UAMS
UNIVERSITY OF ARKANSAS FOR MEDICAL SCIENCES

Department of Neurobiology and Developmental Sciences
Center for Translational Neuroscience
College of Medicine

Neuronal Signals - NBDS 5161
Session 4: Analyzing Synaptic Activity, Membrane and Synaptic Currents

Abdallah HAYAR

Lectures can be downloaded from
http://hayar.net/NBDS5161

Updated Tentative Schedule for Neuronal Signals (NBDS 5161)
One Credit–Hour, Summer 2010
Location: Biomedical Research Building II, 6th floor, conference room,
Time: 9:00 -10:20 am

Session	Day	Date	Topic	Instructor
1	Tue	6/1	Design of an electrophysiology setup	Hayar
2	Thu	6/3	Neural population recordings	Hayar
3	Thu	6/10	Single cell recordings	Hayar
4	Fri	6/11	Analyzing synaptic activity	Hayar
5	Mon	6/14	Data acquisition and analysis	Hayar
6	Wed	6/16	Analyzing and plotting data using OriginLab	Hayar
7	Fri	6/18	Detecting electrophysiological events	Hayar
8	Mon	6/21	Writing algorithms in OriginLab®	Hayar
9	Wed	6/23	Imaging neuronal activity	Hayar
10	Fri	6/25	Laboratory demonstration of an electrophysiology and imaging experiment	Hayar
11	Fri	7/9	Article presentation I: Electrophysiology	Hayar
12	Mon	7/12	Article presentation II: Imaging	Hayar
13	Wed	7/14	Exam and students' survey about the course	Hayar

Student List

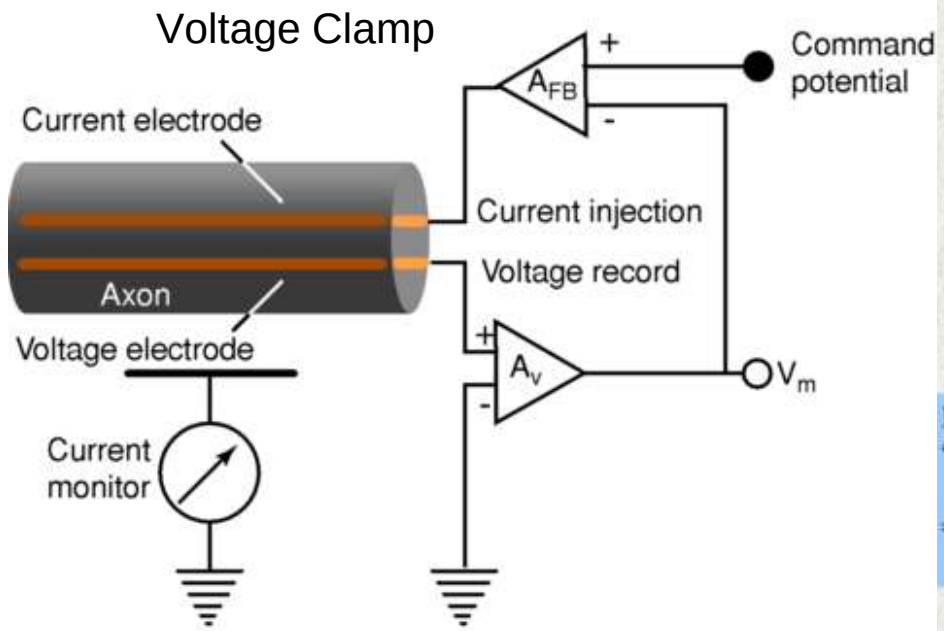
	Name	E-mail	Regular/Auditor	Department	Position
1	Simon, Christen	CSimon@uams.edu	Regular (form signed)	Neurobiology & Developmental Sciences	Graduate Neurobiology – Mentor: Dr. Garcia-Rill
2	Kezunovic, Nebojsa	NKezunovic@uams.edu	Regular (form signed)	Neurobiology & Developmental Sciences	Graduate Neurobiology – Mentor: Dr. Garcia-Rill
3	Hyde, James R	JRHyde@uams.edu	Regular (form signed)	Neurobiology & Developmental Sciences	Graduate Neurobiology – Mentor: Dr. Garcia-Rill
4	Yadlapalli, Krishnapraveen	KYadlapalli@uams.edu	Regular (form signed)	Pediatrics	Research Technologist – Mentor: Dr. Alchaer
5	Pathan, Asif	APATHAN@uams.edu	Regular (form signed)	Pharmacology & Toxicology	Graduate Pharmacology – Mentor: Dr. Rusch
6	Kharade, Sujay	SKHARADE@uams.edu	Regular (form signed)	Pharmacology & Toxicology	Graduate Pharmacology – 4th year - Mentor: Dr. Rusch
7	Howell, Matthew	MHOWELL2@uams.edu	Regular (form signed)	Pharmacology & Toxicology	Graduate Interdisciplinary Toxicology - 3rd year - Mentor: Dr. Gottschall
8	Beck, Paige B	PBBeck@uams.edu	Regular (form signed)	College of Medicine	Medical Student – 2nd Year - Mentor: Dr. Garcia-Rill
9	Atcherson, Samuel R	SRAtcherson@uams.edu	Auditor (form signed)	Audiology & Speech Pathology	Assistant Professor
10	Detweiler, Neil D	NDDETWEILER@uams.edu	Auditor (form not signed)	Pharmacology & Toxicology	Graduate Pharmacology – 1st year
11	Thakali, Keshari M	KMThakali@uams.edu	Unofficial auditor	Pharmacology & Toxicology	Postdoctoral Fellow – Mentor: Dr. Rusch
12	Boursoulian, Feras	FBoursoulian@uams.edu	Unofficial auditor	Neurobiology & Developmental Sciences	Postdoctoral Fellow – Mentor: Dr. Hayar
13	Steele, James S	JSSTEELE@uams.edu	Unofficial auditor	College of Medicine	Medical Student – 1st Year – Mentor: Dr. Hayar
14	Smith, Kristen M	KMSmith2@uams.edu	Unofficial auditor	Neurobiology & Developmental Sciences	Research Technologist – Mentor: Dr. Garcia-Rill
15	Gruenwald, Konstantin	kjoachimg@gmail.com	Unofficial auditor	Neurobiology & Developmental Sciences	High school Student – Mentor: Dr. Hayar
	Yang, Dong	YangDong@uams.edu	Unable to attend	Pediatrics Pulmonary	Research Assistant – Accepted in Neuroscience

Why study voltage clamping?

