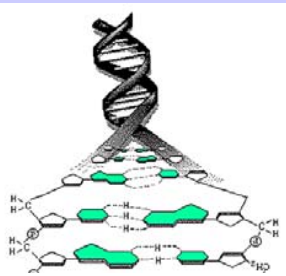
Ритмическая активность буккального аппарата Clione



Ритмическая активность буккальных мотонейронов и механограмма движений крючков и радулы.

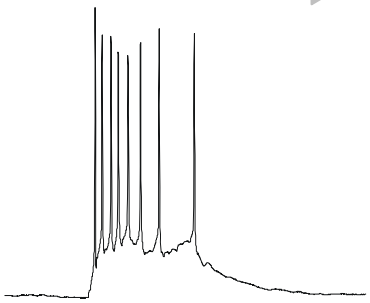


The principal question of developmental neuroscience is how during development 10^{12} neurons establish 10^{15} specific synaptic connections to produce our functional thinking brain



genes

activity

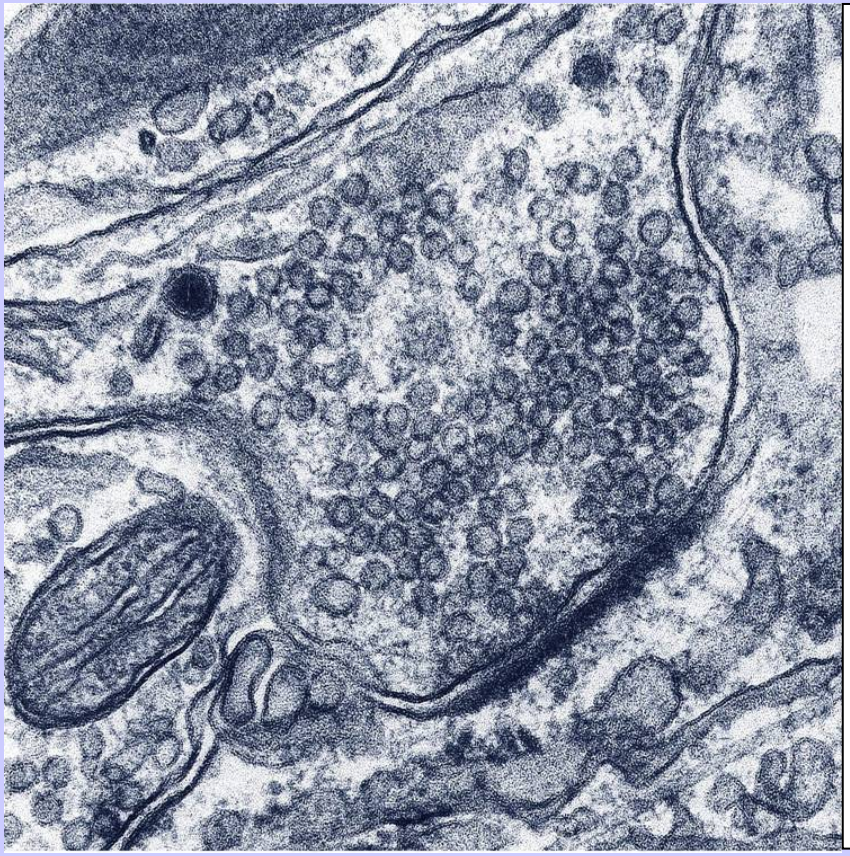


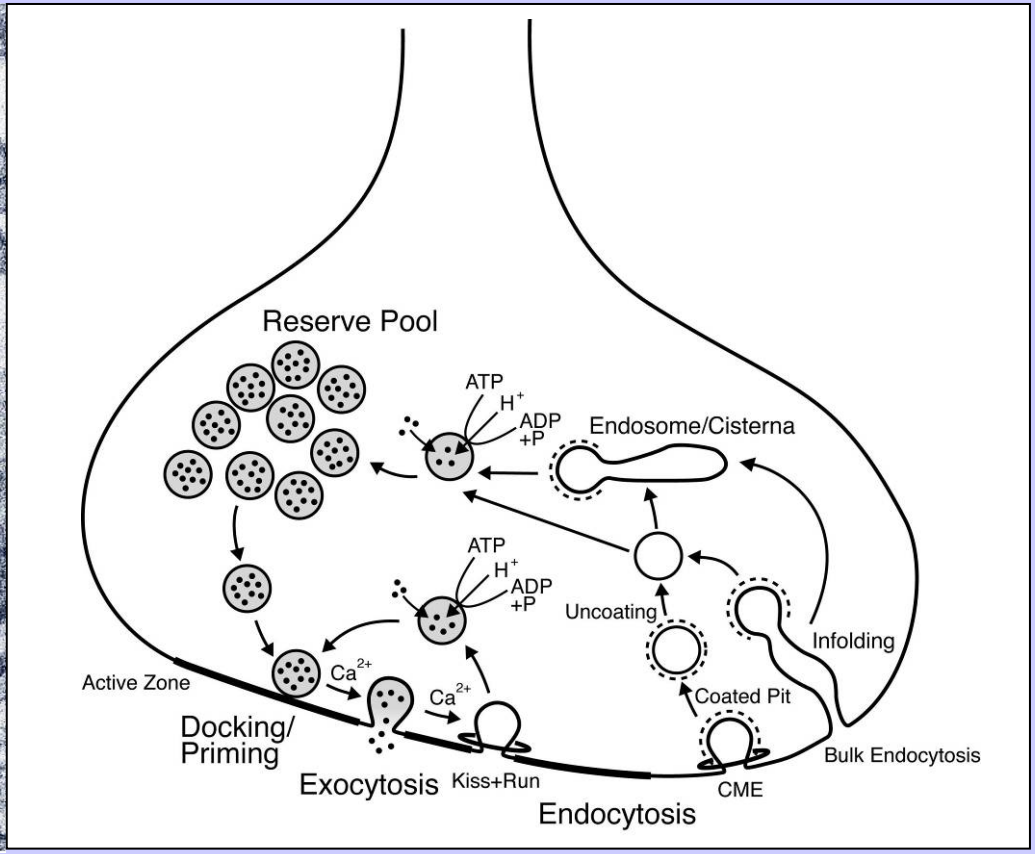
“critical periods”

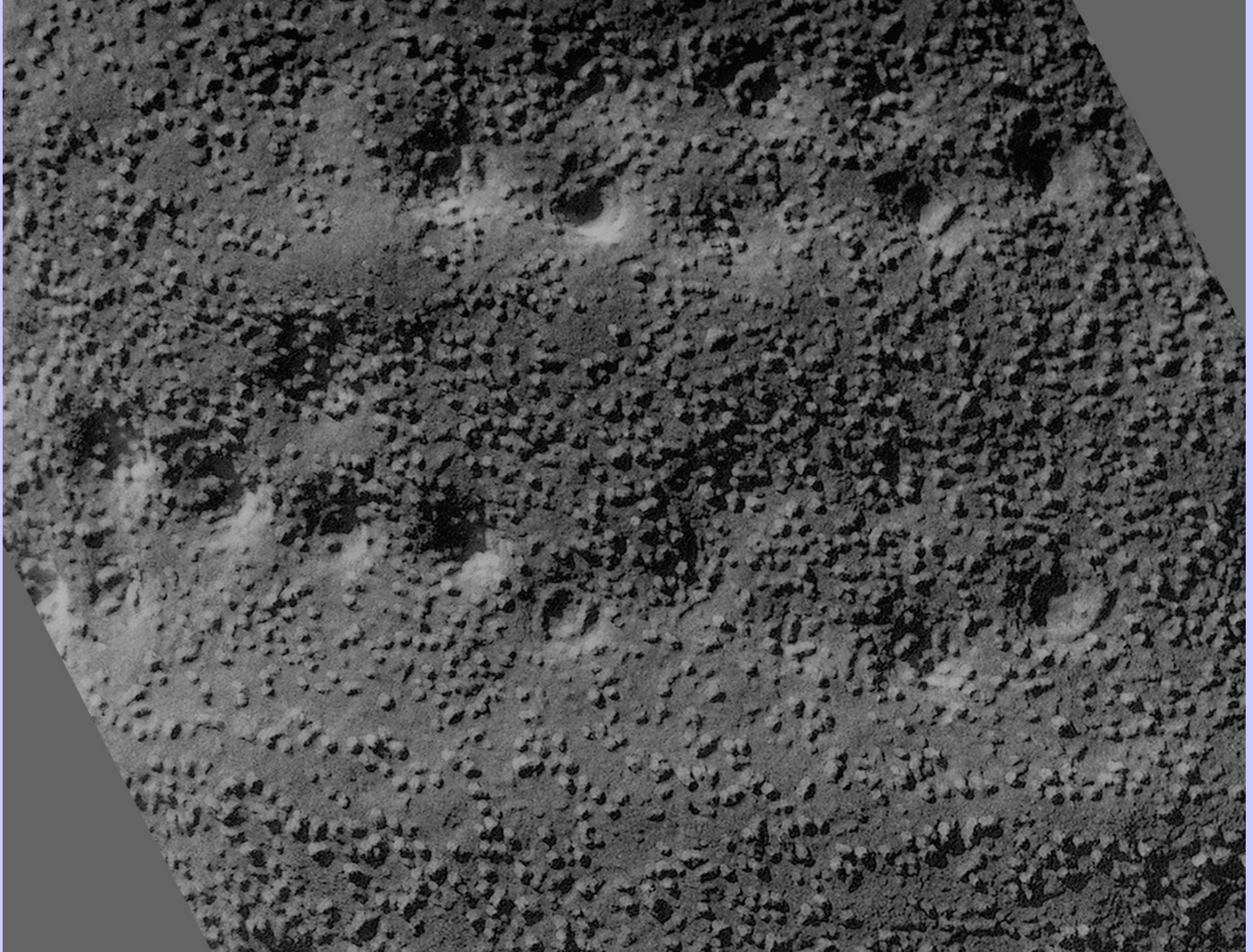
ПРОБЛЕМА: ПЛАСТИЧНОСТЬ СИНАПТИЧЕСКИХ СВЯЗЕЙ В НЕРВНОЙ СИСТЕМЕ

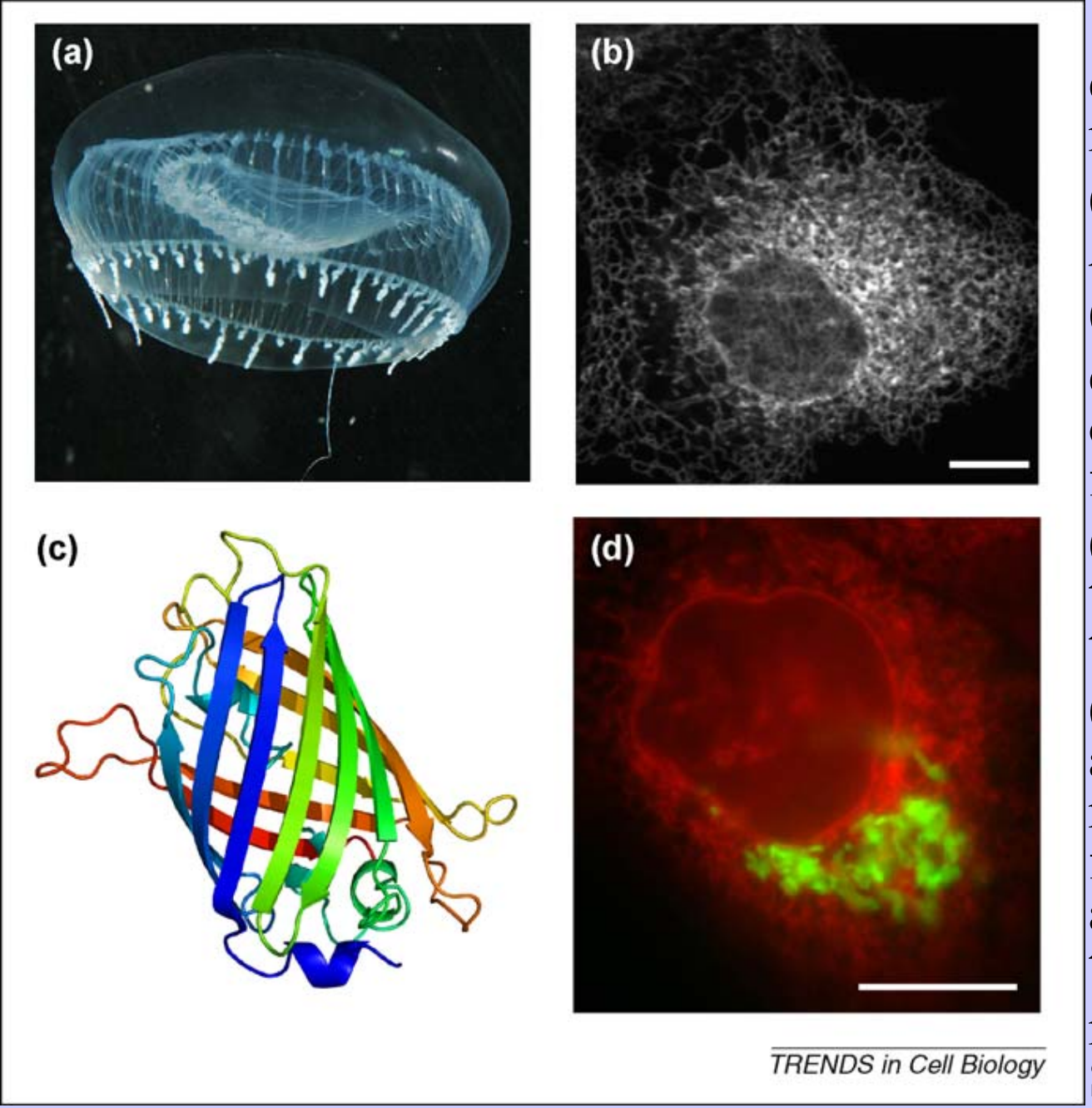
- Как возникает и где хранится информация об изменении активности в синапсах?

CNS synapses contain only about 100 synaptic vesicles. Thus, they have to be quickly recycled locally.









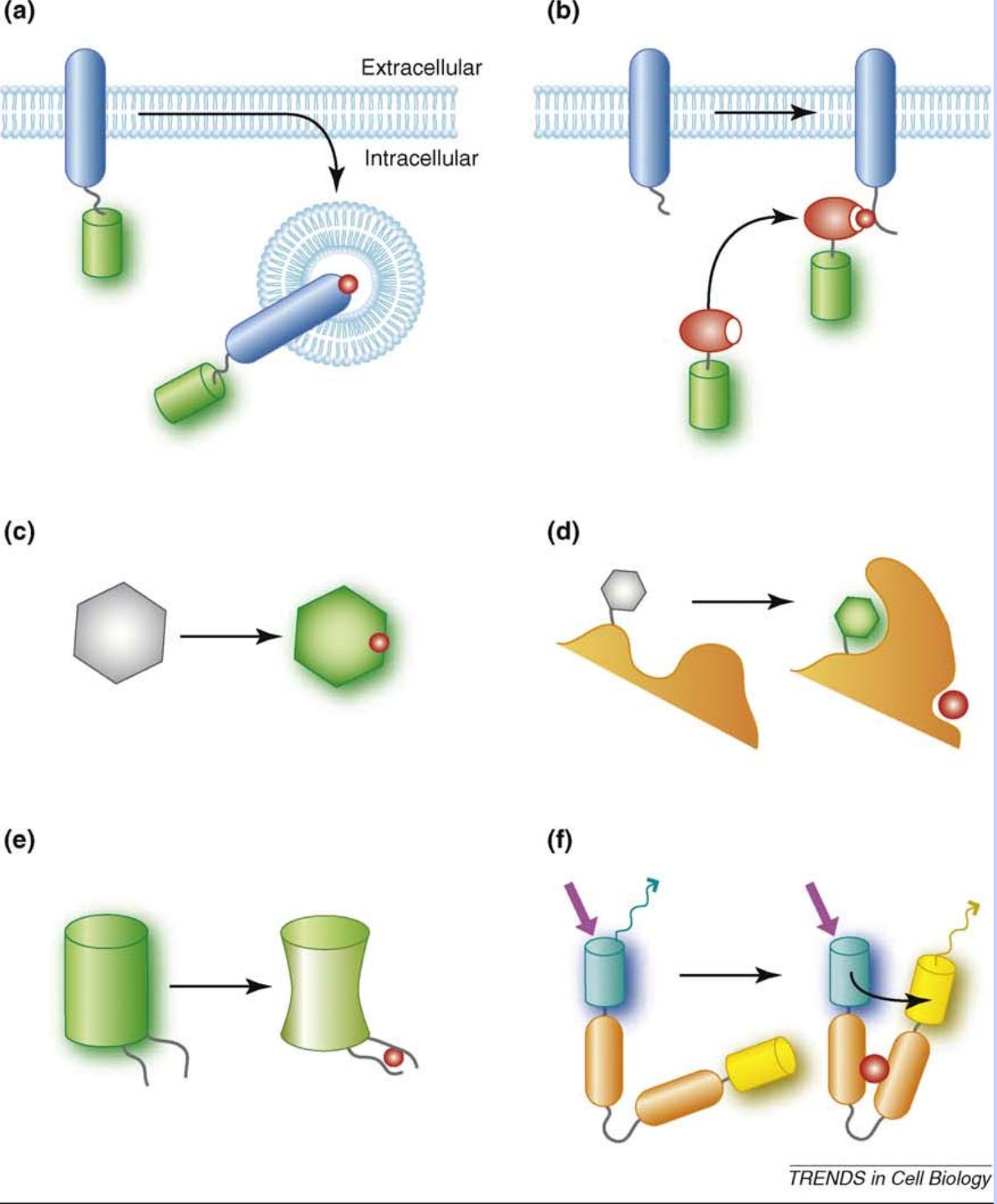
GFP from jellyfish to expression in mammalian cells.

(a) The jellyfish Aequorea victoria.

(b) GFP targeted to the endoplasmic reticulum of a mammalian fibroblast.

(c) Ribbon diagram of the barrel structure typical of FPs.

(d) Co-expression of an endoplasmic reticulum targeted red fluorescent protein and a Golgi complex targeted GFP in a mammalian fibroblast. Scale bars = 10 mm.



Principles of fluorescent probes that report on the distribution and conformation transitions of signaling molecules. (a) Simple tagging of proteins with fluorescent tags (green) allows the monitoring of the distribution and the movements of the protein in cells but does not address conformation transitions.

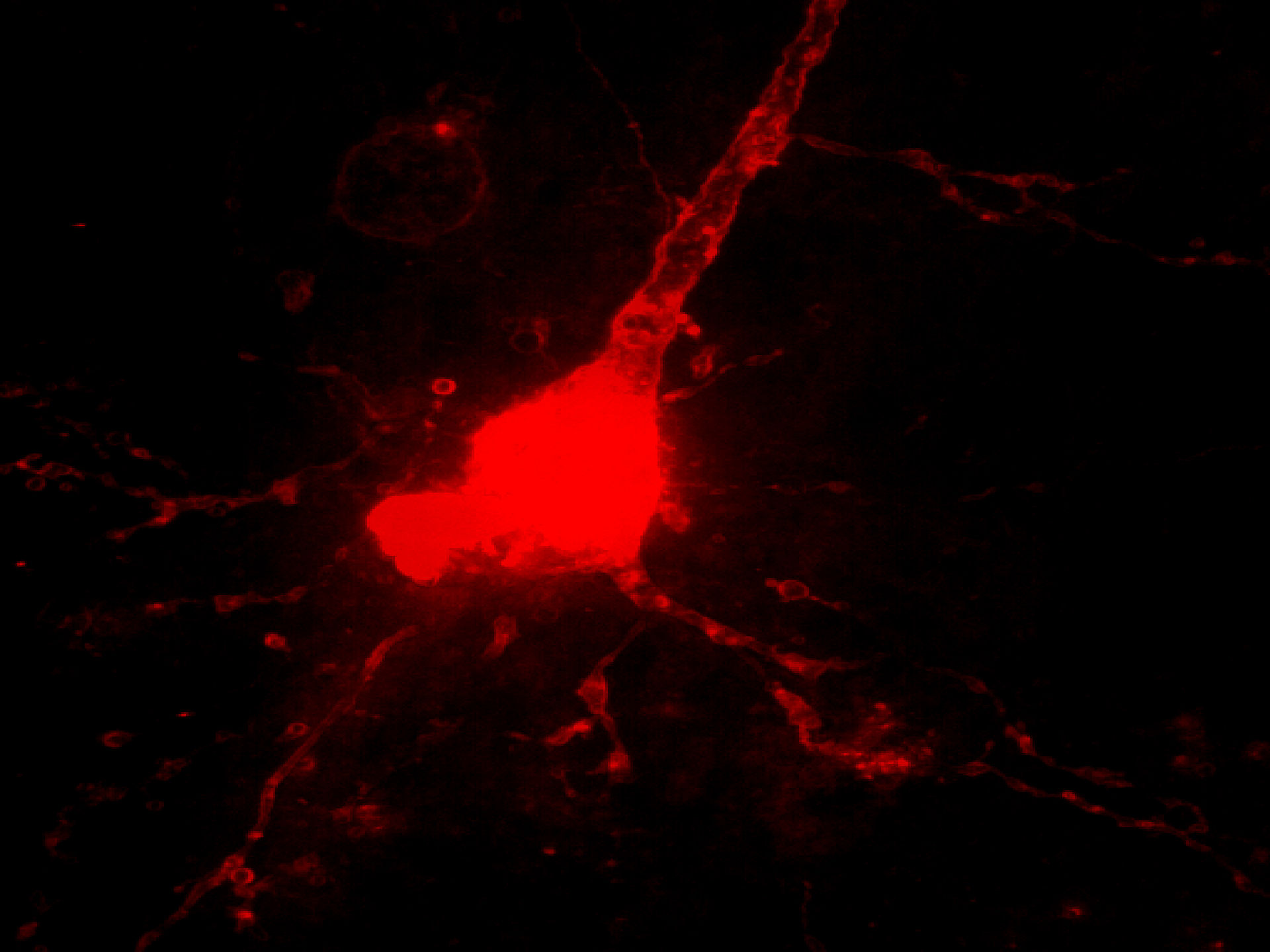
(b) Reporters containing a protein domain (orange) that recognize a specific conformation (such as a phosphorylation event; indicated by a red dot) can be recruited from the cytosol to the membrane upon phosphorylation of a membrane protein. However, the endogenous protein is often not sufficient to make a visible redistribution of the probe.

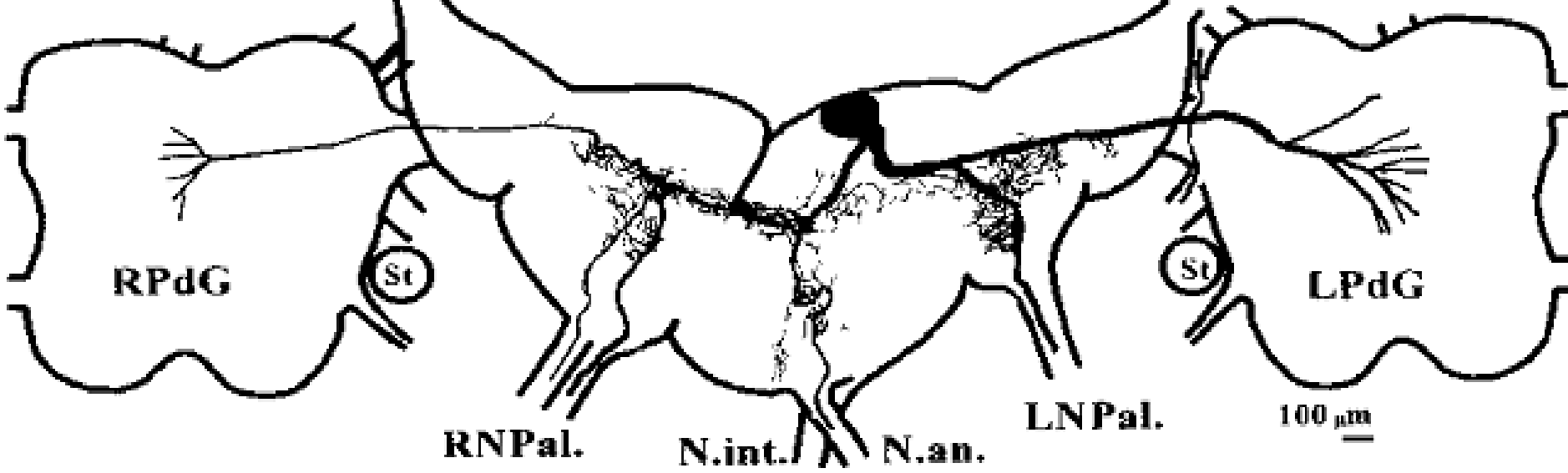
(c) Ideal fluorescent reporters change their properties (intensity or spectrum) upon binding of a ligand, such as the dyes used for Ca2+-measurements.

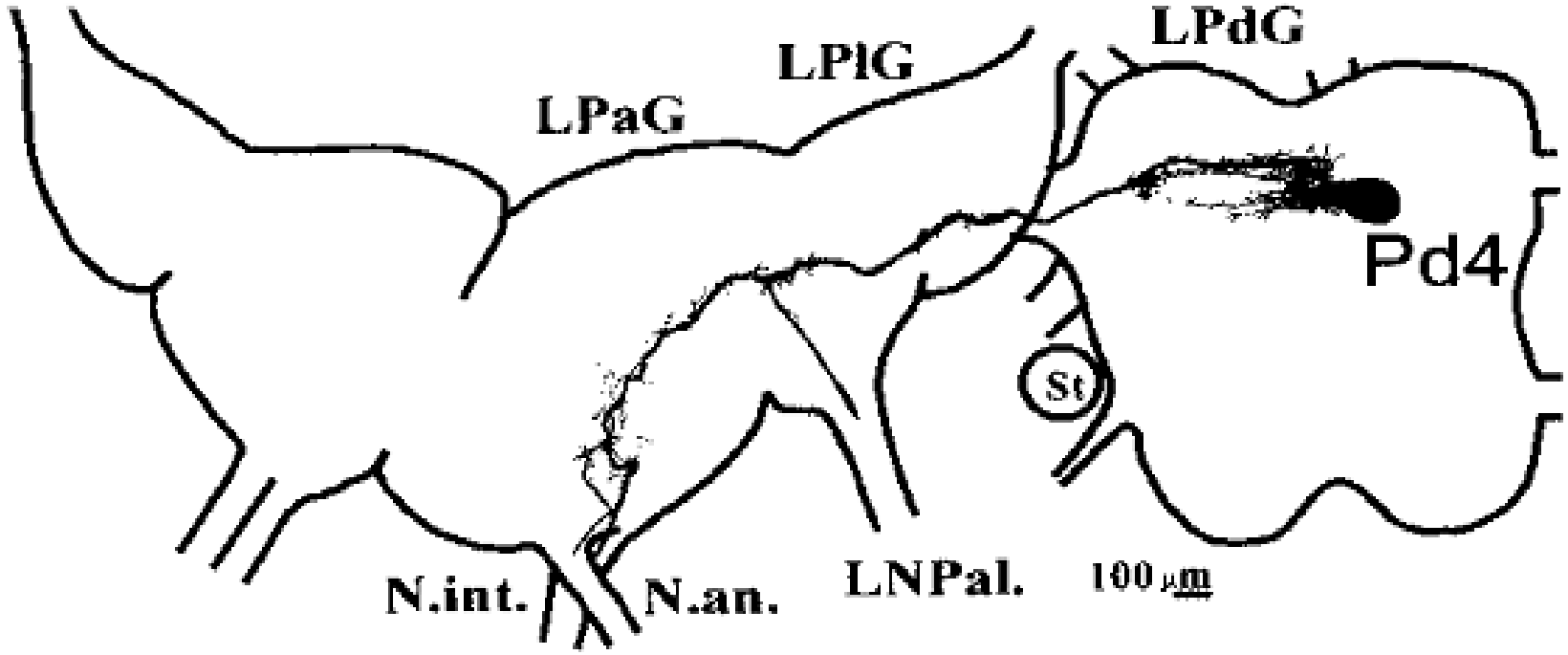
(d) In a similar fashion some fluorescent molecules change their properties when their environment is changed, such as when they enter a more hydrophobic pocket. This allows monitoring of conformation changes or protein-protein interactions with properly placed (conjugated) fluorophores.

(e) In an ideal case, genetically coded fluorescent molecules (mostly circularly permutated GFP variants) can change their fluorescence properties when protein motifs woven into them bind to specific ligands.

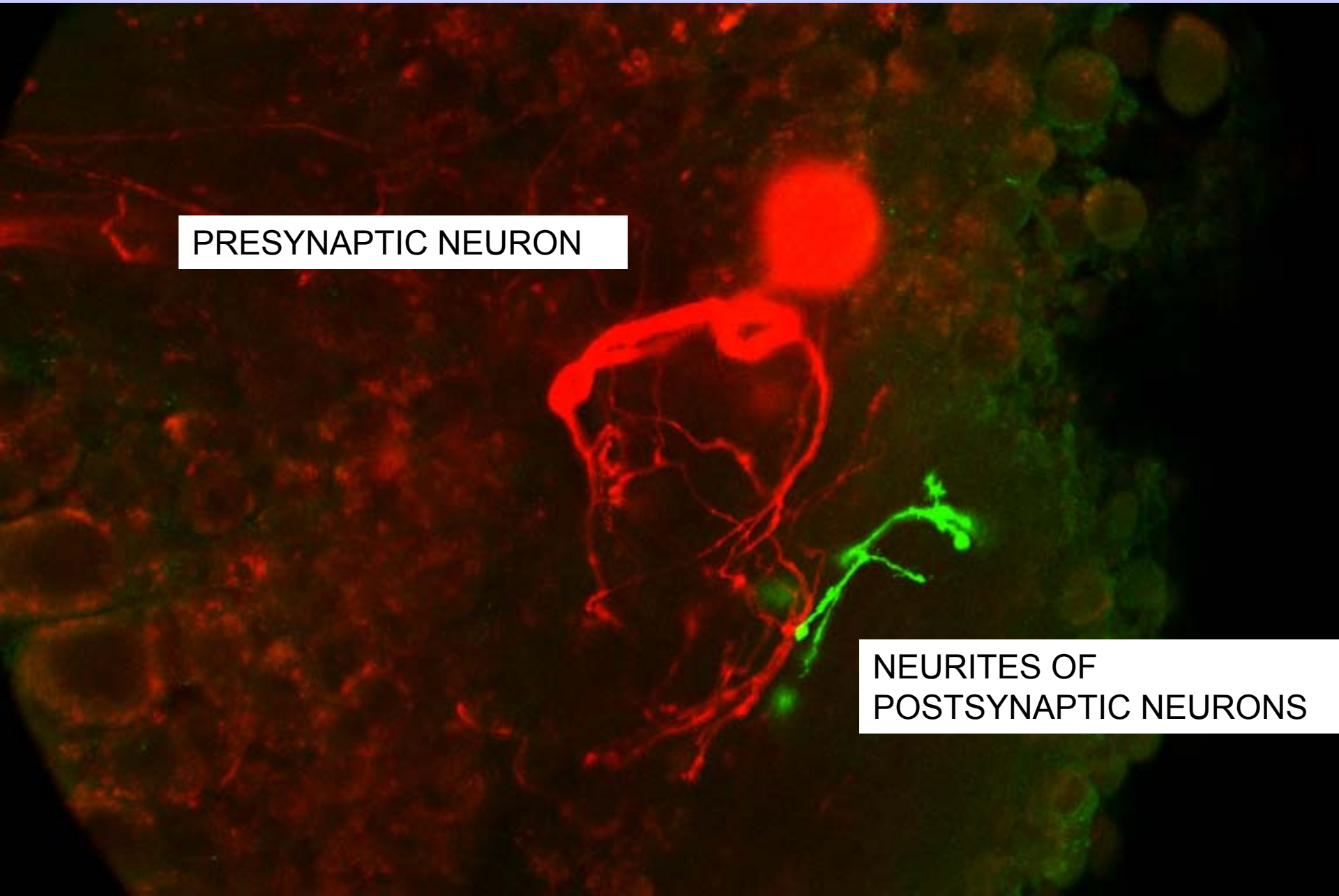
(f) Classical probes based on FRET where the conformation change induced by ligand binding alters the distance or orientation of the two attached fluorescent molecules, so causing a detectable change in FRET efficiency