- Historical: This is the method invented by Hodgkin and Huxley to discover the voltage-dependent behavior of sodium and potassium currents.

- Factual: To understand the voltage and time dependence of sodium and potassium currents underlying the action potential.

- Methodological:
  - The same method, in principle, is still used to study many other types of membrane currents (calcium currents, chloride currents, pump currents, etc.)
  - The same method is used to study the currents that go through single ion channels.



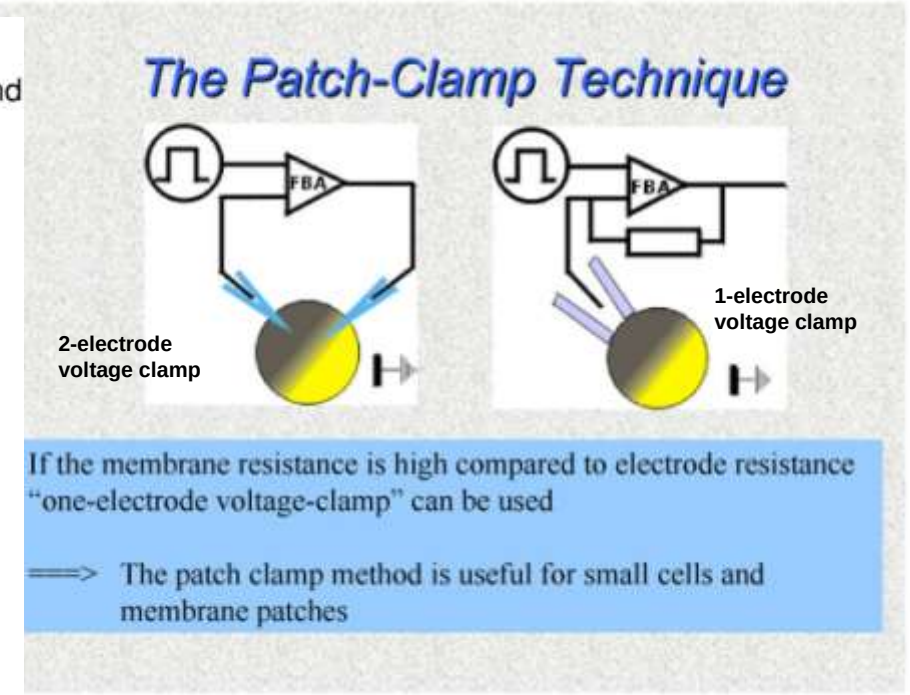
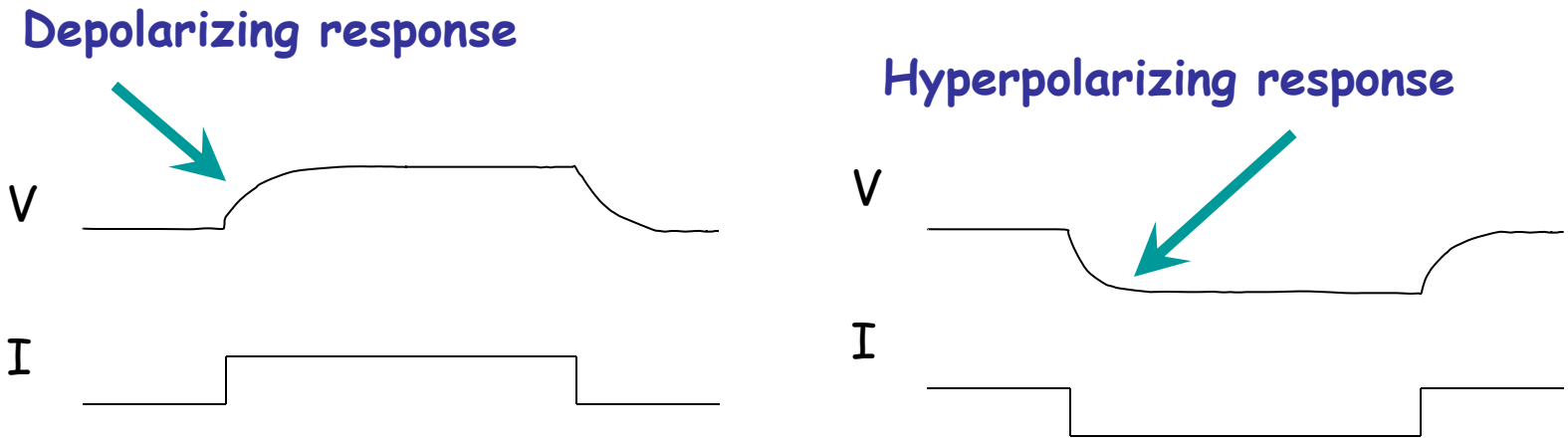


Fig. 5. The voltage-clamp technique keeps the voltage across the membrane constant so that the amplitude and time course of ionic currents can be measured. In the two-electrode voltage-clamp technique, one electrode measures the voltage across the membrane while the other injects current into the cell to keep the voltage constant. The experimenter sets a voltage to which the axon or neuron is to be stepped (the command potential). Current is then injected into the cell in proportion to the difference between the present membrane potential and the command potential. This feedback cycle occurs continuously, thereby clamping the membrane potential to the command potential. By measuring the amount of current injected, the experimenter can determine the amplitude and time course of the ionic currents flowing across the membrane.