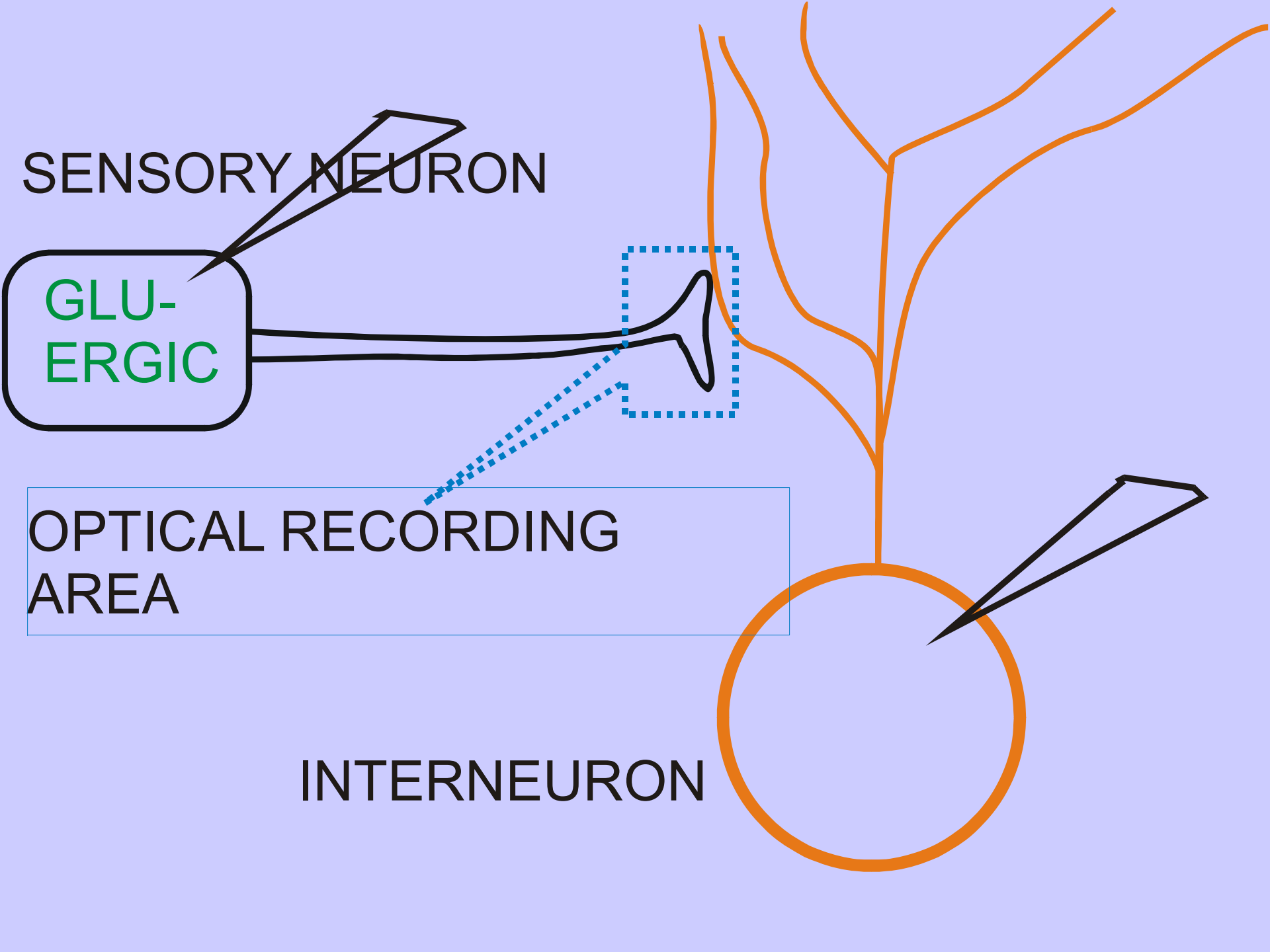


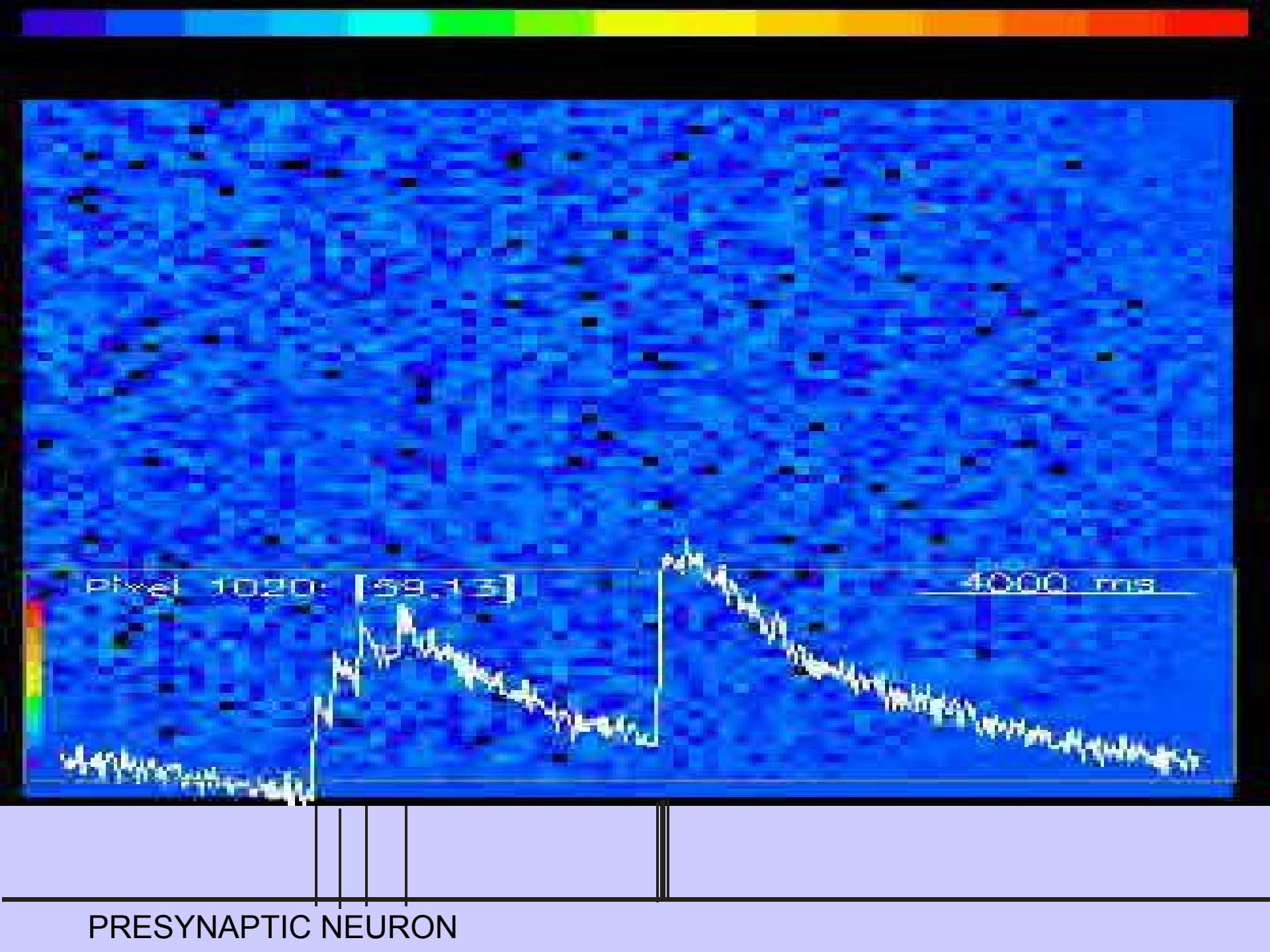

RPdG
St
St
LPdG
RNPal.
N.int.
N.an.
LNPal.
100 µm


LPdG
LPlG
LPaG
Pd4
St
N.int.
N.an.
LNPal.
100 µm

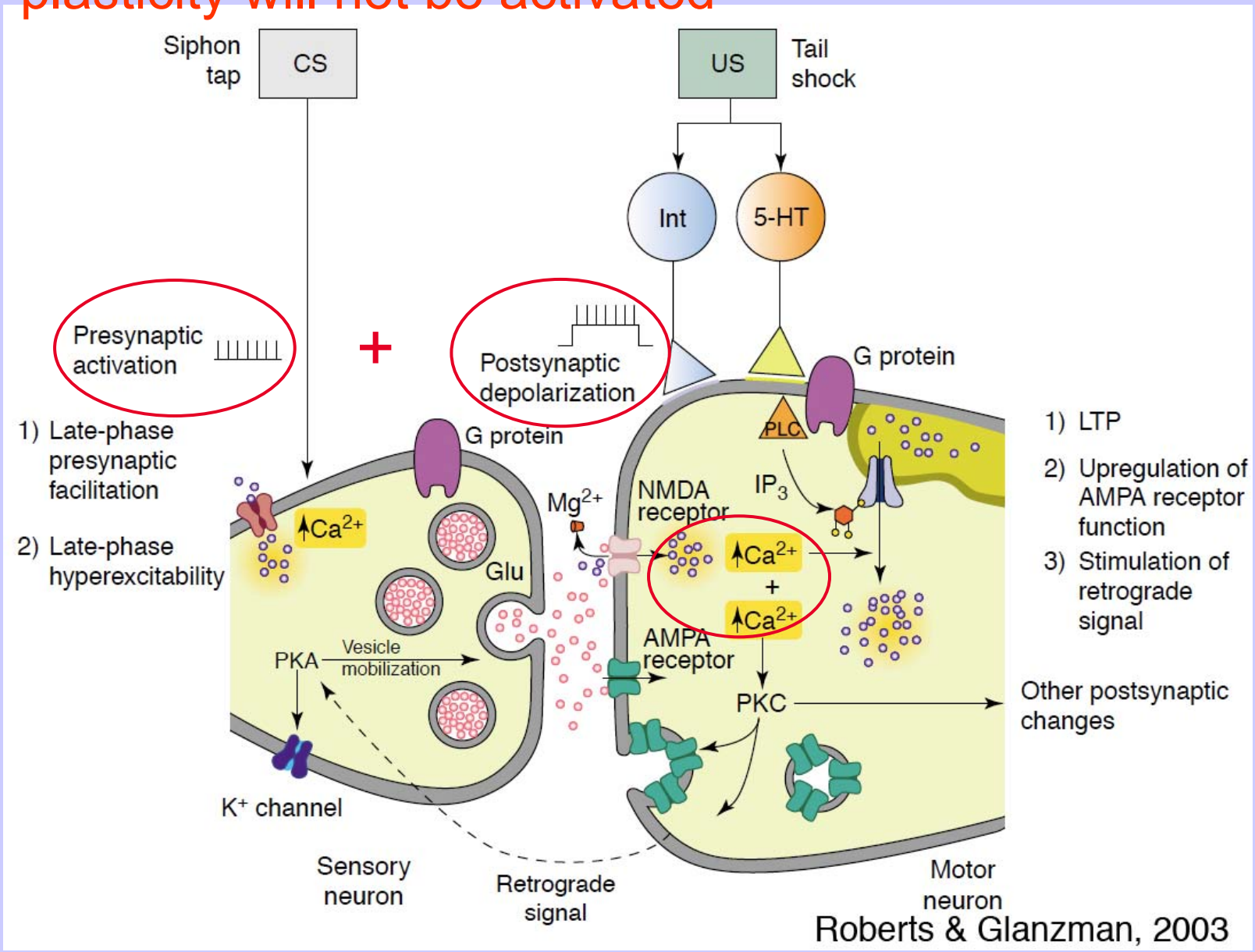
CONFOCAL MICROSCOPY OF SYNAPTIC CONTACT ZONE BETWEEN IDENTIDFIED SNAIL NEURONS



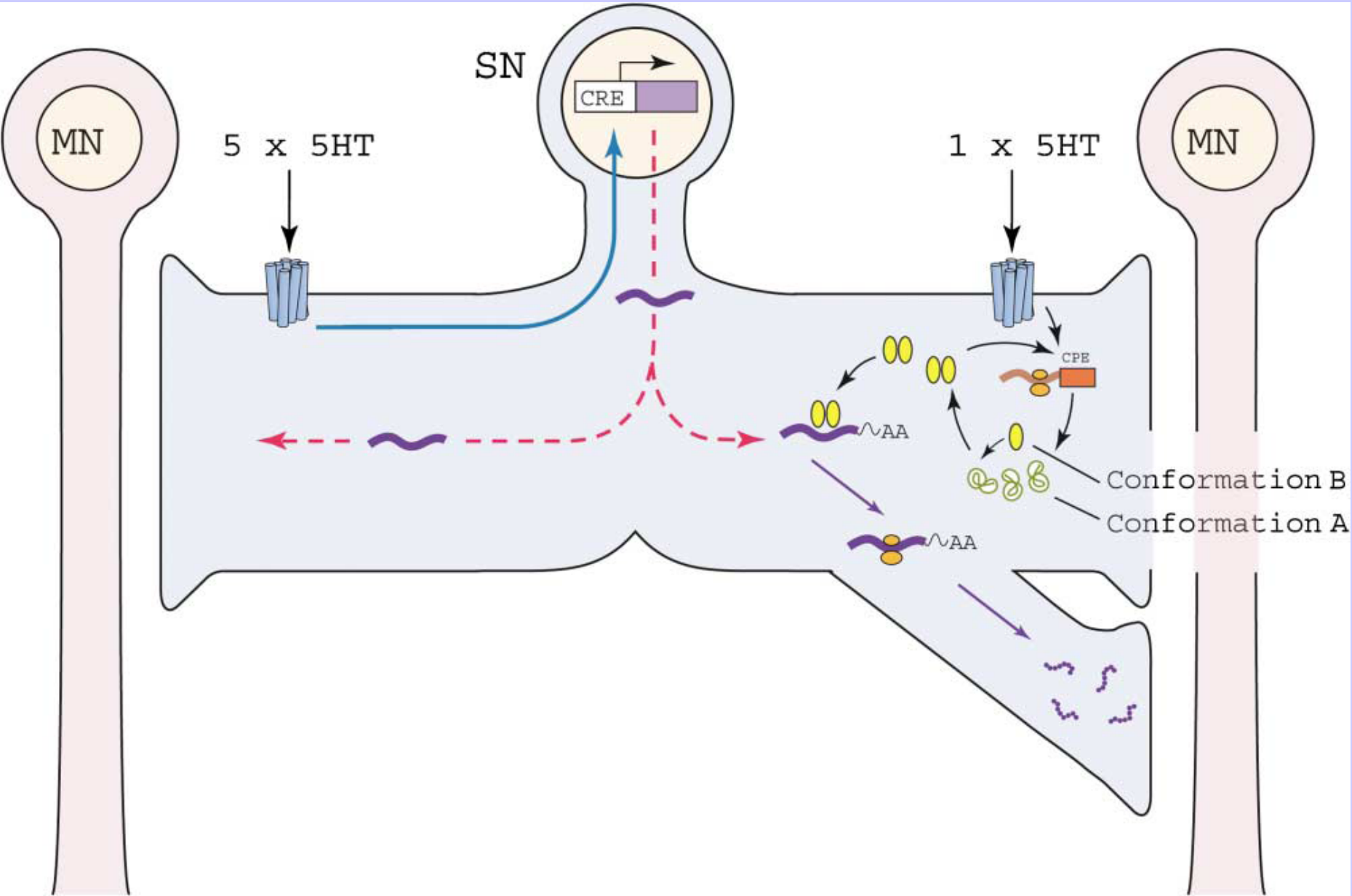

SENSORY NEURON
GLU-
ERGIC
OPTICAL RECORDING
AREA
INTERNEURON

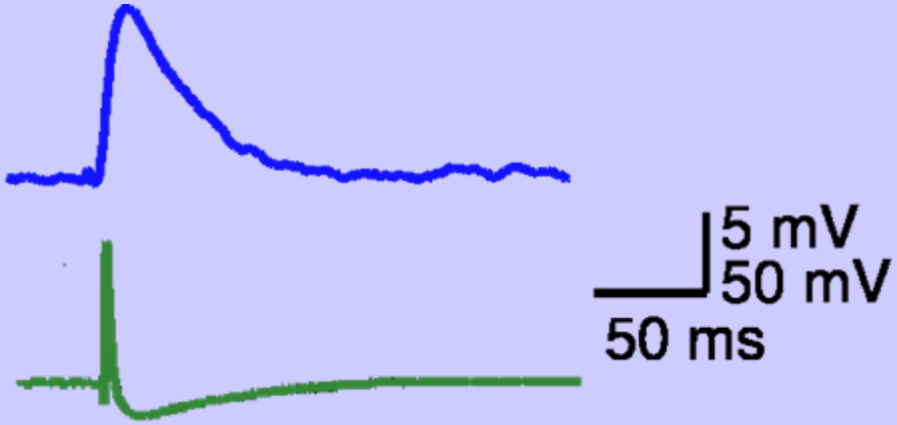

Pixel 1020: [59,13]
4000 ms
PRESYNAPTIC NEURON

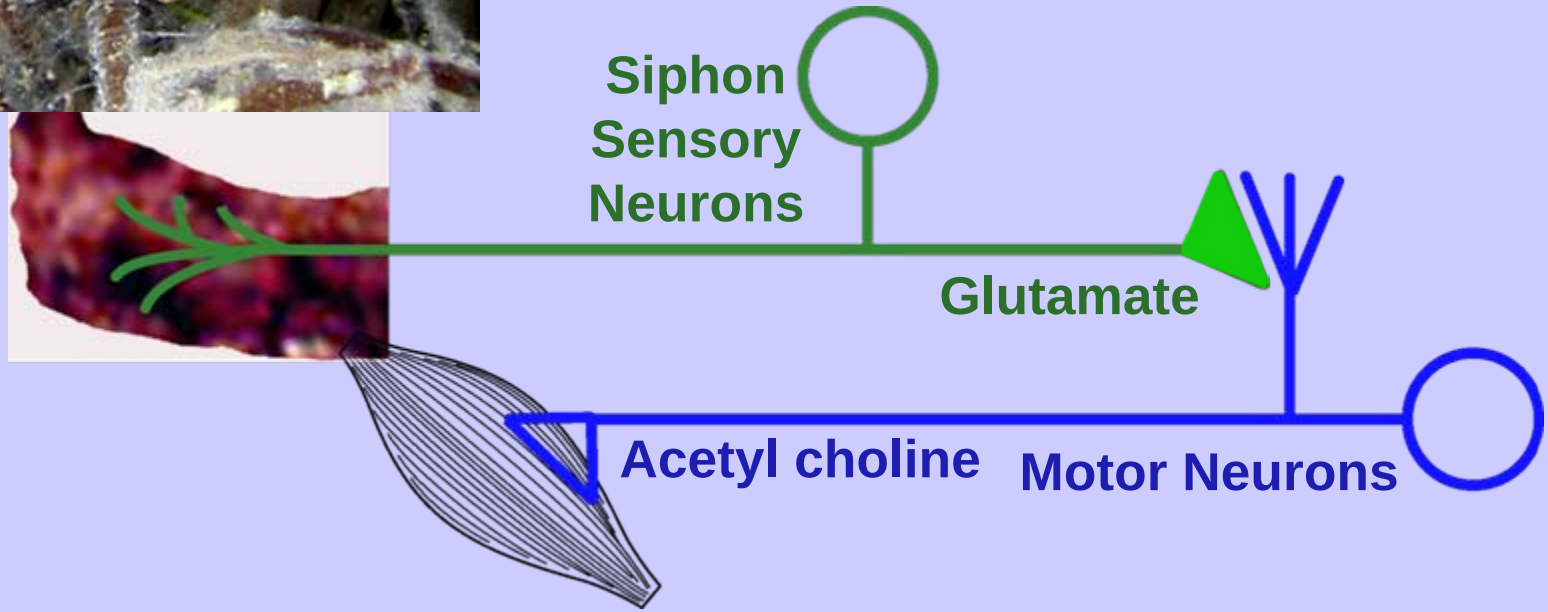
If synapses are gated off, Hebbian mechanisms of plasticity will not be activated



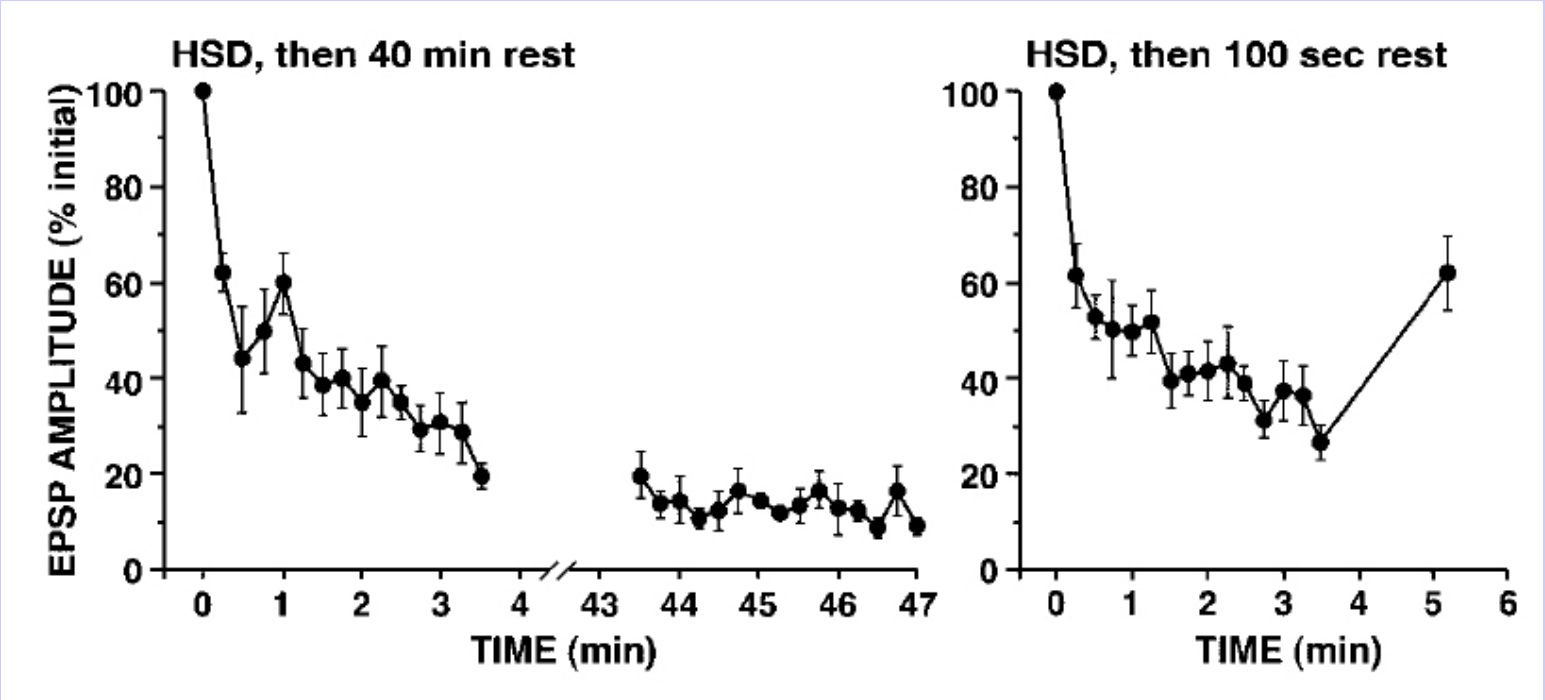
A Prion-Based Model for Self-Perpetuating Synaptic Change




5 mV
50 mV
50 ms

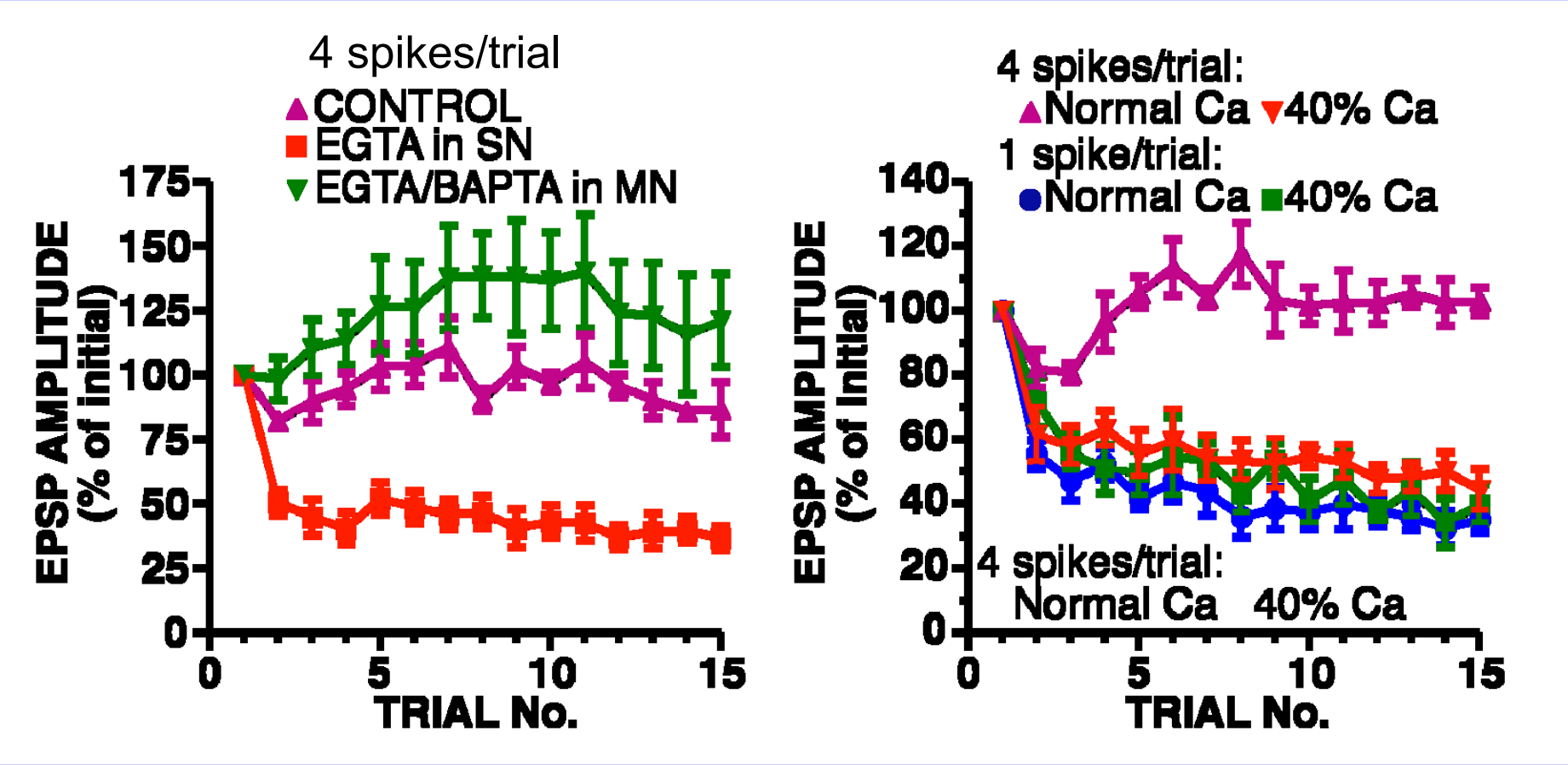

Siphon
Sensory
Neurons
Glutamate
Acetyl choline
Motor Neurons

Synaptic depression can persist tens of minutes after induction



"short term?"

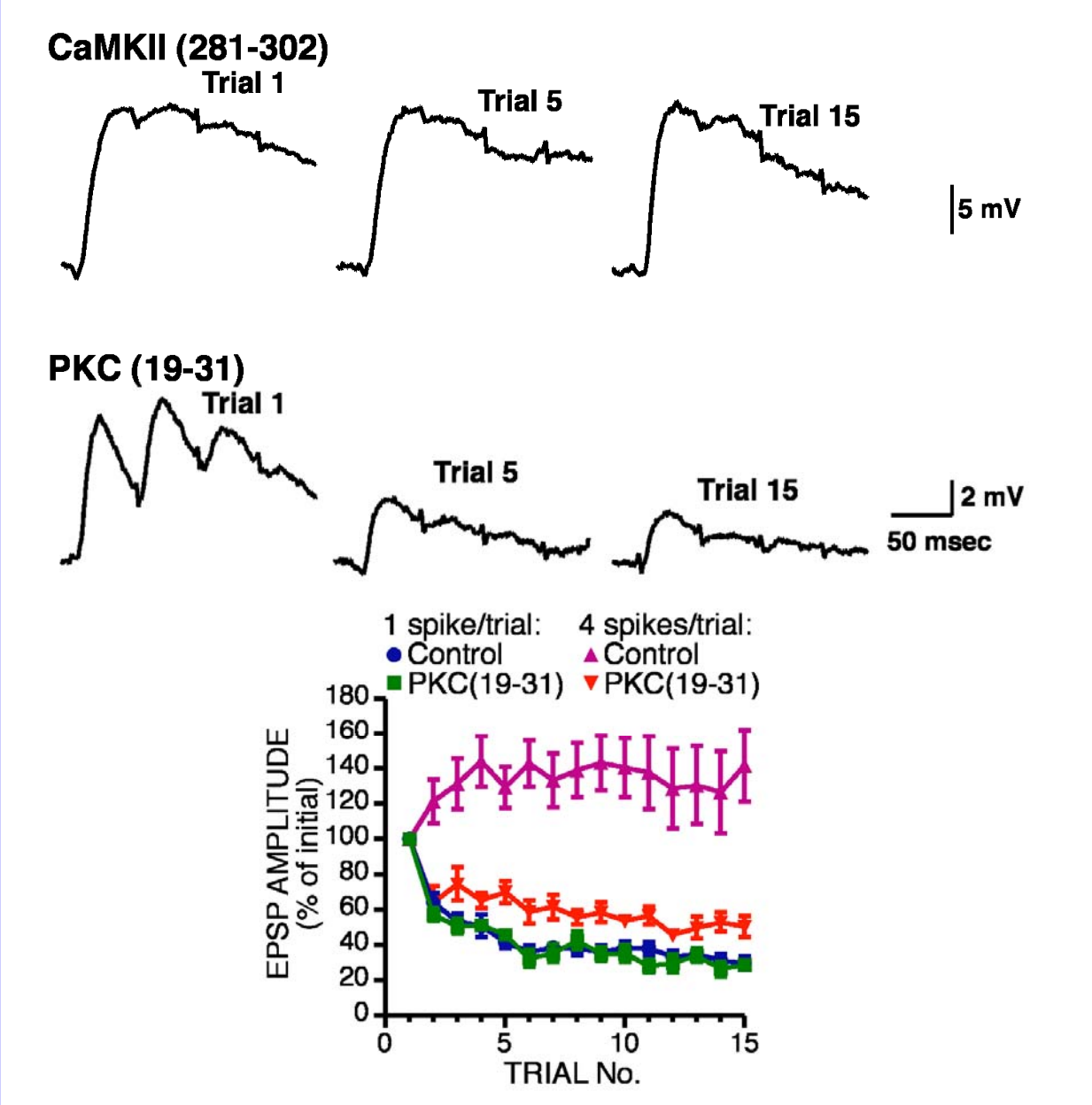
Burst-Dependent Protection is Initiated by Presynaptic Ca influx



Chelating postsynaptic Ca did not affect burst-dependent protection

Burst-Dependent Protection requires more Ca than HSD

Burst-Dependent Protection is Mediated by Presynaptic PKC

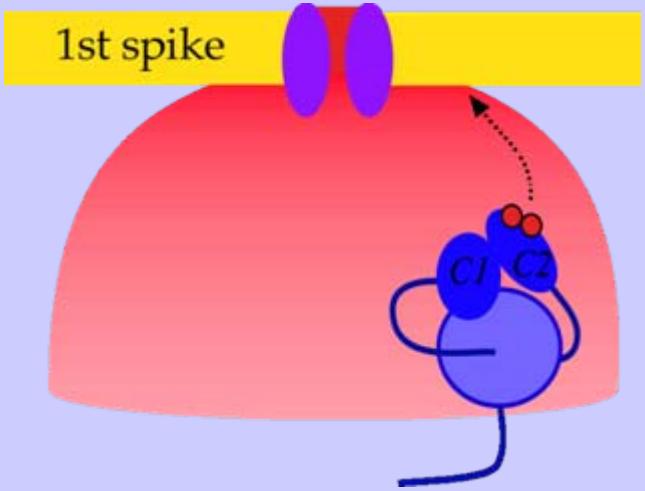


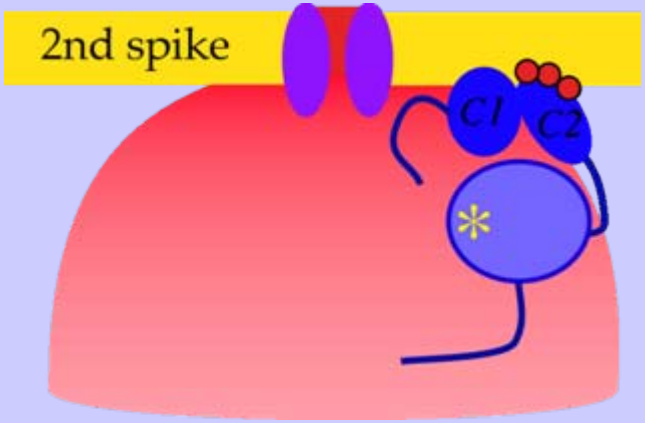
1. HSD is initiated by Ca influx during action potential,
2. but is independent of release

3. Homosynaptic depression involves a switching off, or silencing, of release sites, which can persist for tens of minutes

3. Brief bursts of spikes in the presynaptic SN protect against synaptic depression via a Ca- and PKC-dependent mechanism

   (= Burst-dependent protection)

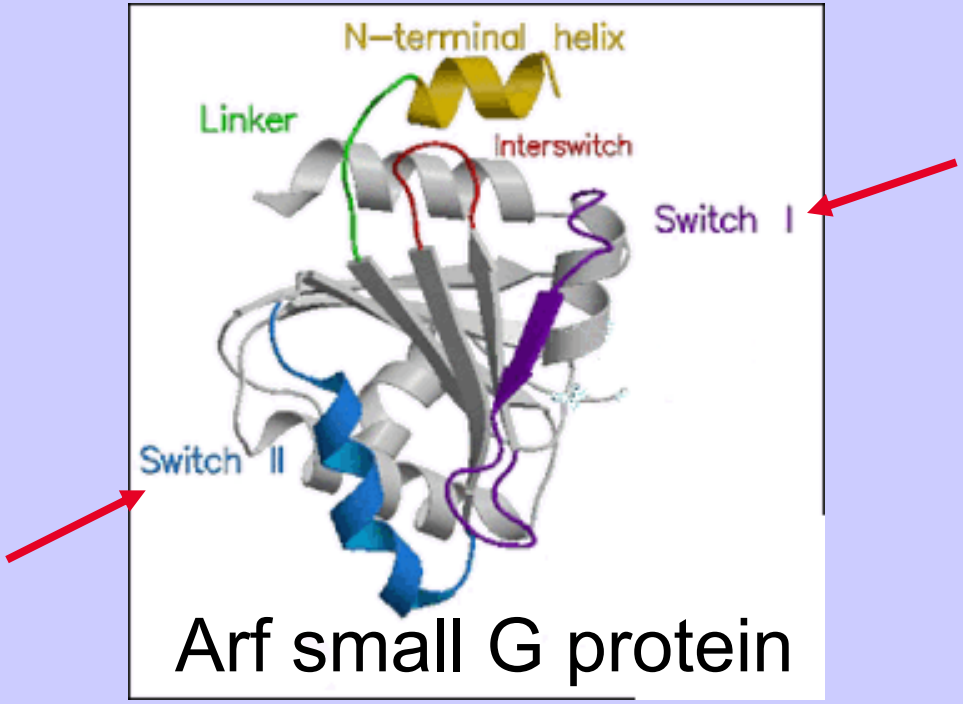




Additional Ca binding site, Ca ion coordinated by phospholipid

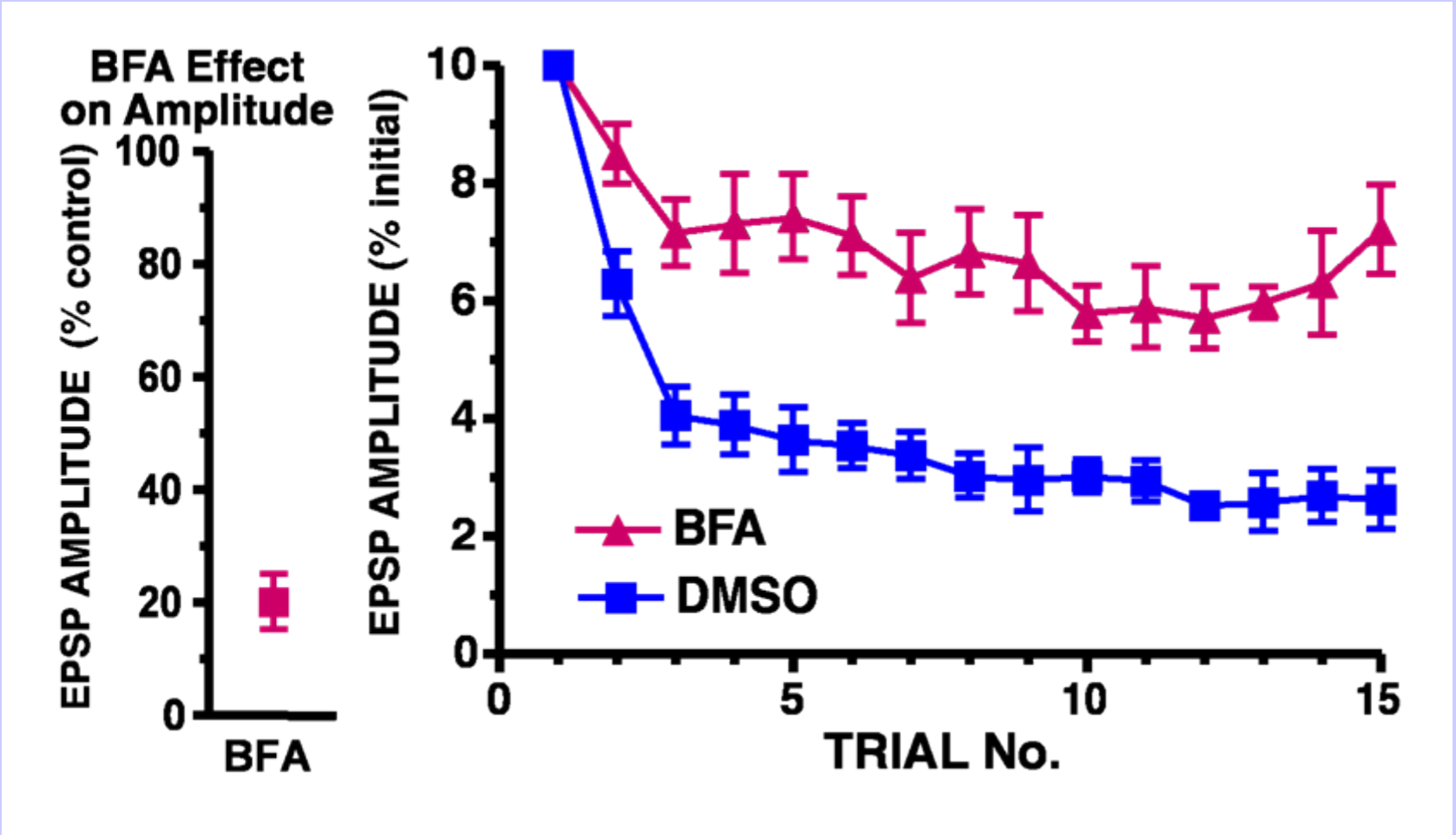
Lauren Jones
Leighton Izu
Andreea Negroiu
Qin Wan

- What is the Molecular Switch that mediates Homosynaptic Depression and that PKC



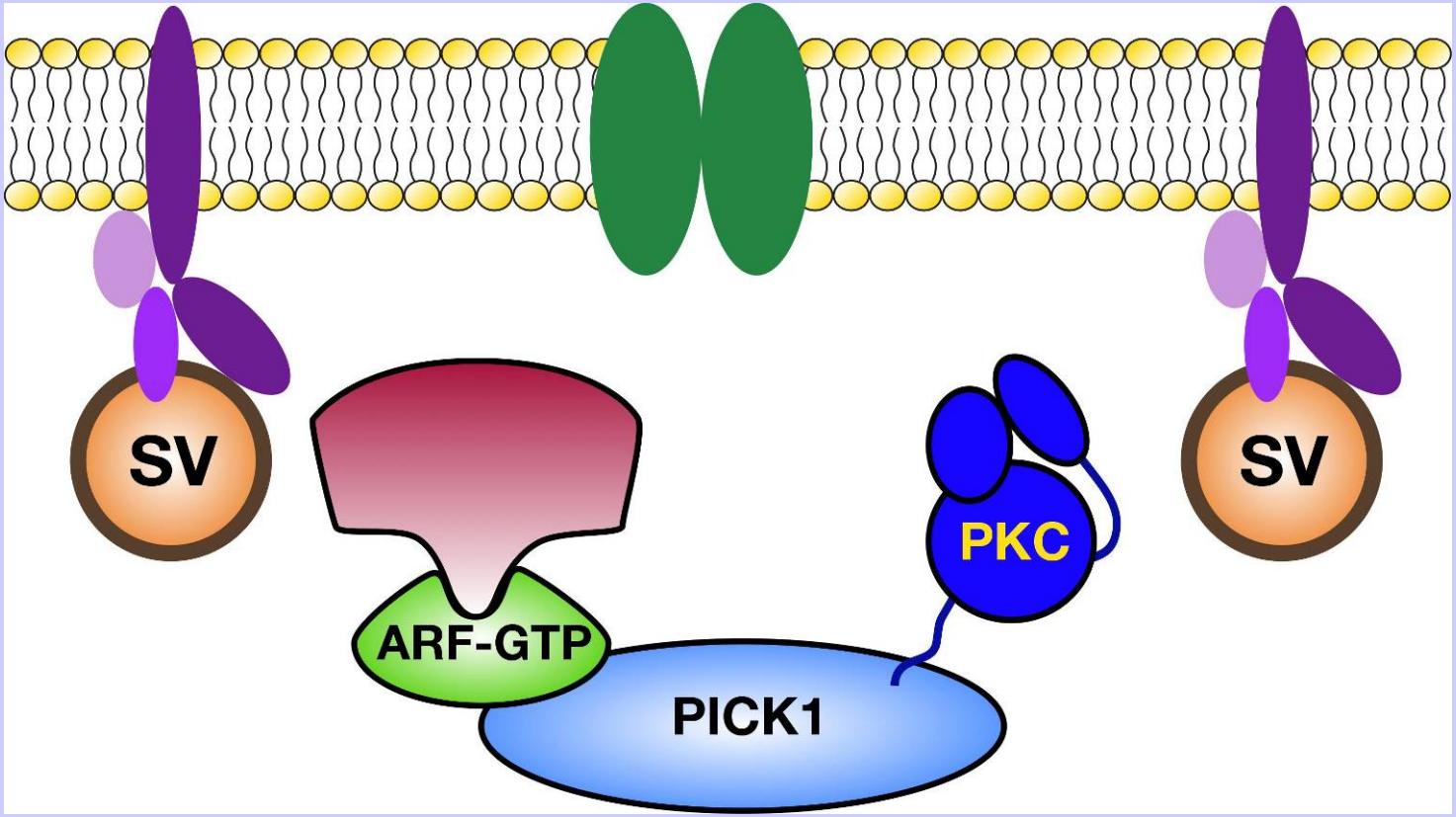
Arf small G protein

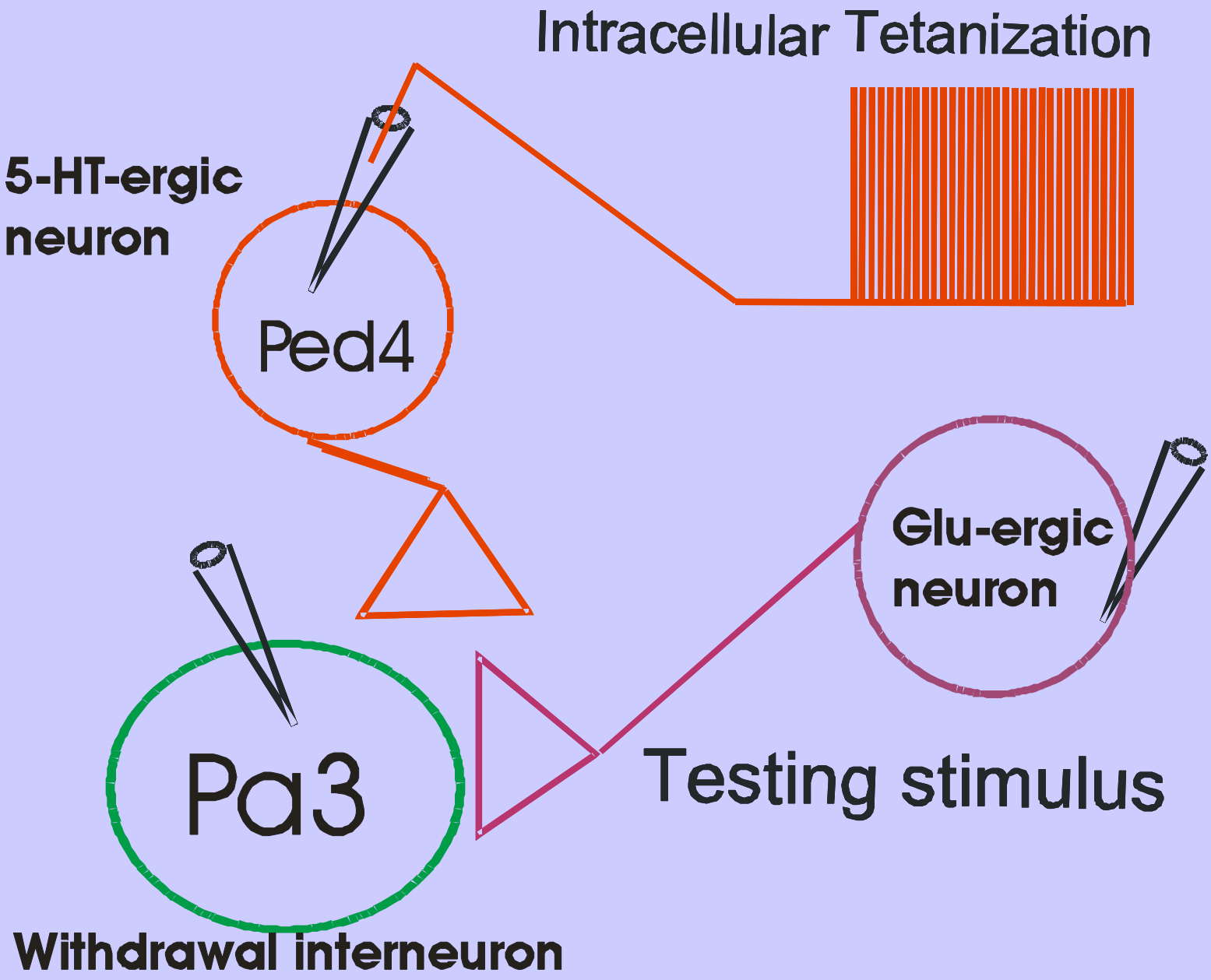
Brefeldin A inhibits guanine nucleotide exchange factors for Arf (small G protein)



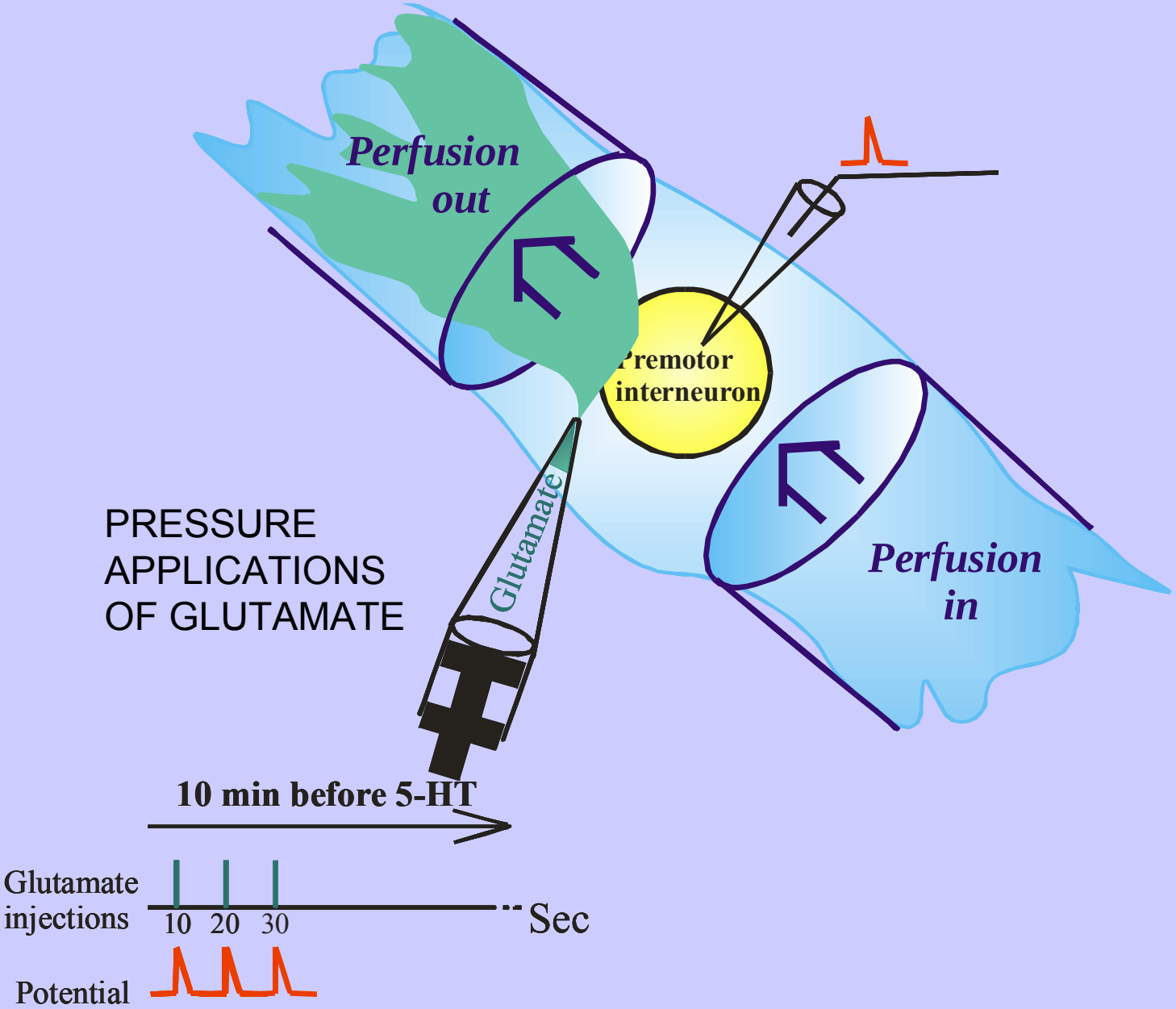
Brefeldin A (inhibits activators of ARF) mimicked & occluded HSD

PICK1 may provide a scaffold, localizing both PKC Apl-I and Arf to release sites at synapse

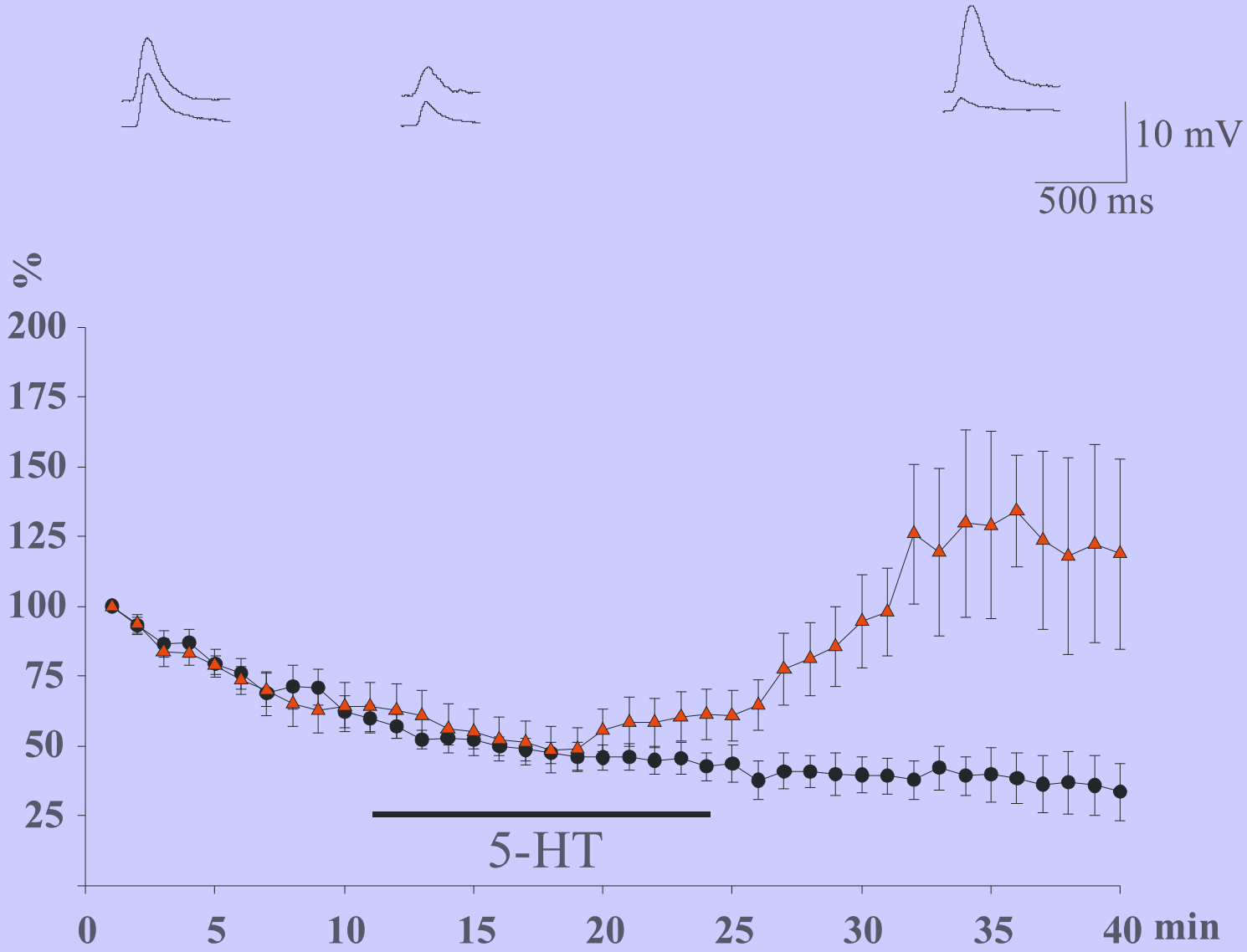


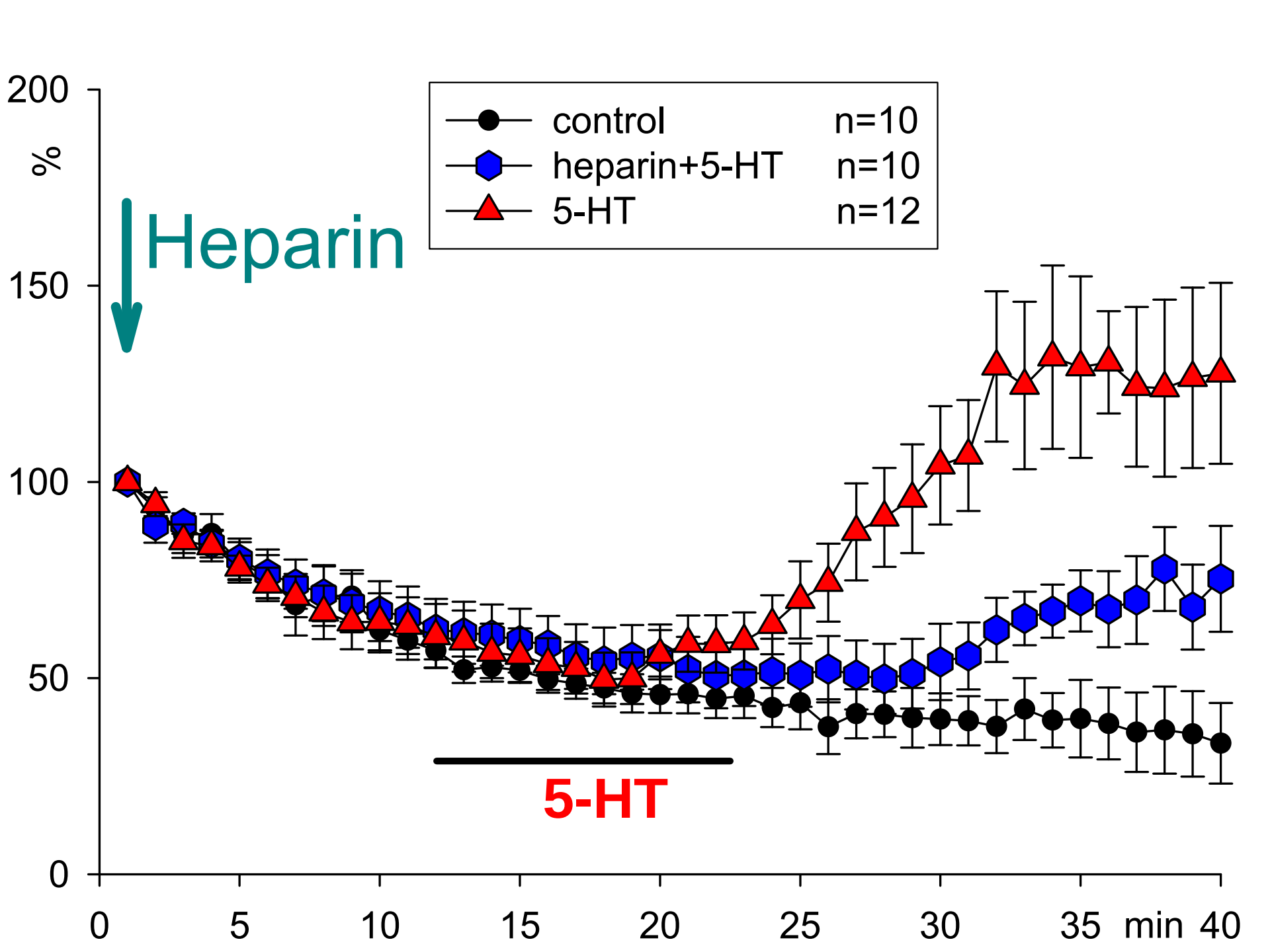

Intracellular Tetanization
5-HT-ergic neuron
Ped4
Glu-ergic neuron
Pa3
Testing stimulus
Withdrawal interneuron