Voltage clamping: 3 principles

(1) Injecting positive current into the cell depolarizes the cell (injecting negative current hyperpolarizes it).



(2) When current is injected into the cell, it takes some time to hyperpolarize/depolarize the cell because the cell's capacitance must be charged/discharged.

(3) When there is no net flow of ions into the cell, the membrane potential doesn't change.

Voltage Clamp

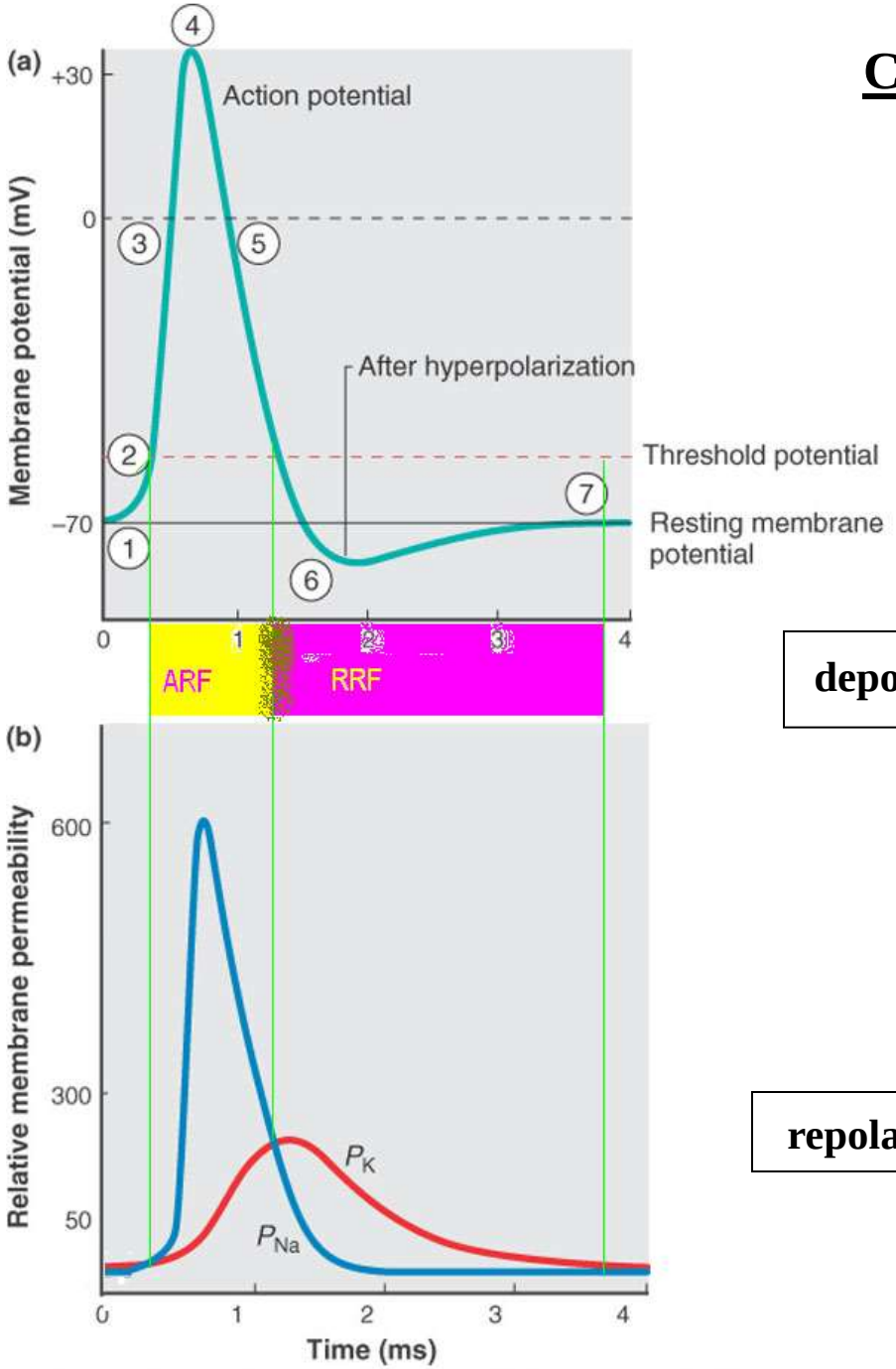
The voltage clamp is used by electrophysiologists to measure the ion currents across a neuronal membrane while holding the membrane voltage at a set level. Neuronal membranes contain many different kinds of ion channels, some of which are voltage gated. The voltage clamp allows the membrane voltage to be manipulated independently of the ionic currents, allowing the current-voltage relationships of membrane channels to be studied

- Measure V_M = Inside - Outside
- Choose Clamp potential (V_C)
- Calculate $V_C - V_M$
- Inject current = $\gamma (V_C - V_M)$

- If $V_C > V_M$, current is positive
  - Membrane potential increases
  - $V_C - V_M$ decreases
- If $V_C < V_M$, current is negative
  - Membrane potential decreases
  - $V_C - V_M$ decreases

- Inward current
  - Negative current injected to maintain membrane potential.
  - Negative current is compensating inward flow of positive ions
  - Transient current
- Outward current
  - Positive current injected to compensate for outward flow of positive ions
  - Persistent current