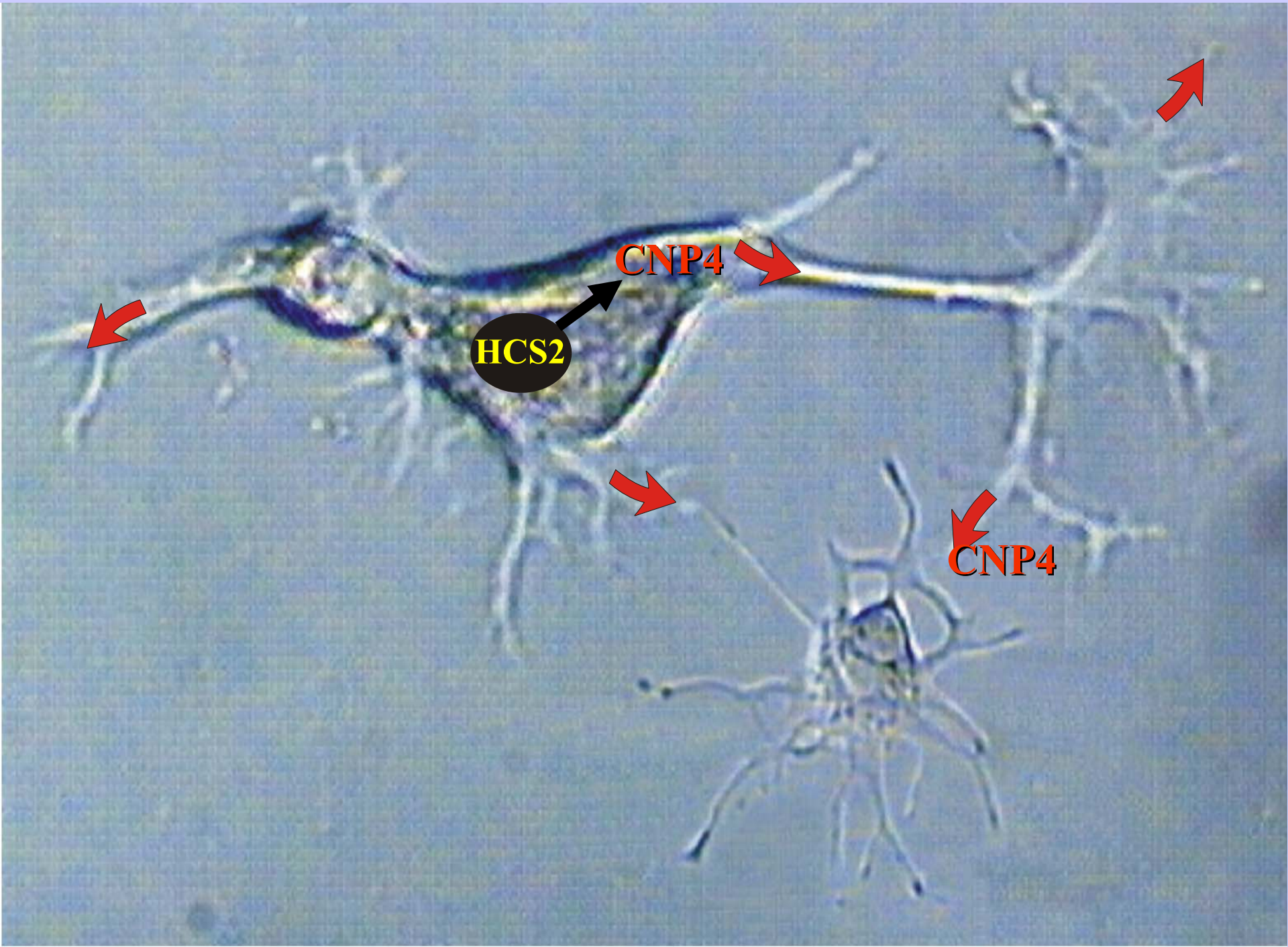
ARTIFICIAL SYNAPSE MODEL



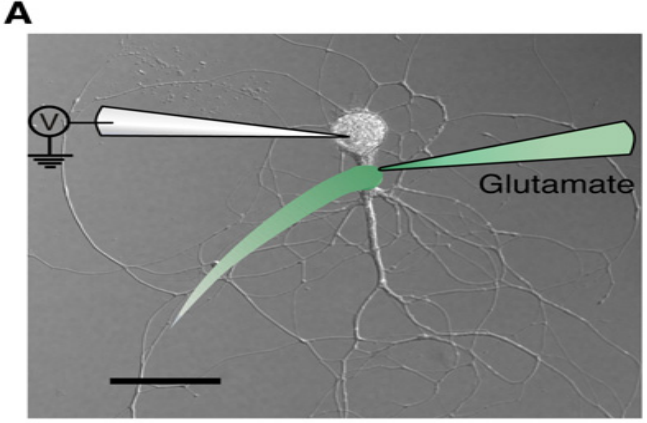
5-HT APPLICATION FACILITATES THE GLU-PSPs

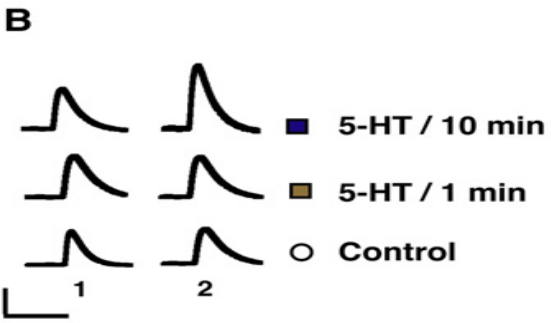


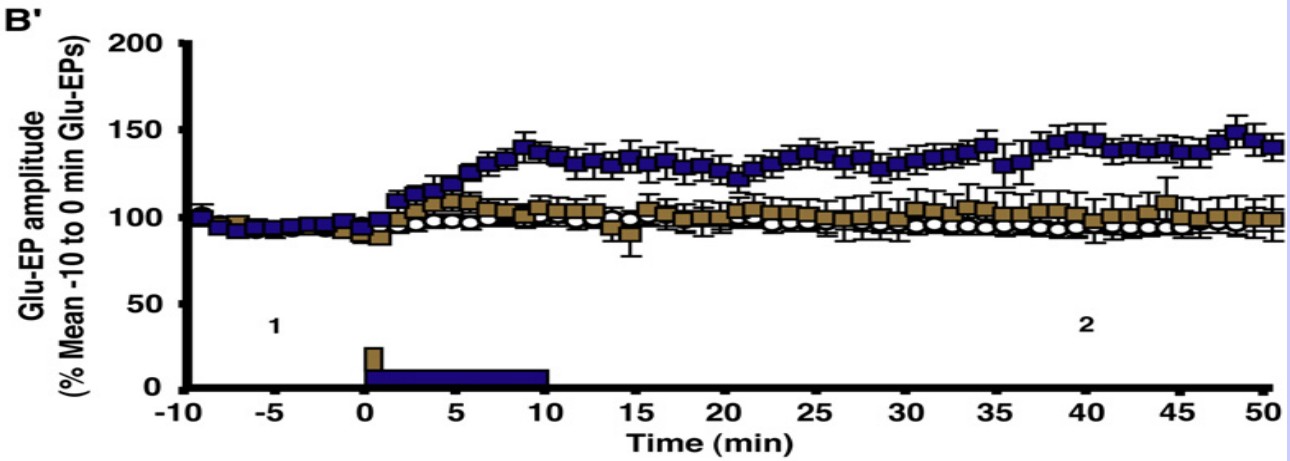

200
%
150
100
50
0
Heparin
control n=10
heparin+5-HT n=10
5-HT n=12
5-HT
0
5
10
15
20
25
30
35
min
40

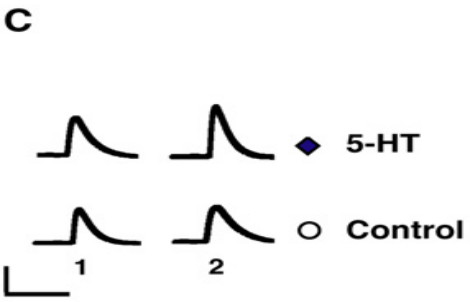

CNP4
HCS2
CNP4

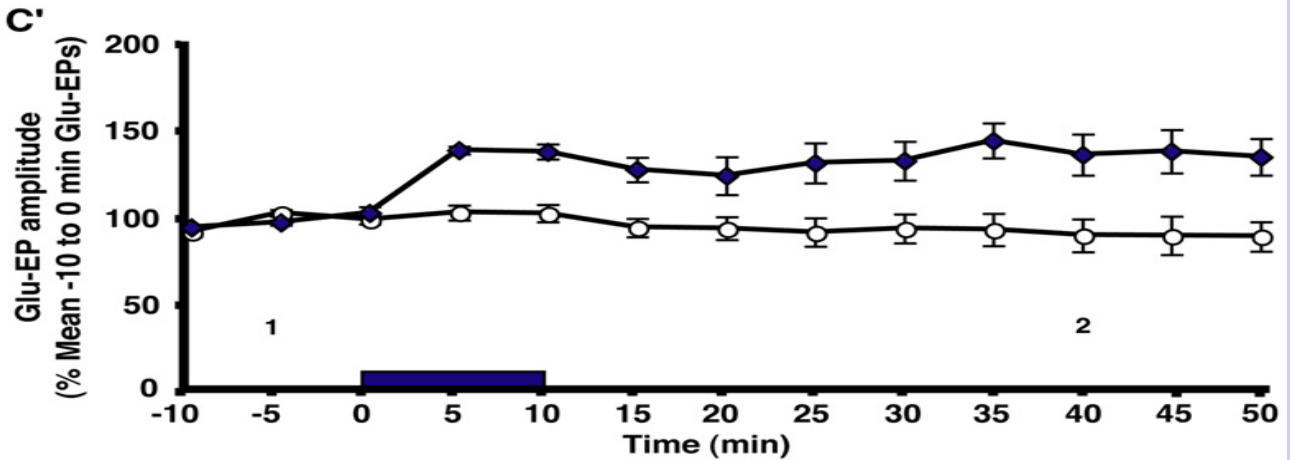
10 Minutes, But Not 1 Minute, of 5-HT Stimulation Causes Prolonged Enhancement of the Glutamate-Evoked Response in Motor Neurons



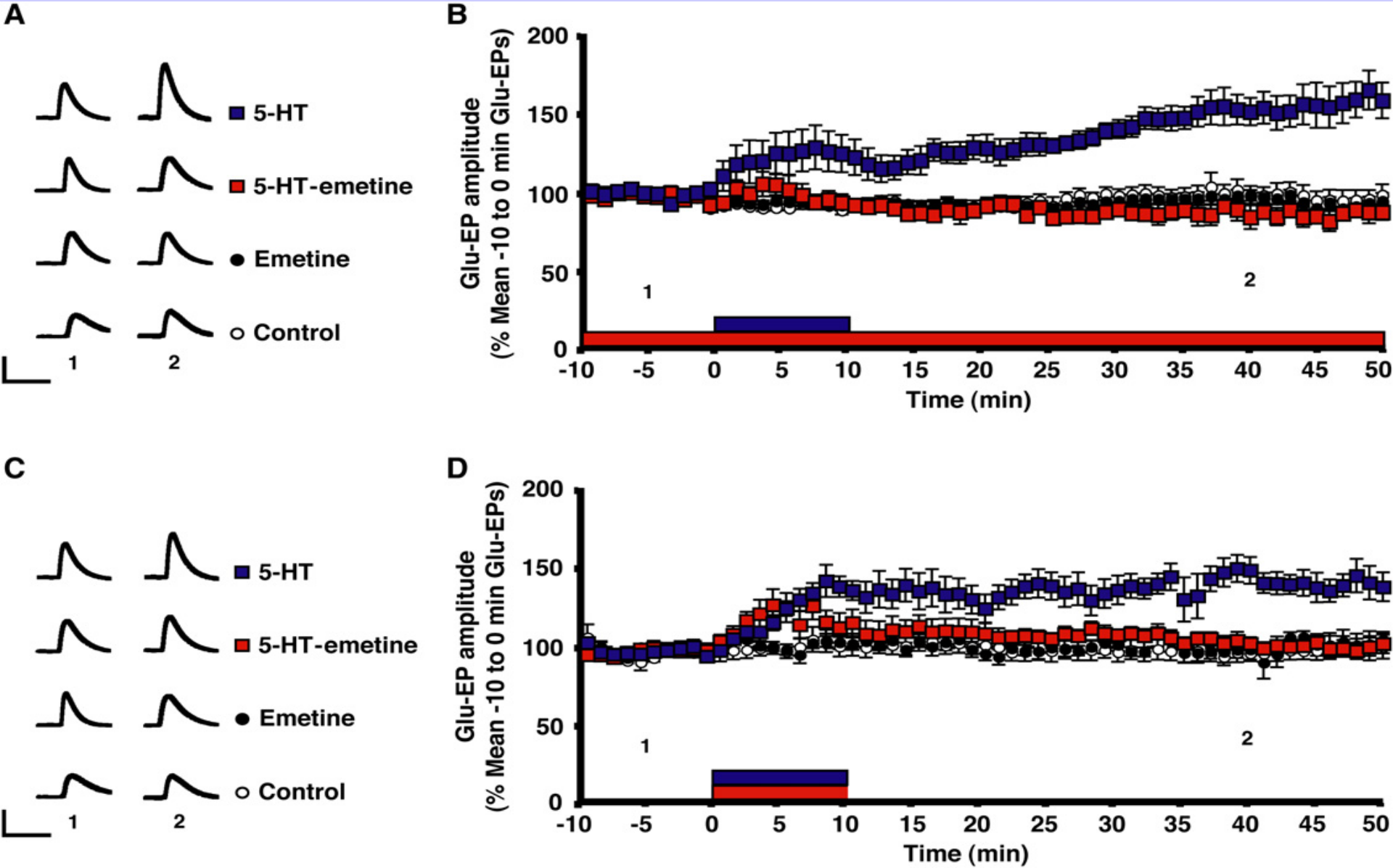


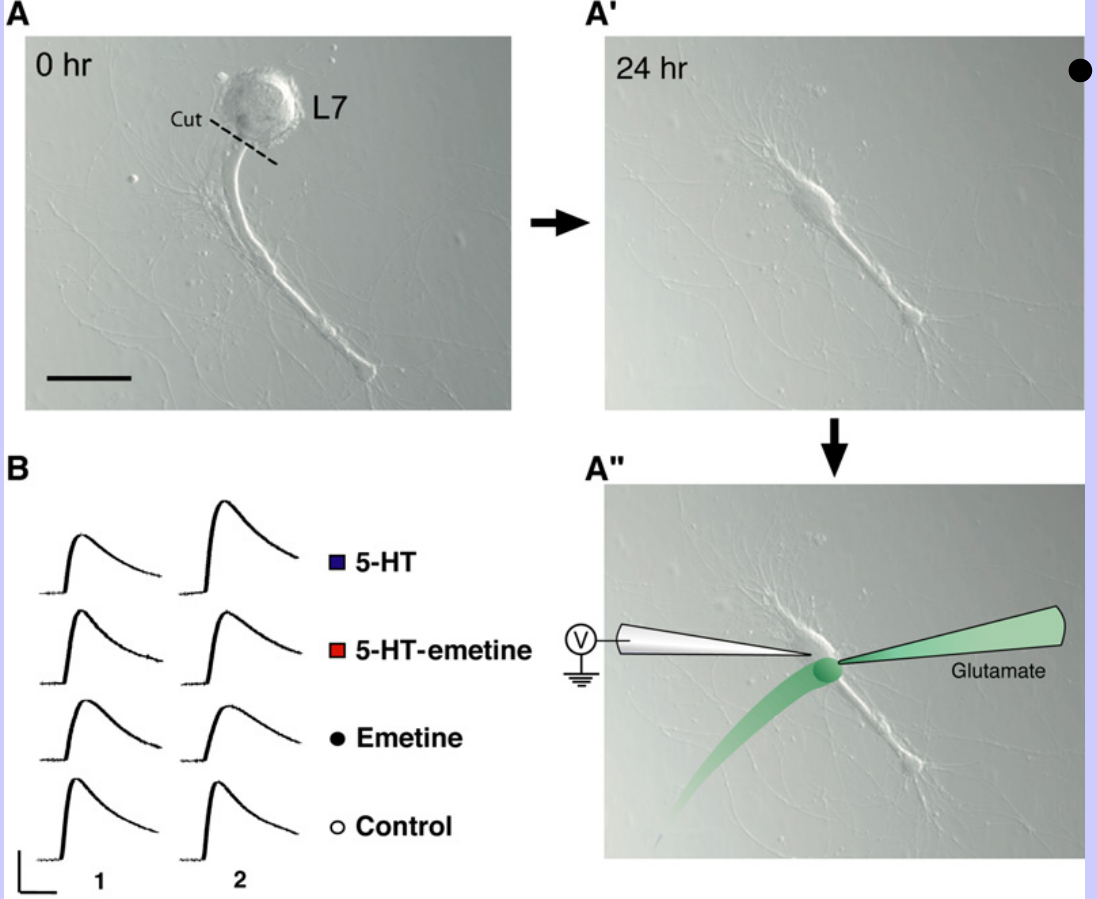


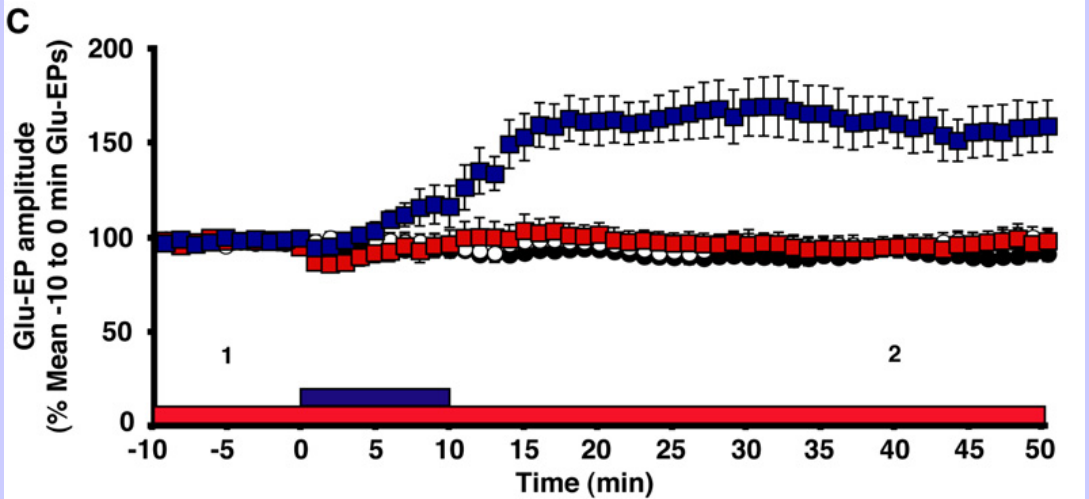




- # Rapid Protein Synthesis Is Required for Enhancement of the Glutamate Response in Motor Neurons