Comparison of Na^+ and K^+ Currents following a Depolarization



Na^+ influx

opens Na^+ channels

depolarization

opens K^+ channels

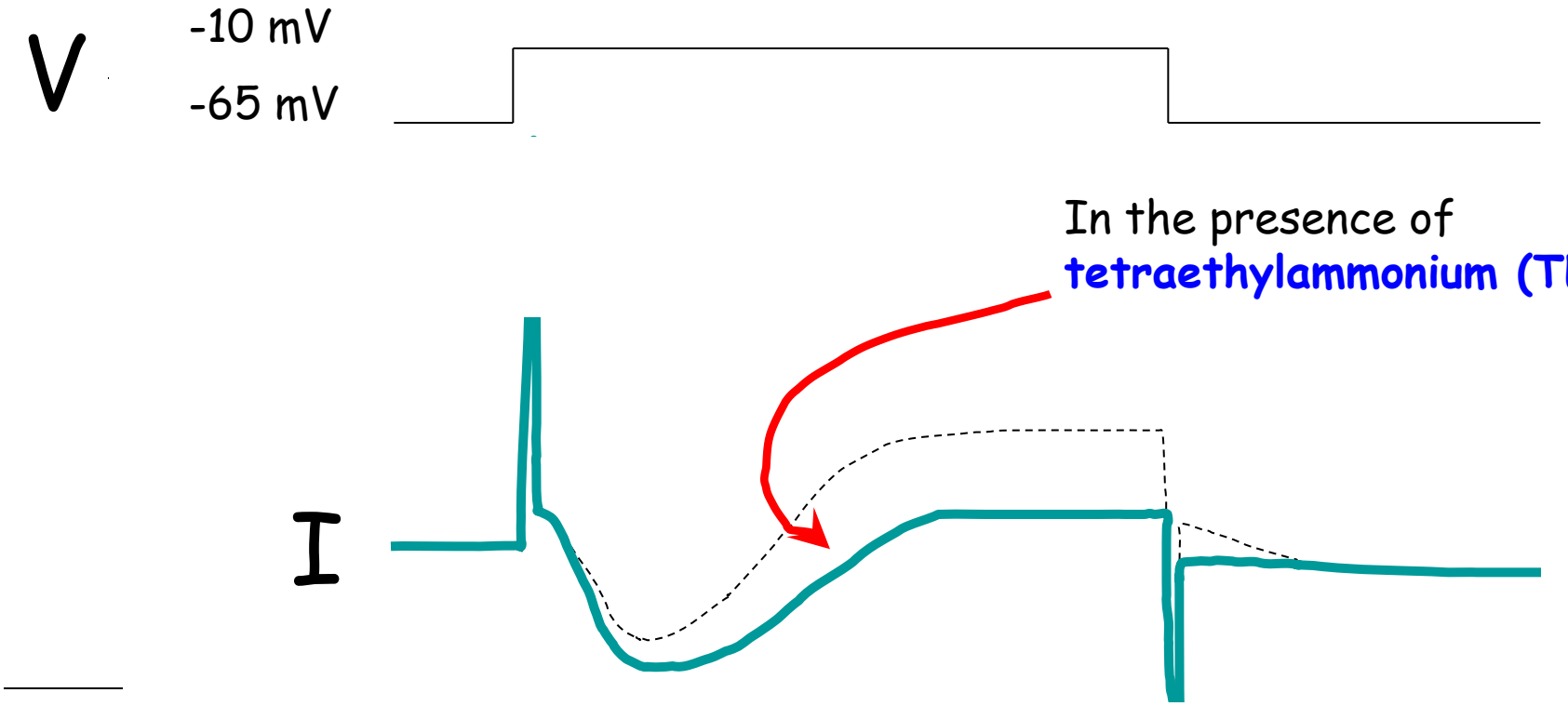
K^+ efflux

repolarization

closes K^+ channels

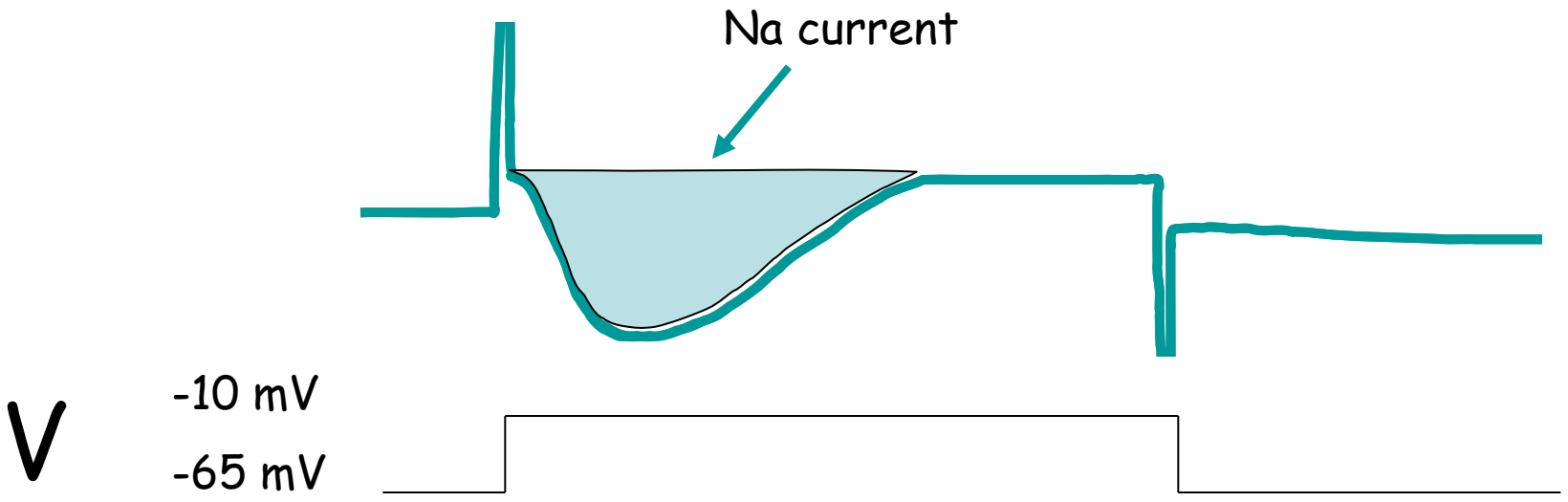
Voltage clamp: what is the behavior of voltage dependent sodium current?

The pharmacological method: Block the potassium current with a drug: **tetraethylammonium**. The voltage-dependent current that remains is the voltage-dependent sodium current.



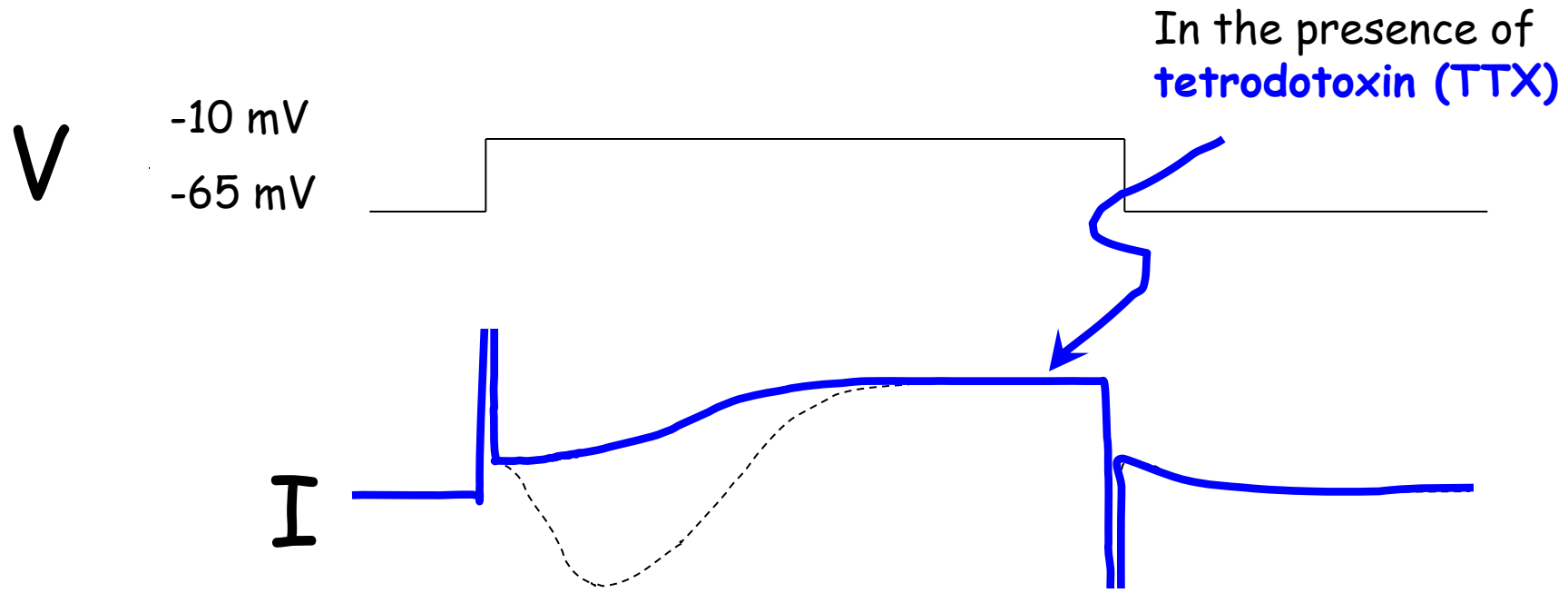
Voltage clamp: what is the behavior of voltage dependent sodium current?

The pharmacological method: Block the potassium current with a drug: **tetraethylammonium**. The voltage-dependent current that remains is the voltage-dependent sodium current.
Note: even with a constant voltage, the sodium current first increases, and then automatically, while the depolarization is maintained, the current decreases (inactivation)



Voltage clamp: what is the behavior of voltage dependent potassium current?