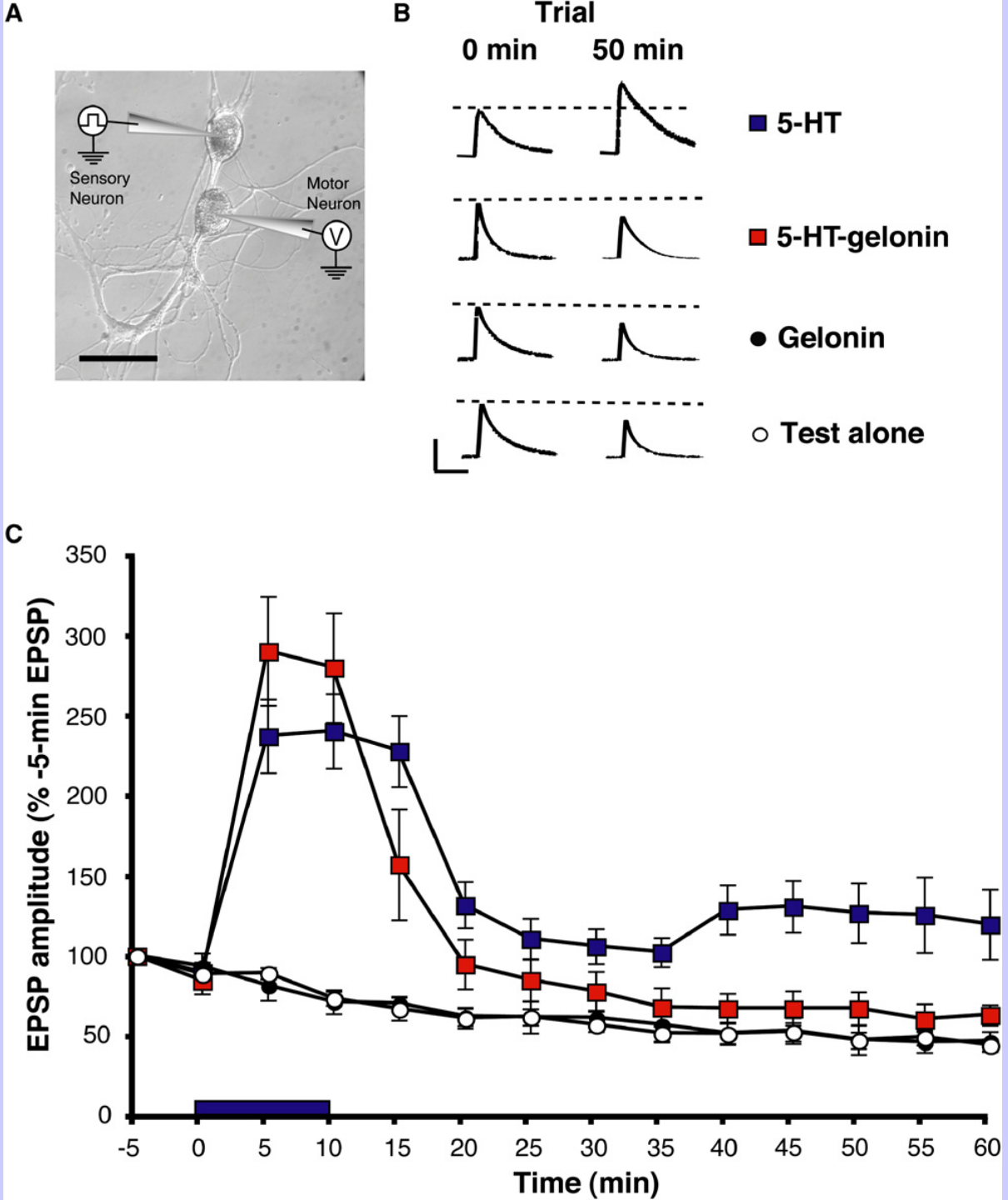


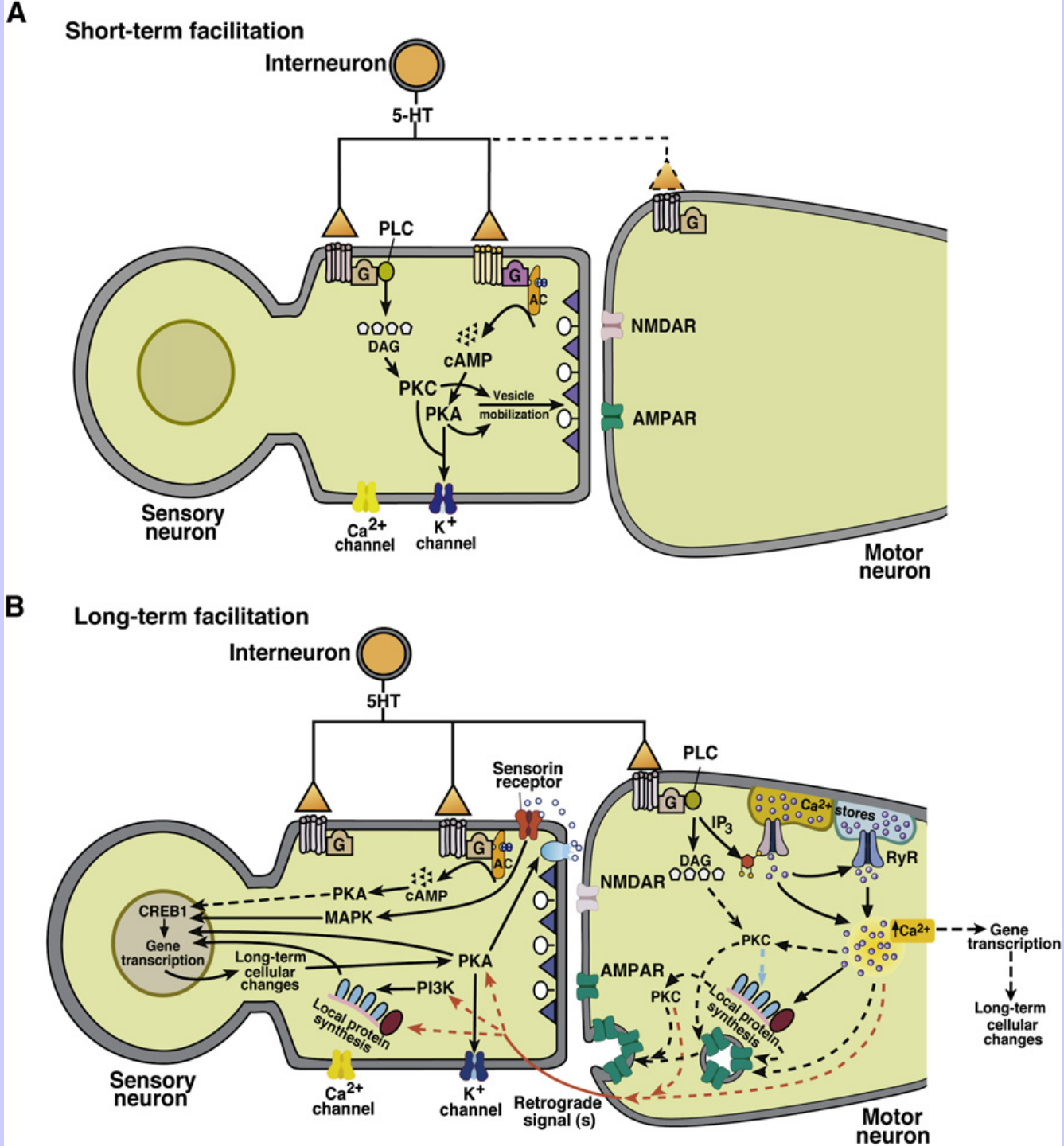




- Emetine Blocks Enhancement of the Glutamate-Evoked Response in a Surgically Isolated Motor Neurite

- Postsynaptic Inhibition of Protein Synthesis Blocks Persistent Synaptic Facilitation

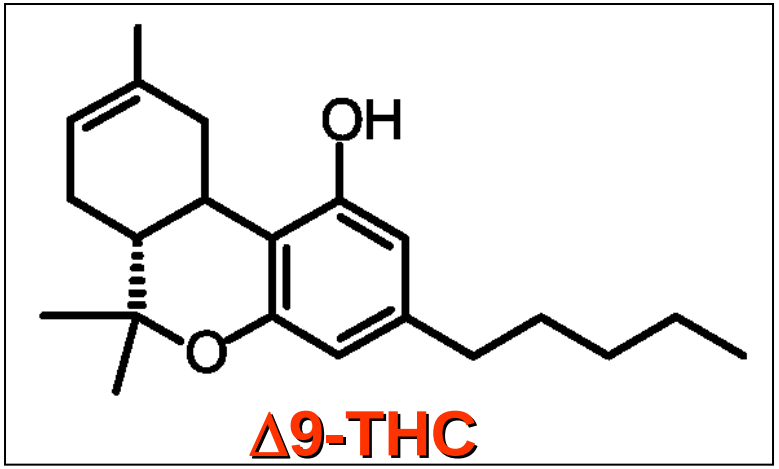




Cellular Models for Different Temporal Phases of Facilitation in Aplysia.

Cellular mechanisms of learning and memory have been highly conserved during evolution

Effects of Cannabinoids

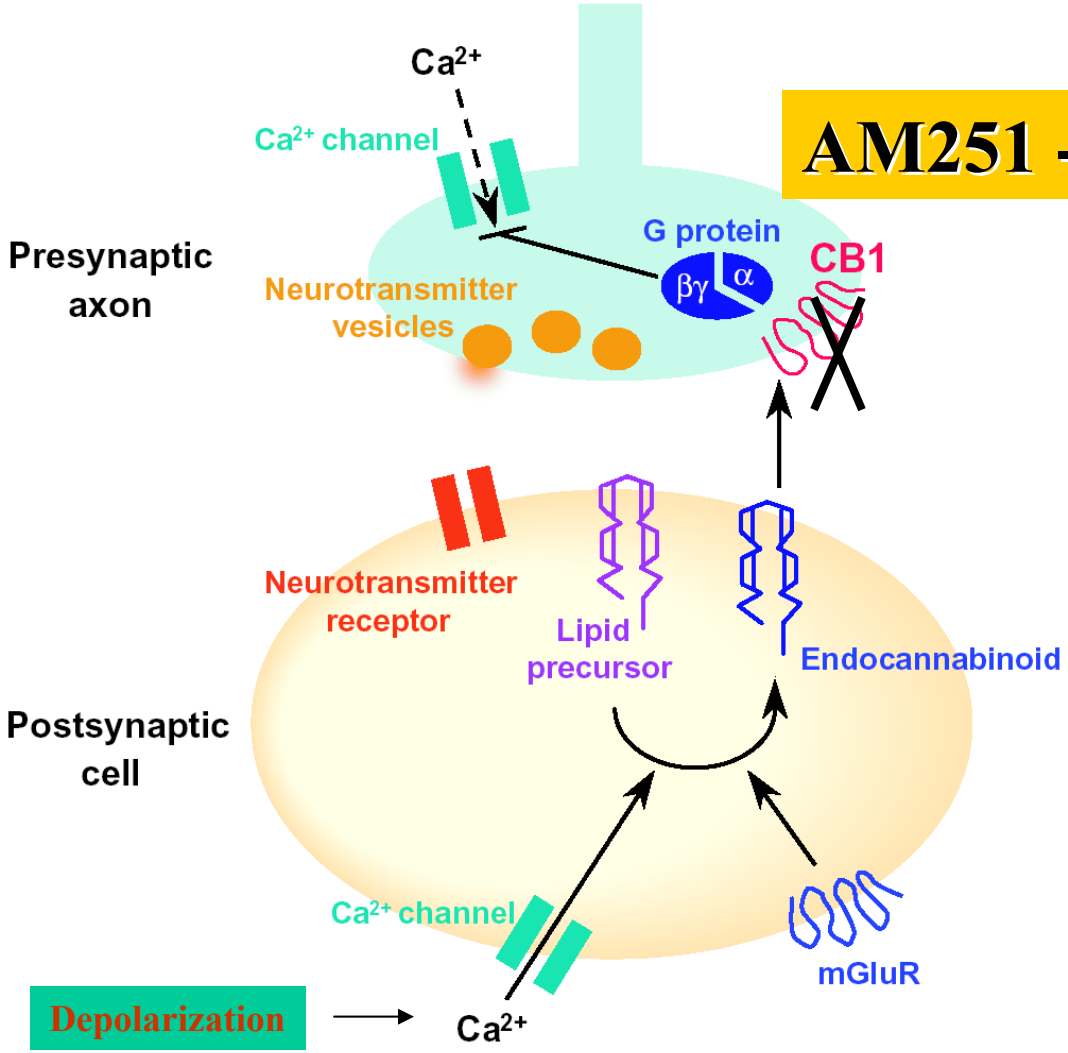


Δ9-THC

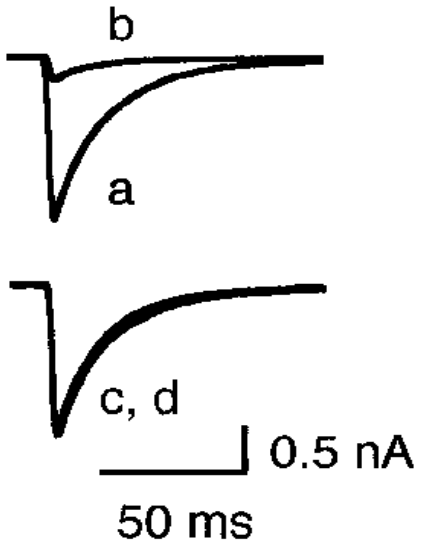
Δ9-Tetrahydrocannabinol (Δ9-THC)
the major psychoactive ingredient of *Cannabis sativa* (marijuana, hashish)

- Motor function
- Tremor, decreased body temperature
- Pain sensitivity
- Memory & cognition
- Euphoria

Ca^{2+}-induced retrograde signaling by endocannabinoids

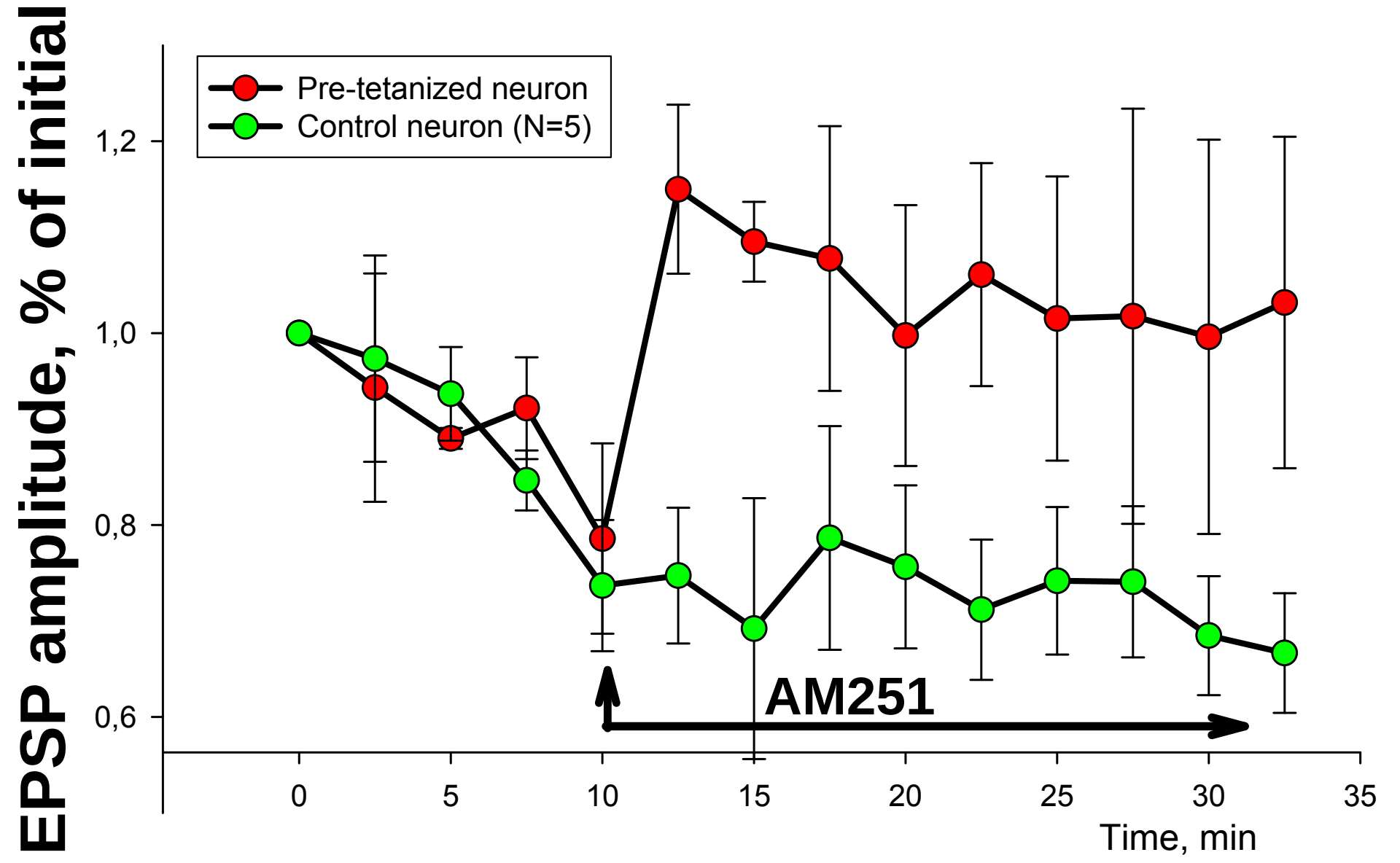


AM251 - Antagonist

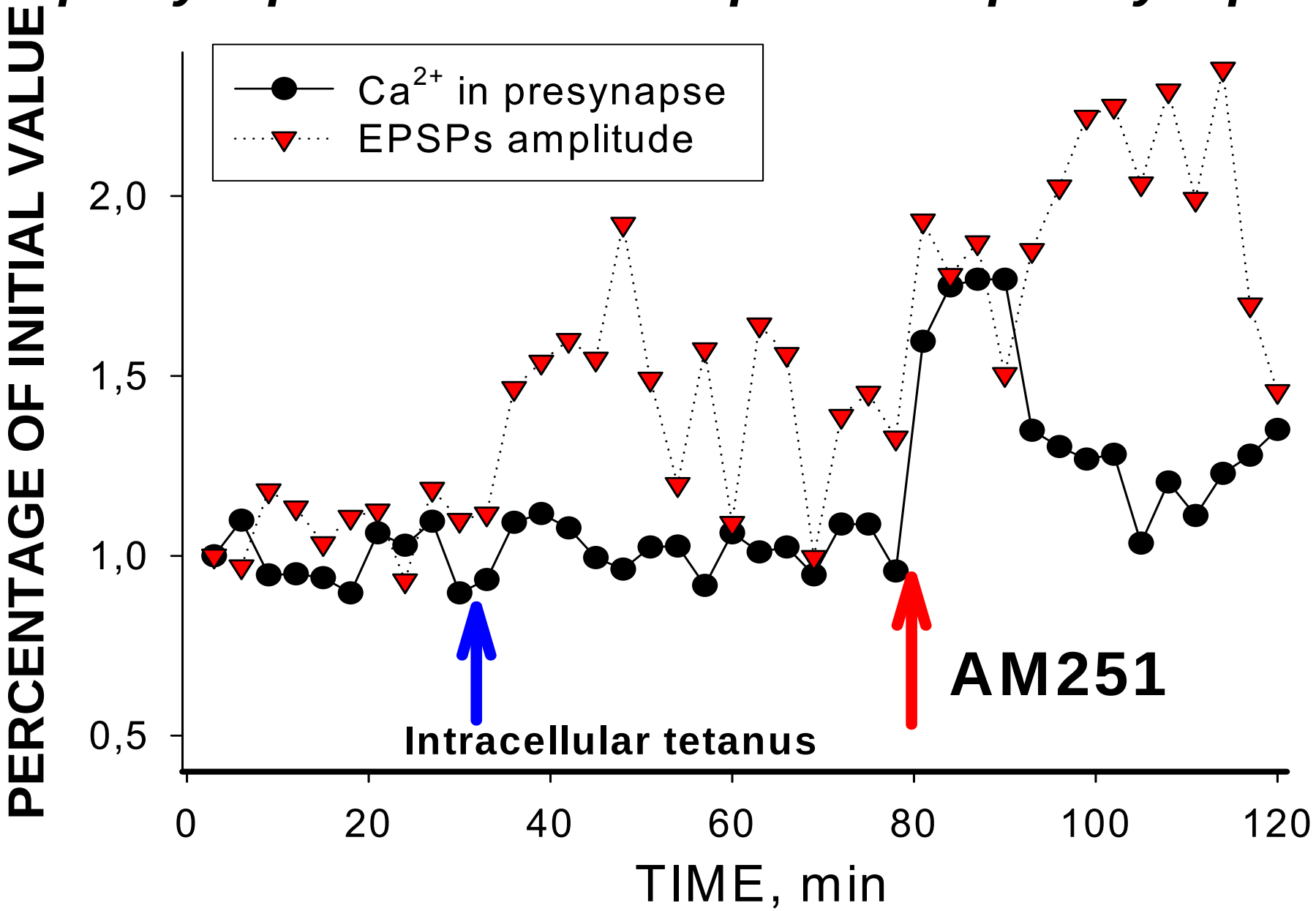


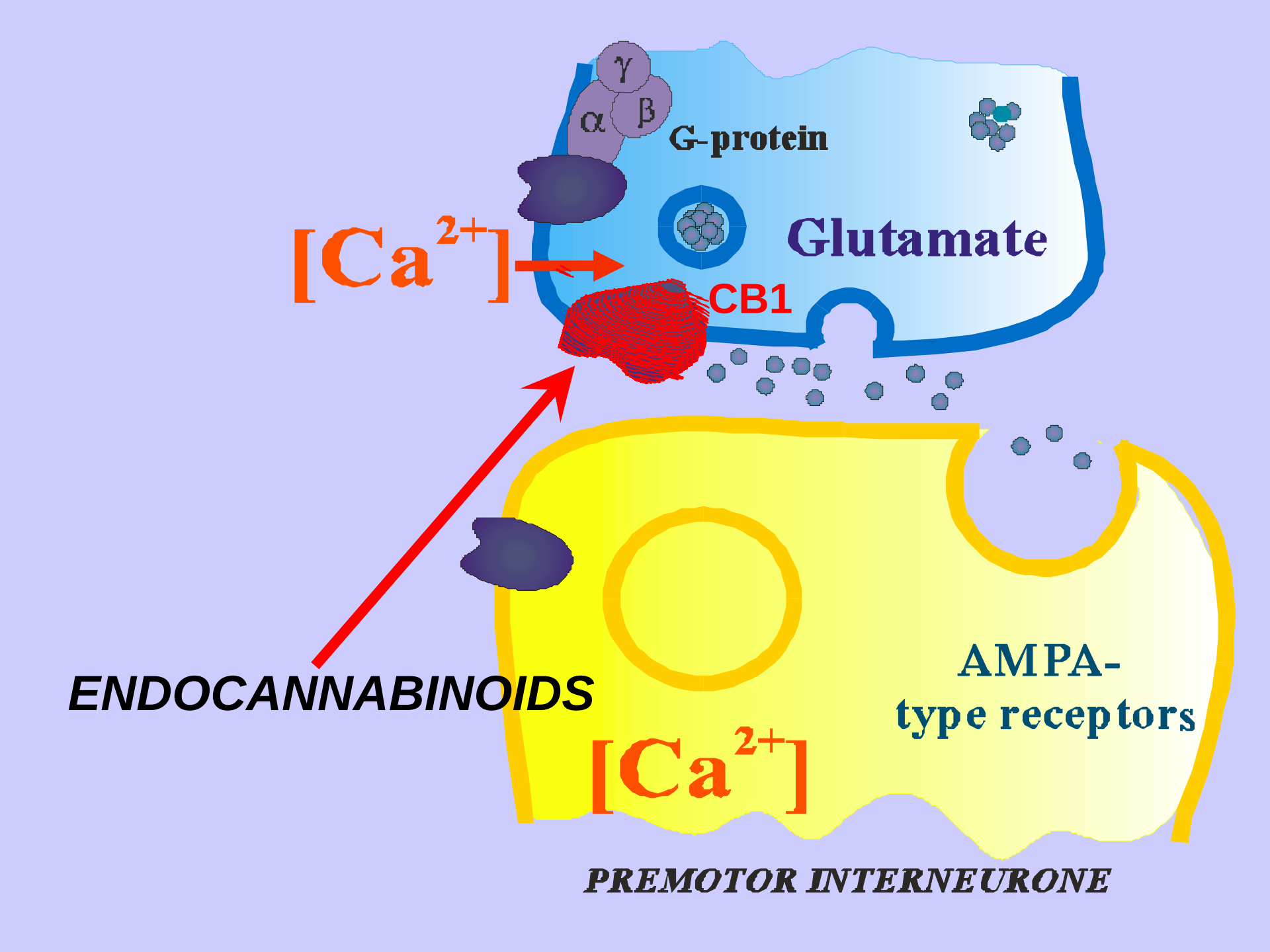
Wilson & Nicoll, 2002

EFFECT OF CB1 RECEPTOR BLOCKER AM251 ON EPSP AMPLITUDE IN CONTROL AND PRE-TETANIZED NEURONS

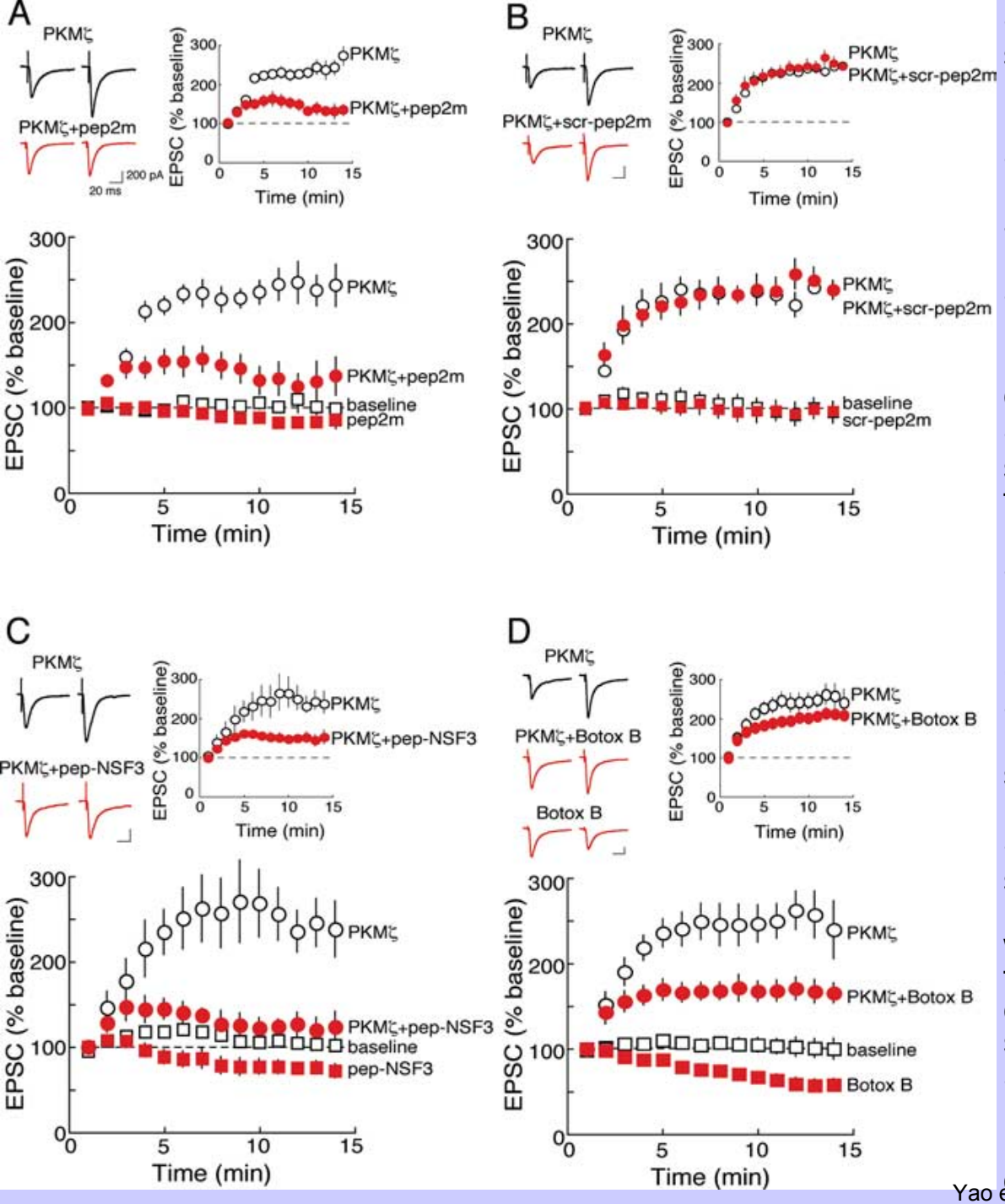


Simultaneous recording of Ca^{2+} concentration in presynapse and EPSP amplitude in postsynapse




γ
α
β
G-protein
[Ca2+]
Glutamate
CB1
ENDOCANNABINOIDS
AMPA-
type receptors
[Ca2+]
PREMOTOR INTERNEURONE

- Persistent phosphorylation by the atypical protein kinase C isoform PKMz was shown to be required for maintaining long-term potentiation (LTP) in hippocampus and for sustaining hippocampus-dependent spatial memory (Pastalkova et al. 2006).