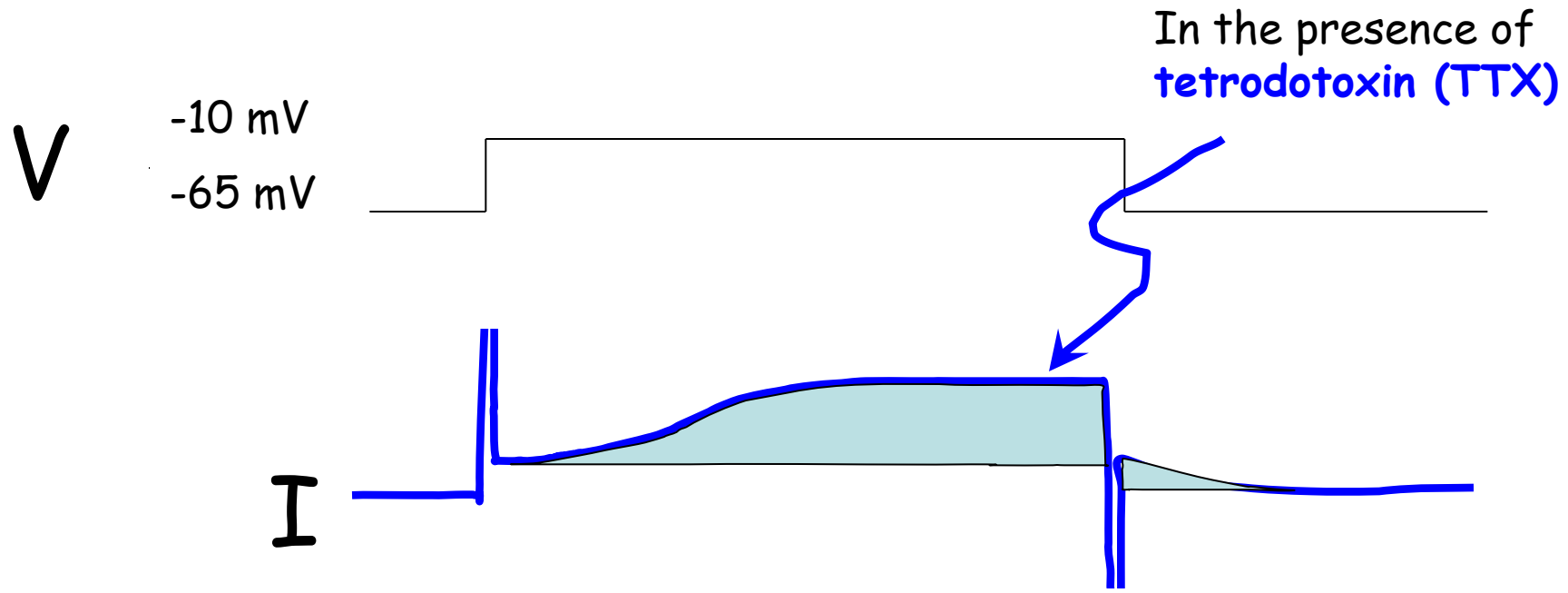
Pharmacological method: Block the voltage-dependent sodium current with **tetrodotoxin**. The current that remains is the voltage-dependent potassium current.



Note: (1) the potassium current is slower to activate than the sodium current. Therefore, sometimes called "delayed current"
(2) the potassium current is maintained for as long as the depolarization is maintained. (only closes after repolarization)

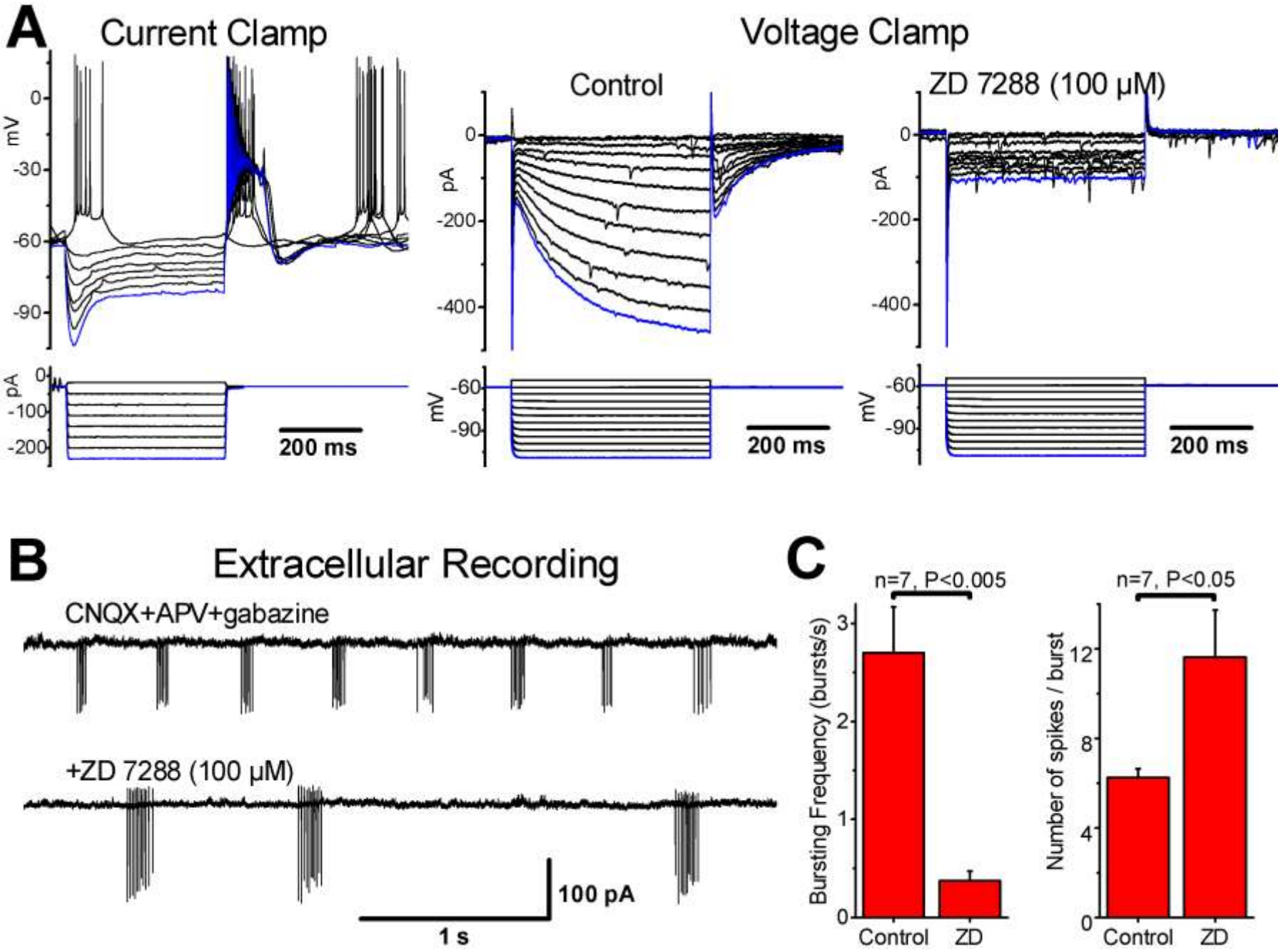
Voltage clamp: what is the behavior of voltage dependent potassium current?