•PKM enhances AMPAR-mediated synaptic transmission through NSF/GluR2 interactions. ***A***, Postsynaptic perfusion

•of PKM through a whole-cell recording pipette enhances AMPAR responses at Schaffer collateral/commissural-CA1 pyramidal

•cell synapses (black open circles); pep2m (100 M) together with PKM blocks AMPAR potentiation (red filled circles). pep2m

•alone has minimal effect on baseline synaptic transmission at 0.067 Hz (red filled squares) compared with baseline recordings

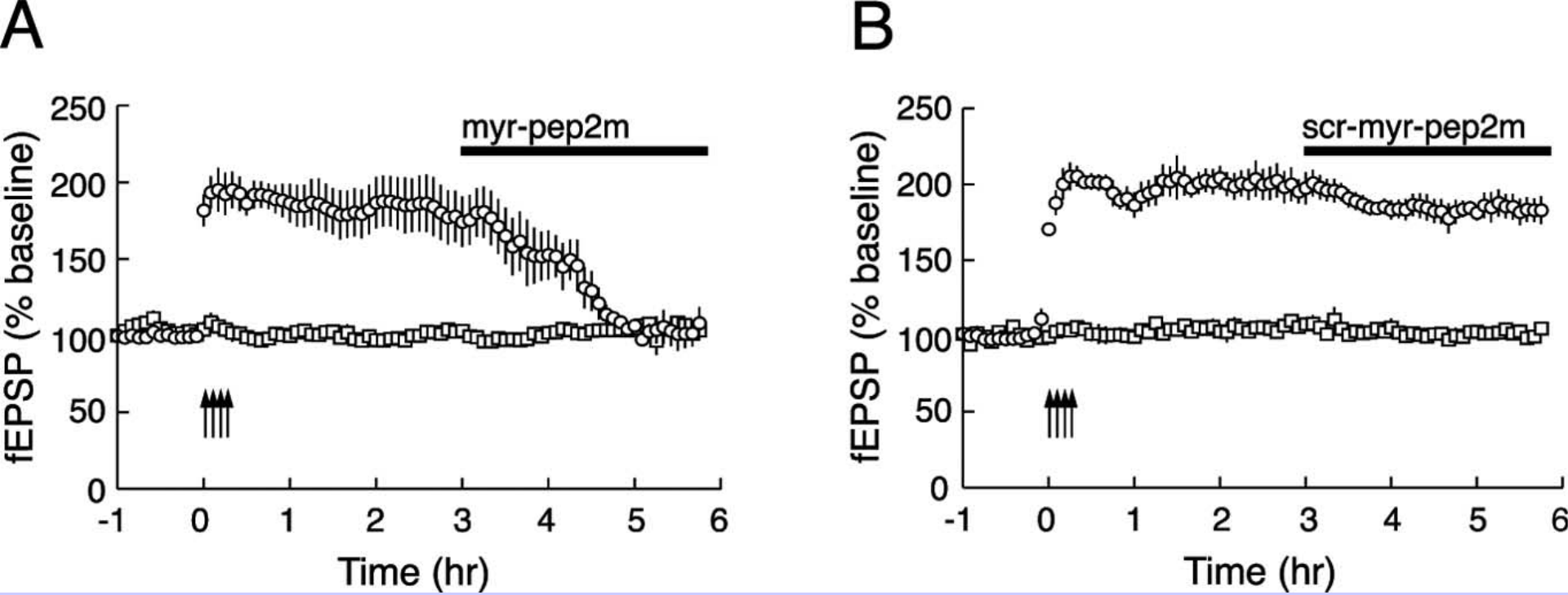
•without pep2m (black open squares). Left insets for all panels show representative traces recorded1 min (left) and13 min

•(right) after cell breakthrough. Right insets for all panels show the subtraction of baseline responses from responses with PKM

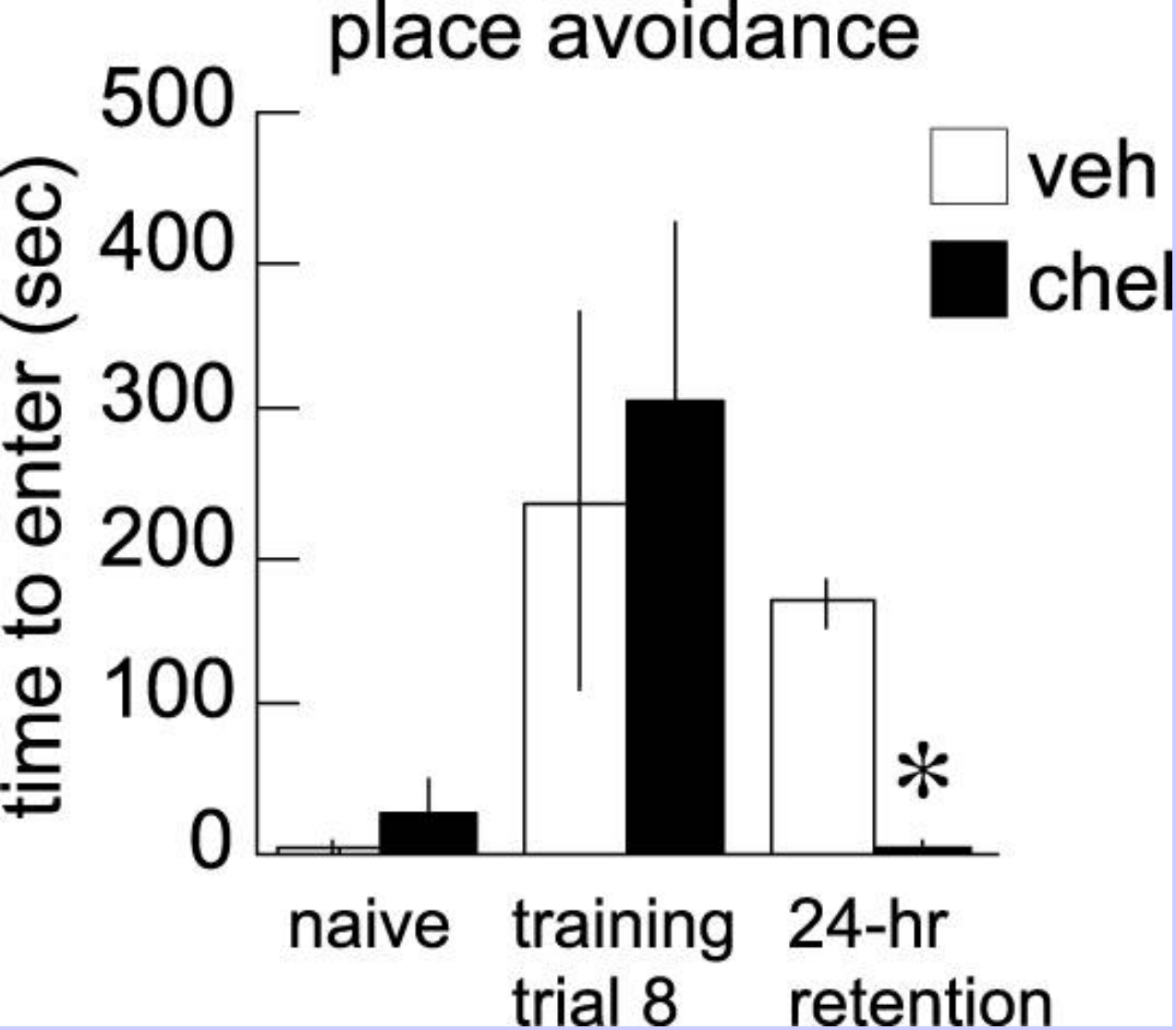
•alone (black open circles) and the subtraction of baseline responses in the presence of the agent from responses with PKM together with the agent (red filled circles). The number of experiments for each condition is five to six.

•***B***, Inactive scrambled version of pep2m(100 M) has no effect onPKM - mediated potentiation ofAMPARresponses (*n*4 –5).

Yao et al., The Journal of Neuroscience, July 30, 2008 • 28(31)

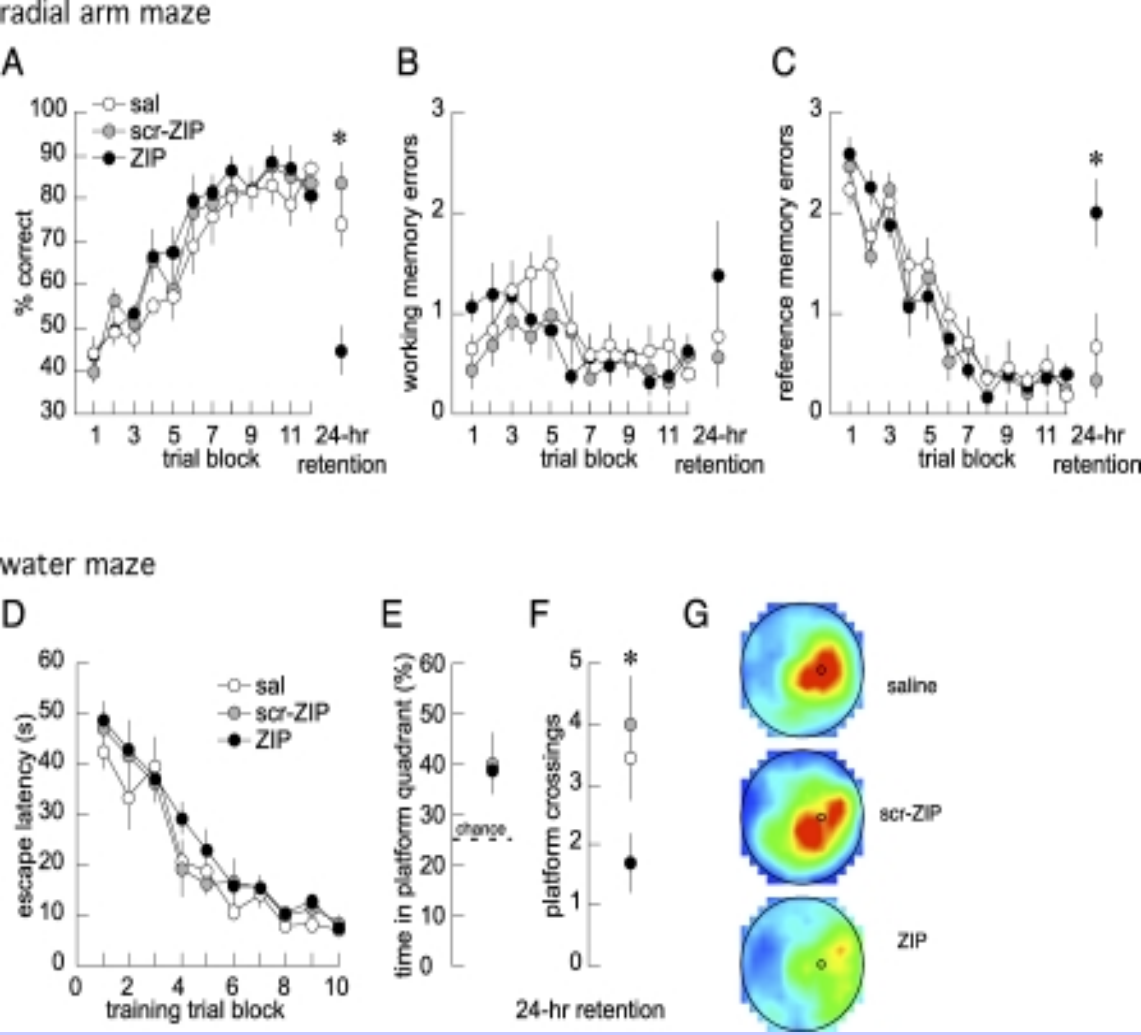


- NSF/GluR2 interactions mediate the persistence of late-LTP. ***A***, myr-pep2m (10 M) reverses late-LTP when applied
- 3 h after tetanic stimulation (open circles). The inhibitor has no effect on an independent pathway simultaneously recorded within
- each slice (open squares) (*n*4). ***B***, An inactive version of myr-pep2m (scr-myr-pep2m; 10 M) has no effect on



•Chelerythrine in DH Disrupts Place Avoidance Memory

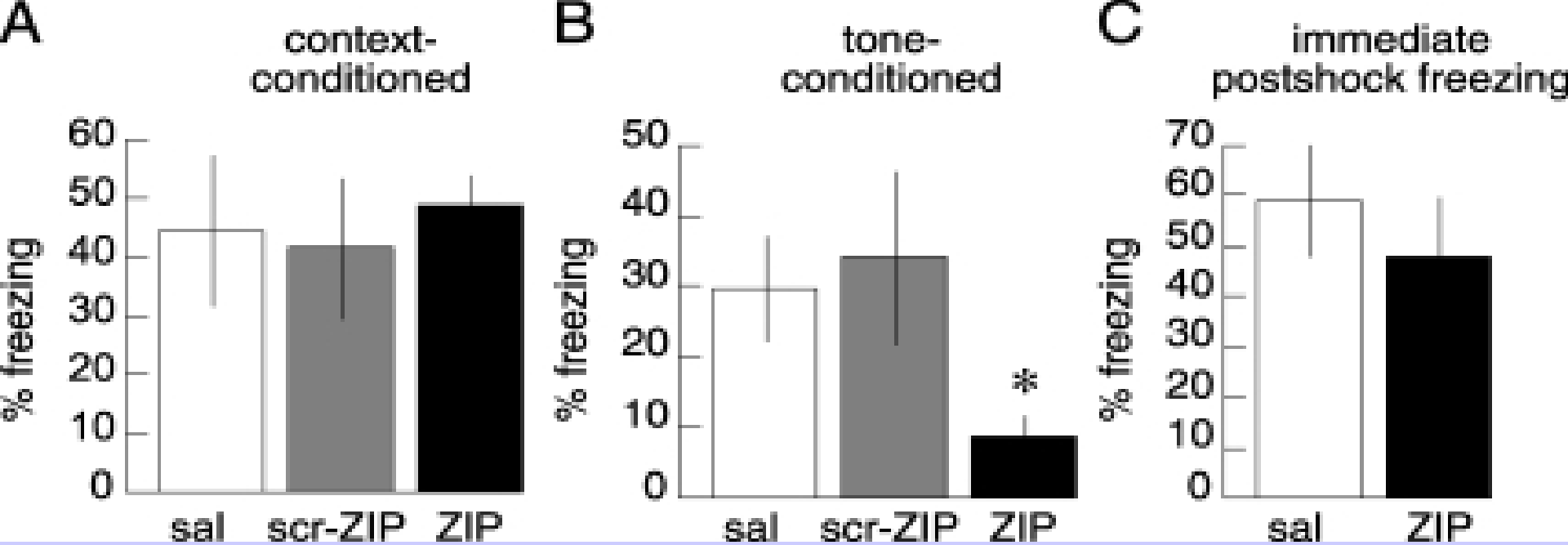
•Naive rats rapidly entered the shock zone on the first training trial but learned to avoid the location for several minutes by the eighth training trial. Chelerythrine (chel) or vehicle (veh) was injected in the DH 2 h before testing retention of 24-h memory. Chelerythrine, but not saline, eliminated retention of the memory, causing avoidance to drop to the level of when the rats were naive ($F_{2,21} = 14.2$; $p = 0.0001$; an asterisk (*) indicates $p \leq 0.05$



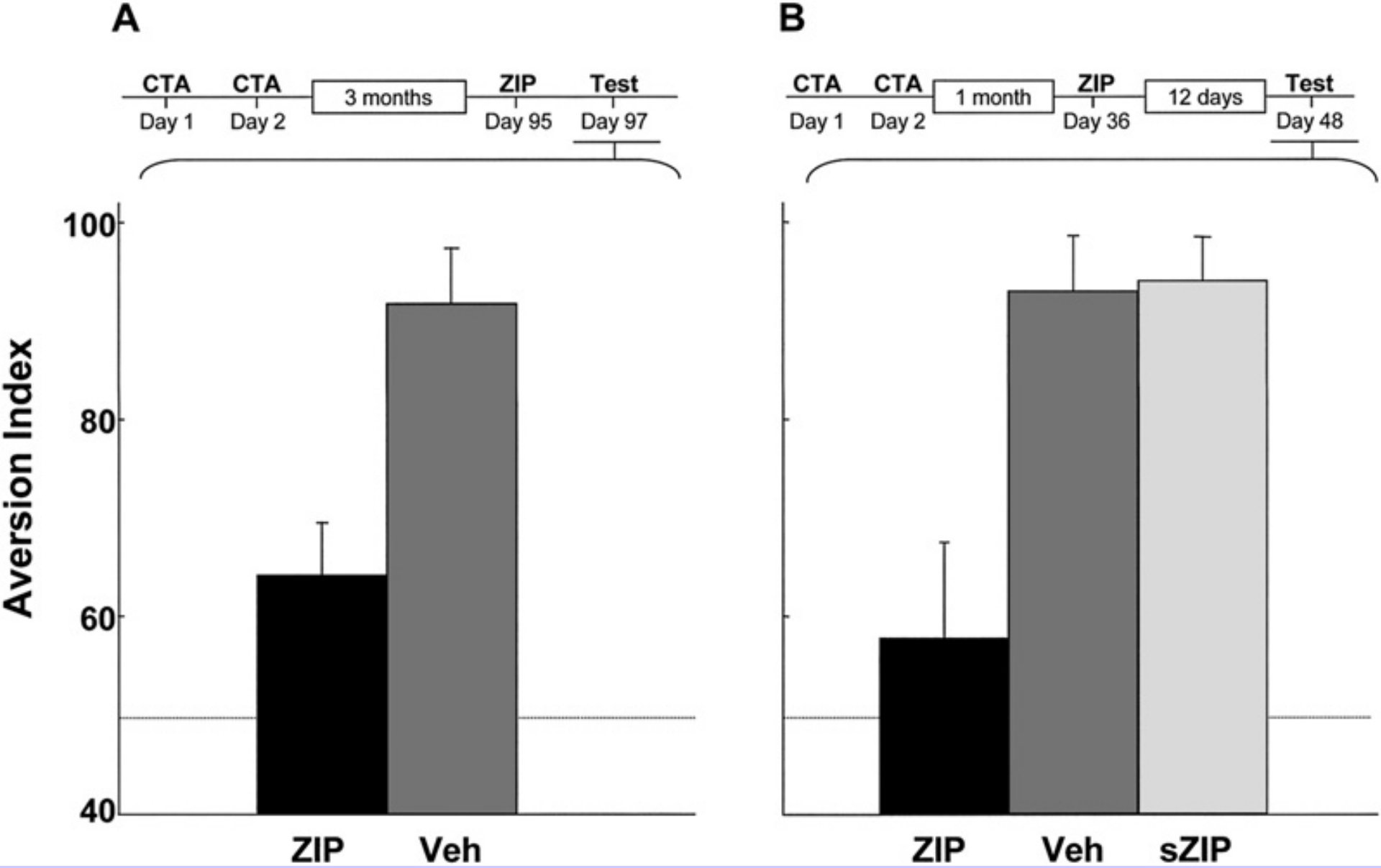
•ZIP in DH Disrupts Spatial Memory

•(A) Performance of the eight-arm radial maze task. Learning across 6 d (ten trials per day) was followed by a single retention trial after a 24-h interval. Two hours before the retention trial, each rat received a bilateral DH injection of either saline (sal, $n = 9$), the control peptide (scr-ZIP, $n = 9$), or ZIP ($n = 8$). The ZIP injection impaired overall performance ([A]; $F2,23 = 14.80$; $p = 10-5$) by increasing reference memory errors ([C]; $F2,23 = 9.30$; $p = 0.001$) without increasing working memory errors ([B]; $F2,23 = 1.16$; $p = 0.33$).

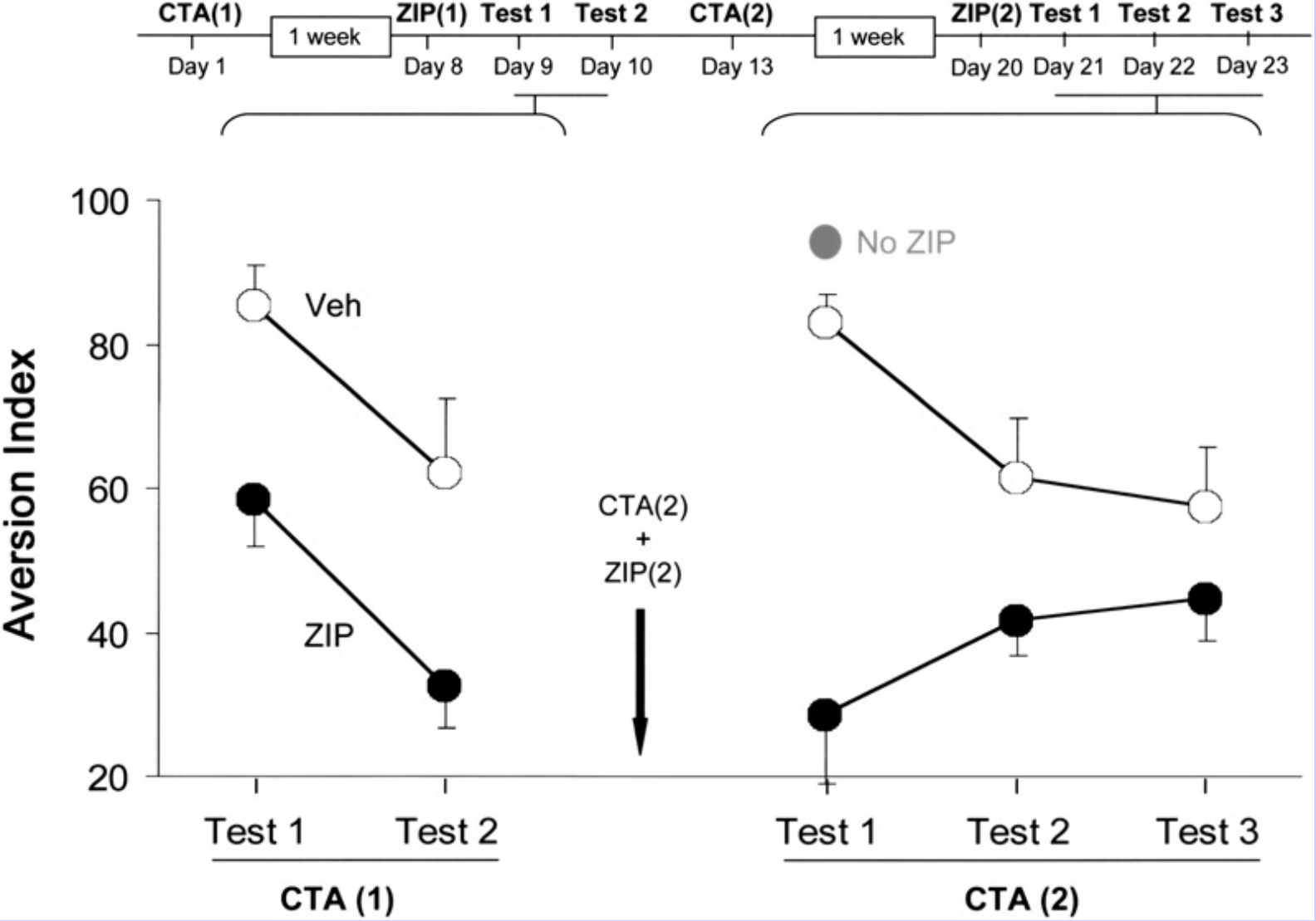
•(D–G) Performance of the water maze task (D) during training (two four-trial blocks per day) and (E–G) during the unreinforced swim retention test after a 24-h interval. Each rat received a bilateral DH infusion of saline ($n = 7$), scr-ZIP ($n = 7$), or ZIP ($n = 10$) 2 h before the retention test. (E) Percent time in the target quadrant, (F) number of times the position of the escape platform was crossed, and (G) the color-coded time-in-location map for each treatment group during the



- ZIP in BLA Disrupts Classically Conditioned Fear Memory
- (A) Retention of context-conditioned fear 26 h after bilateral DH injection of saline (sal, $n = 4$), inactive (scr-ZIP, $n = 7$), or active ZIP ($n = 6$). ZIP did not impair retention of contextual fear ($F2,14 = 0.15$; $p = 0.86$).
- (B) Retention of tone-conditioned fear after 22-h posttraining bilateral BLA injections. Retention was tested 2 h (sal, $n = 6$; scr-ZIP $n = 3$; ZIP $n = 10$) or 24 h (sal, $n = 5$; scr-ZIP $n = 4$; ZIP $n = 8$) after the injection. ZIP impaired retention of tone-conditioned fear ($F2,33 = 4.93$; $p = 0.01$).
- (C) Immediate postshock freezing after bilateral BLA

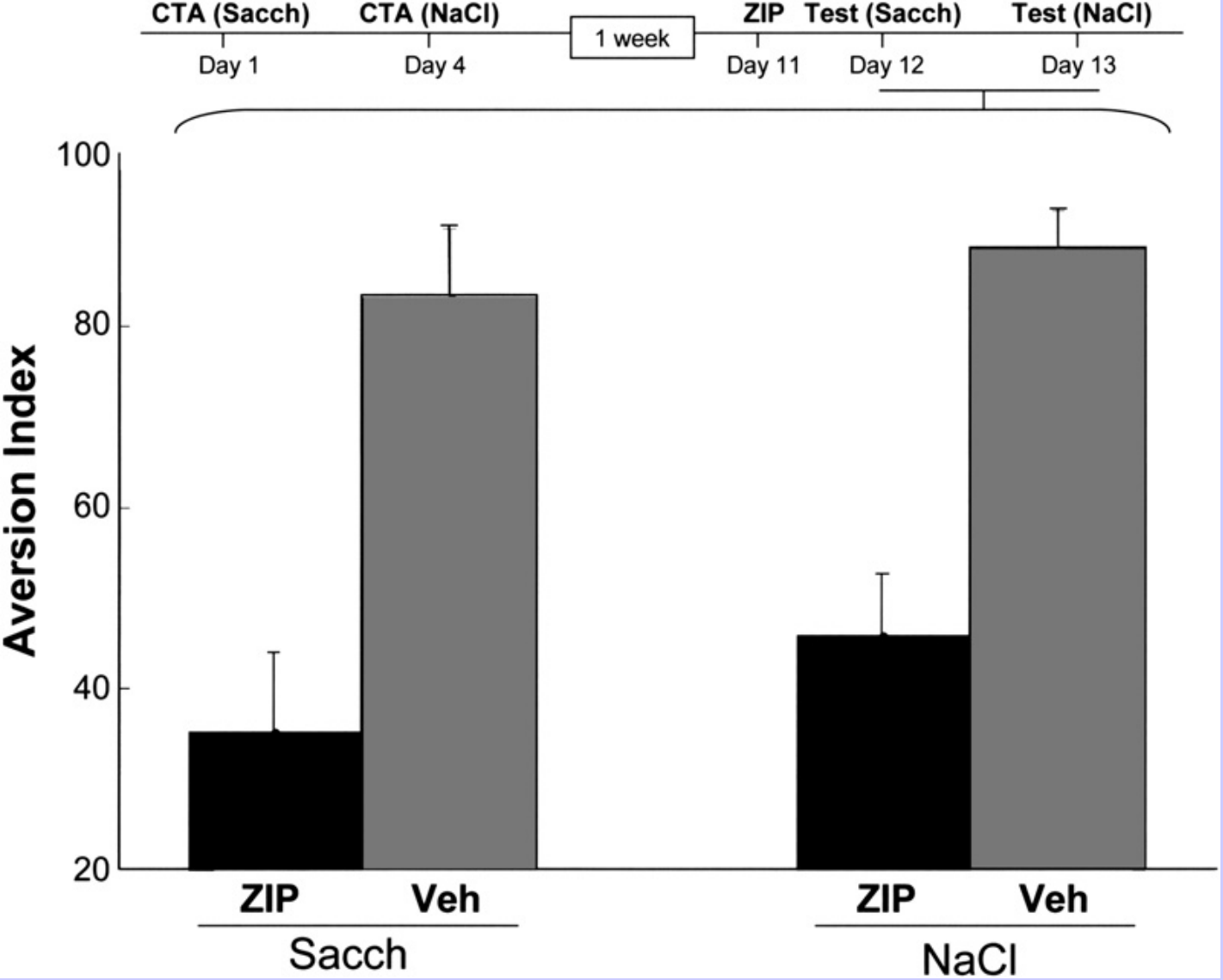


•Effect of ZIP on very long-term CTA memory in the insular cortex. (A) ZIP/vehicle were
•administered 3 mo after training, and memory was tested 2 d later. The dashed line indicates equal
•preference for the CS and water, i.e., AI = 50. (B) ZIP/vehicle/scrambled ZIP were administered 1 mo



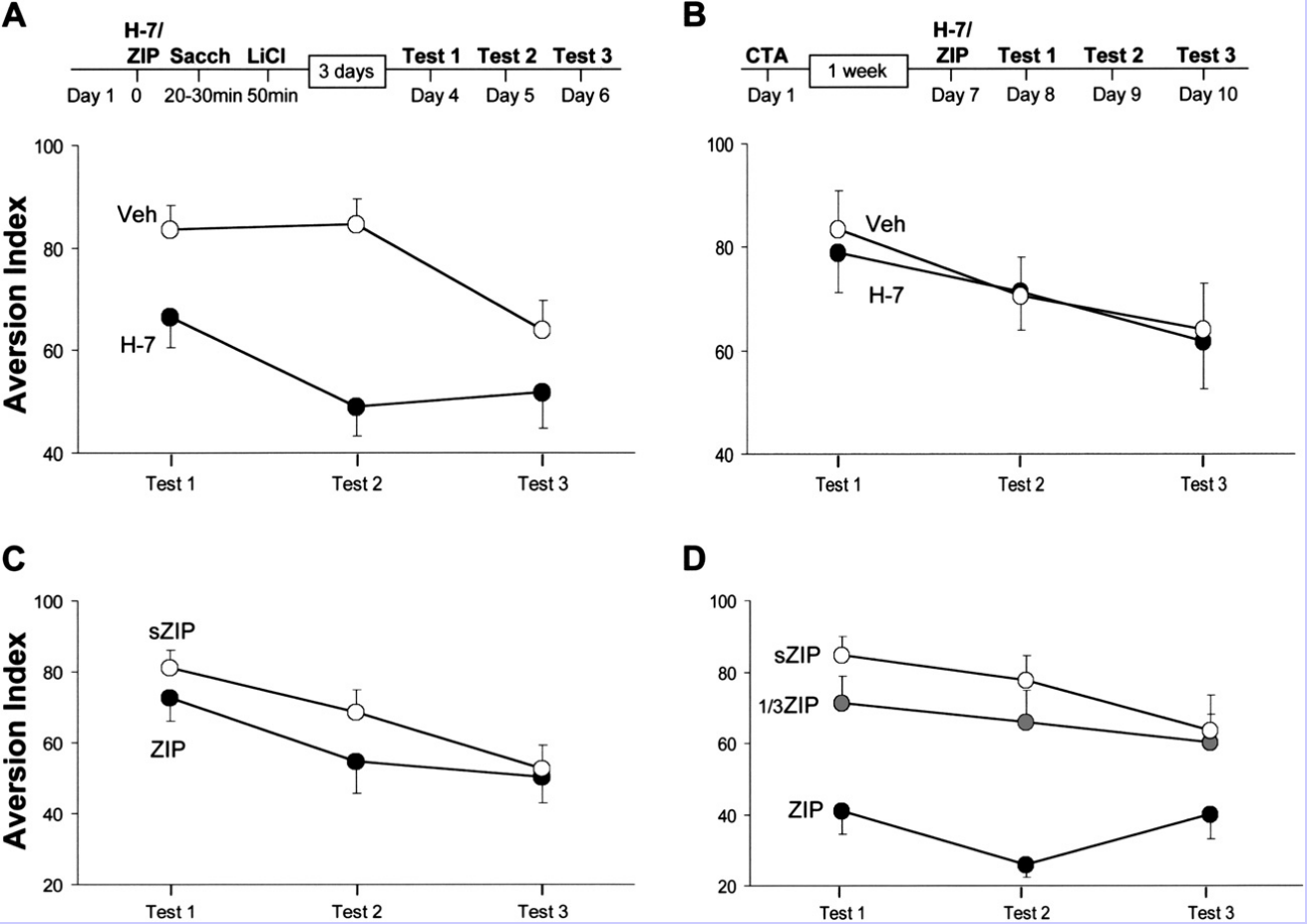
Once erased and reacquired, CTA can be erased again by ZIP in the IC

•Memory erased by ZIP can be reacquired and re-erased in cortex. ZIP/vehicle were
•administrated a week after CTA training (CS = saccharin), followed by two tests. ZIP blocked the
•memory. Three days later, rats underwent CTA training again to the same CS, after which the control
•and ZIP groups were reinjected with vehicle and ZIP, respectively; two rats from the ZIP group were not
•reinjected (denoted as No ZIP). When tested a day later, the Vehicle and No ZIP groups had



A single application of ZIP abolishes long-term memory of multiple associations involving different taste qualities

A single application of ZIP can abolish multiple associations of different taste qualities. Rats were trained on CTA using saccharin as CS, and 3 d later to CTA using NaCl as CS. One week later, the groups were microinfused into the IC with either ZIP or vehicle. Rats were tested on saccharin and NaCl, 1 and 2 d later, respectively.



Unlike ZIP, the serine/threonine protein kinase inhibitor H-7 disrupts learning but has no effect on long-term memory once established

- Double-dissociation of the effect of ZIP and of a general serine/threonine kinase inhibitor relatively ineffective toward PKMz on learning and memory of CTA.

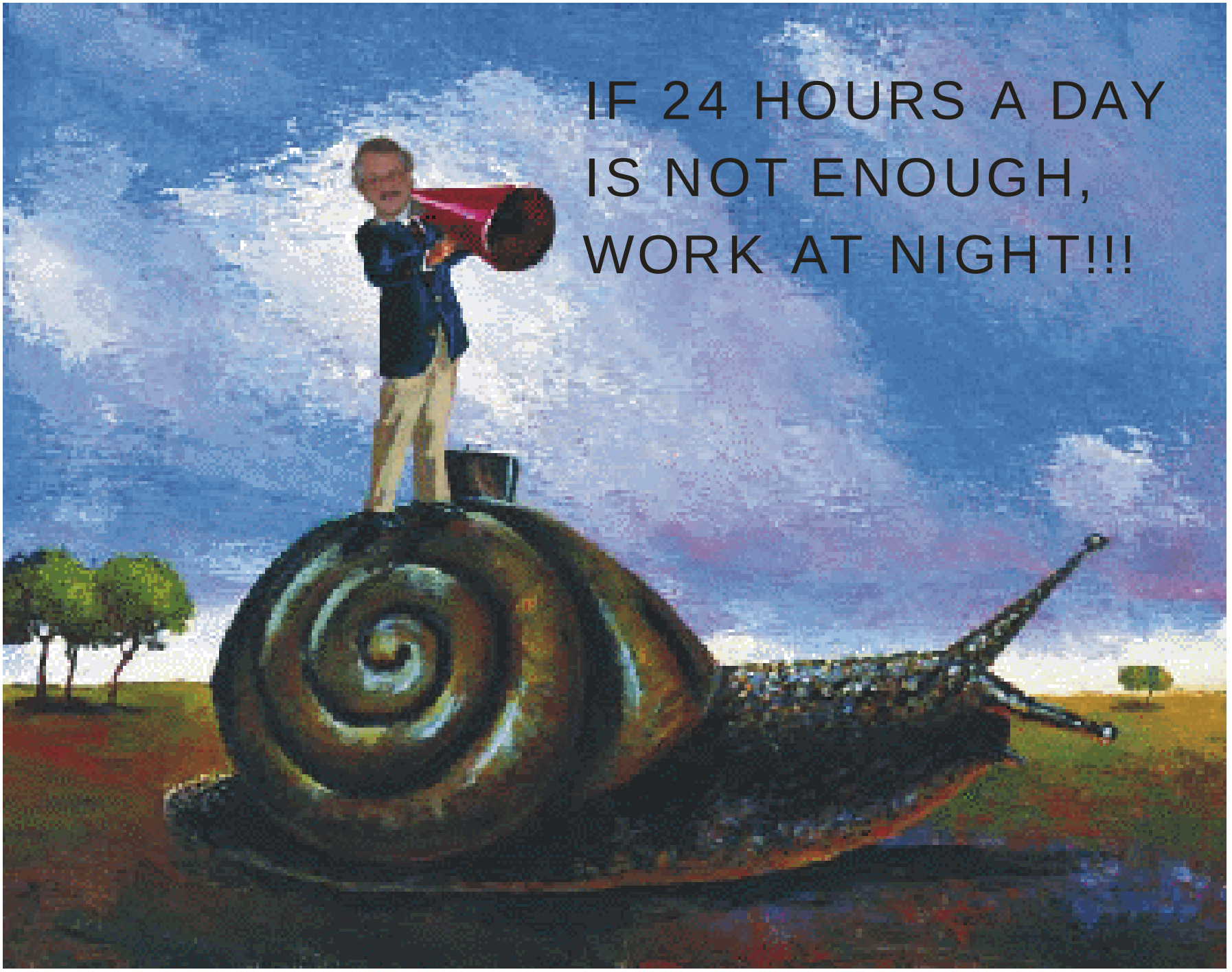
(A) Microinfusion of the serine/threonine protein kinase inhibitor H-7 into the IC 20 min before exposure to taste in CTA training. Memory was tested 3 d later.

(B) (B) Microinfusion of H-7 1 wk after CTA training. Memory was tested a day later.

(C) (C) Microinfusion of ZIP/sZIP 20 min before learning, under the same conditions in which H-7 was administrated in C.

(D) (D) Microinfusion into the IC of different concentrations of ZIP (3.3 or 10 nmol/mL, denoted 1/3ZIP and ZIP, respectively) and of sZIP (10 nmol/mL), a week after CTA training, under the same conditions in which H-7 was administrated in B. As can be

- Results suggest that the cellular mechanism targeted by ZIP consolidates within hours to a few days, but once this happens, the memory trace does not seem to consolidate further to lose this sensitivity to the amnesic agent. In other words, at least up to a few months after encoding, PKMz remains a critical component of the machinery that keeps memory going in cortex.

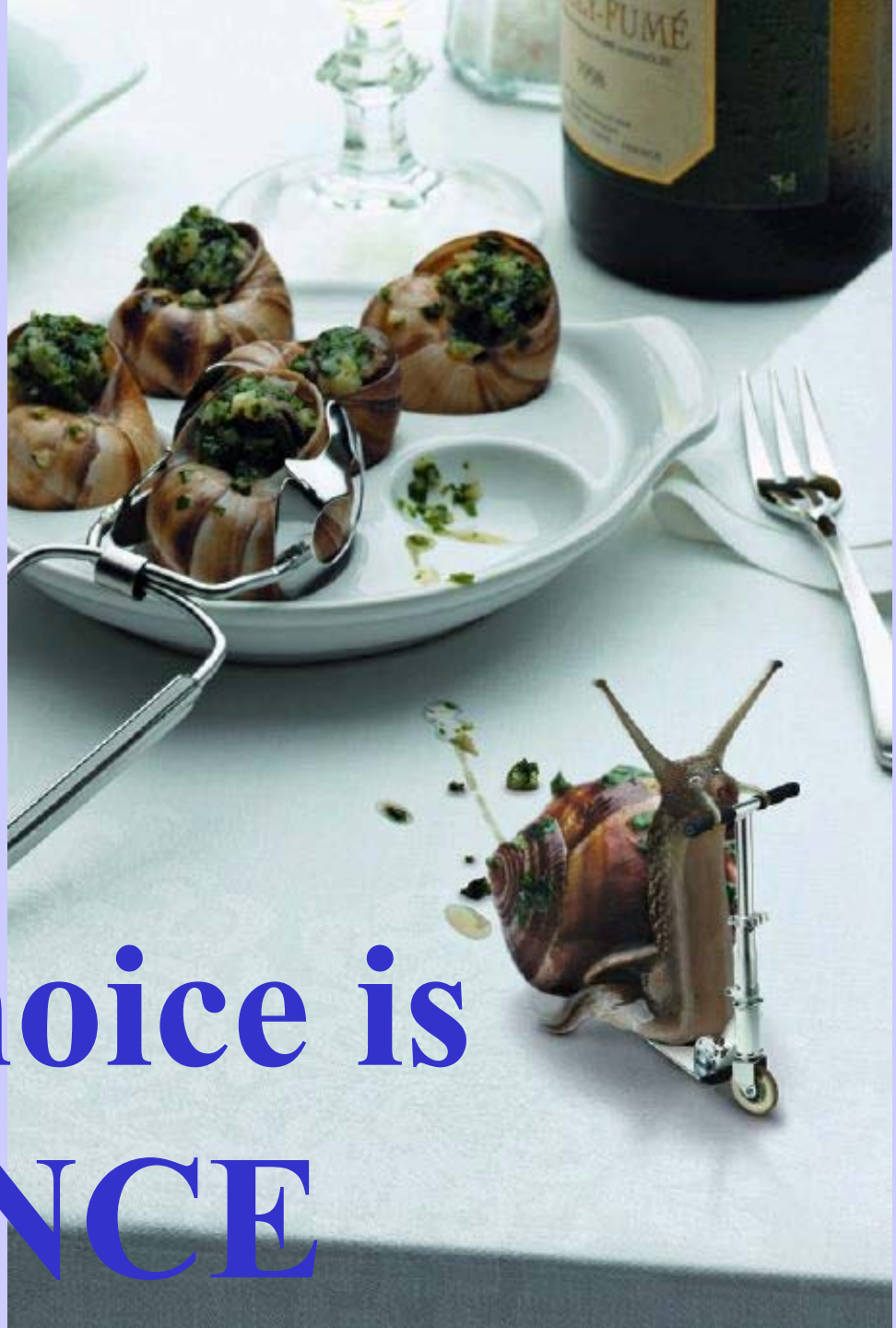

IF 24 HOURS A DAY
IS NOT ENOUGH,
WORK AT NIGHT!!!

CONCEPTS IN NEUROBIOLOGY

- It is impossible to do Science without concepts
- It is impossible to make a breakthrough in Science if the Concept is not broken

Recipe from Russian cuisine

- Regard concepts as disposable element
- Operate only with experimental data
- If the concept belongs to your boss - relax, and let the boss to have fun
- If you like conceptual thinking, or it is obligatory, go to the extreme



My choice is SCIENCE