Pharmacological method: Block the voltage-dependent sodium current with tetrodotoxin. The current that remains is the voltage-dependent potassium current.



Note: (1) the potassium current is slower to activate than the sodium current. Therefore, sometimes called "delayed current"
(2) the potassium current is maintained for as long as the depolarization is maintained. (only closes after repolarization)

Bursting persists after blockade of I_h current

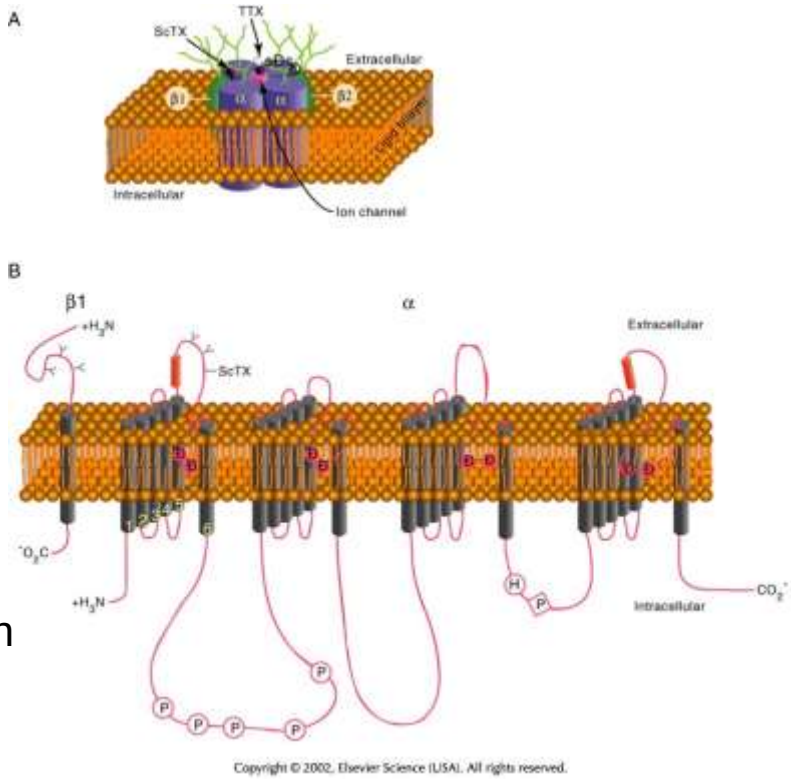


Voltage Dependent Channels

- Diversity of firing patterns produced by myriad voltage dependent channels
- Channels differ by
  - Ion selectivity (e.g. K, Na, Ca)
  - Distribution (Dendrites, soma, axon)
  - Sensitivity to drugs
  - Activation and Inactivation properties:
  - Activation: Turning on of current with depolarization
  - De-activation: Turning off of current with repolarization
  - Inactivation: Turning off of current with sustained depolarization
  - De-inactivation: Removal of inactivation (block) by repolarization

Sodium Currents

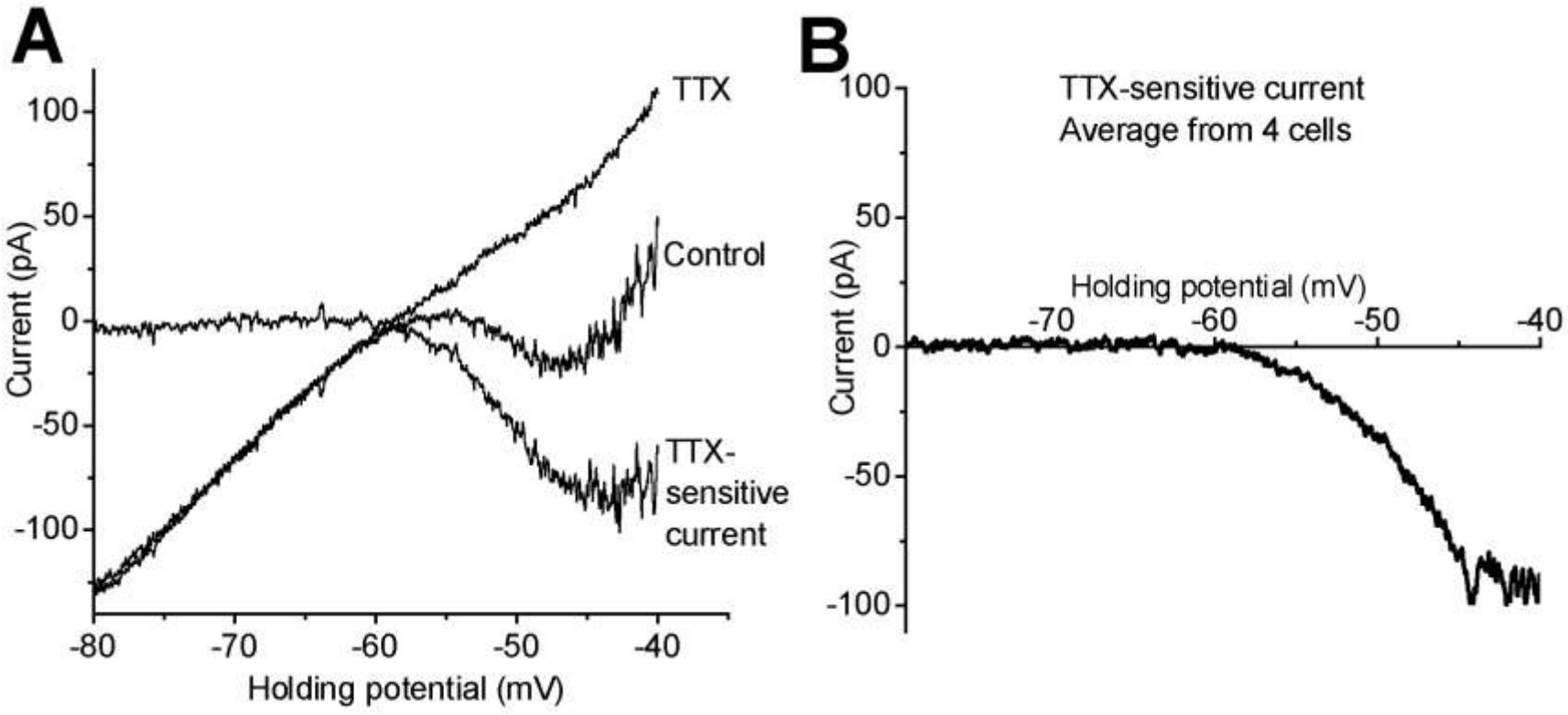
- Transient, I_{NaF}
  - Responsible for Action Potential
- Persistent, I_{NaP}
  - Threshold near resting potential
  - Origin:
    - Window current, or
    - Different gating mode of I_{NaF}, or
    - Separate channel protein



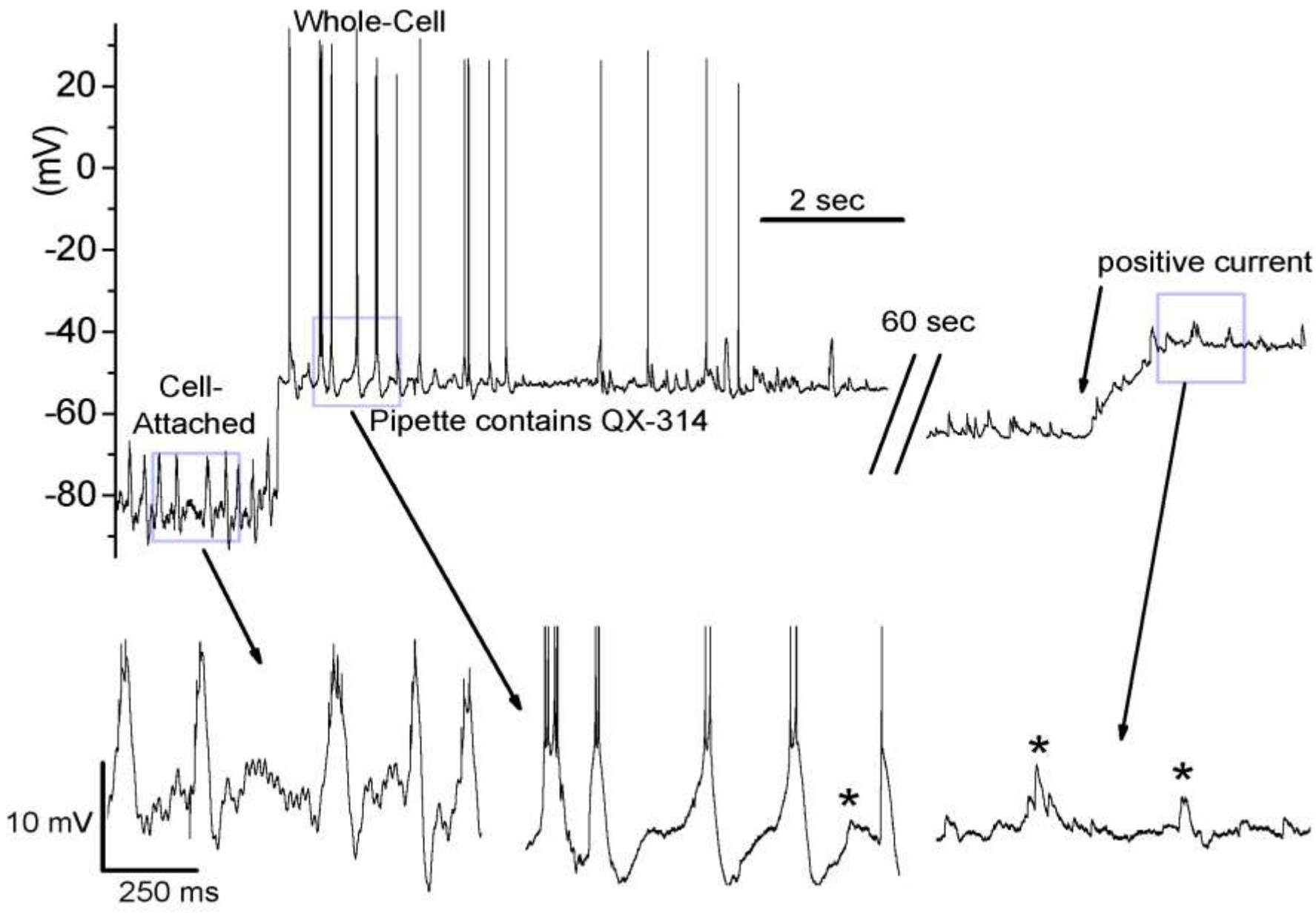
Function of Persistent current
-Enhancement of subthreshold synaptic potentials
-Depolarization activates INaP, => more depolarization
-Hyperpolarization de-activates INaP, which produces more hyperpolarization

Plateau potential
-Prolonged potential that remains after synaptic inputs or current injection is removed
-Contributes to persistent firing

Persistent sodium current is activated by a slow depolarizing voltage ramp (> -60 mV) and is blocked by Tetrodotoxin (TTX)



Bursting is mediated by sodium channel activation and is blocked by intracellular application of a sodium channel blocker QX-314



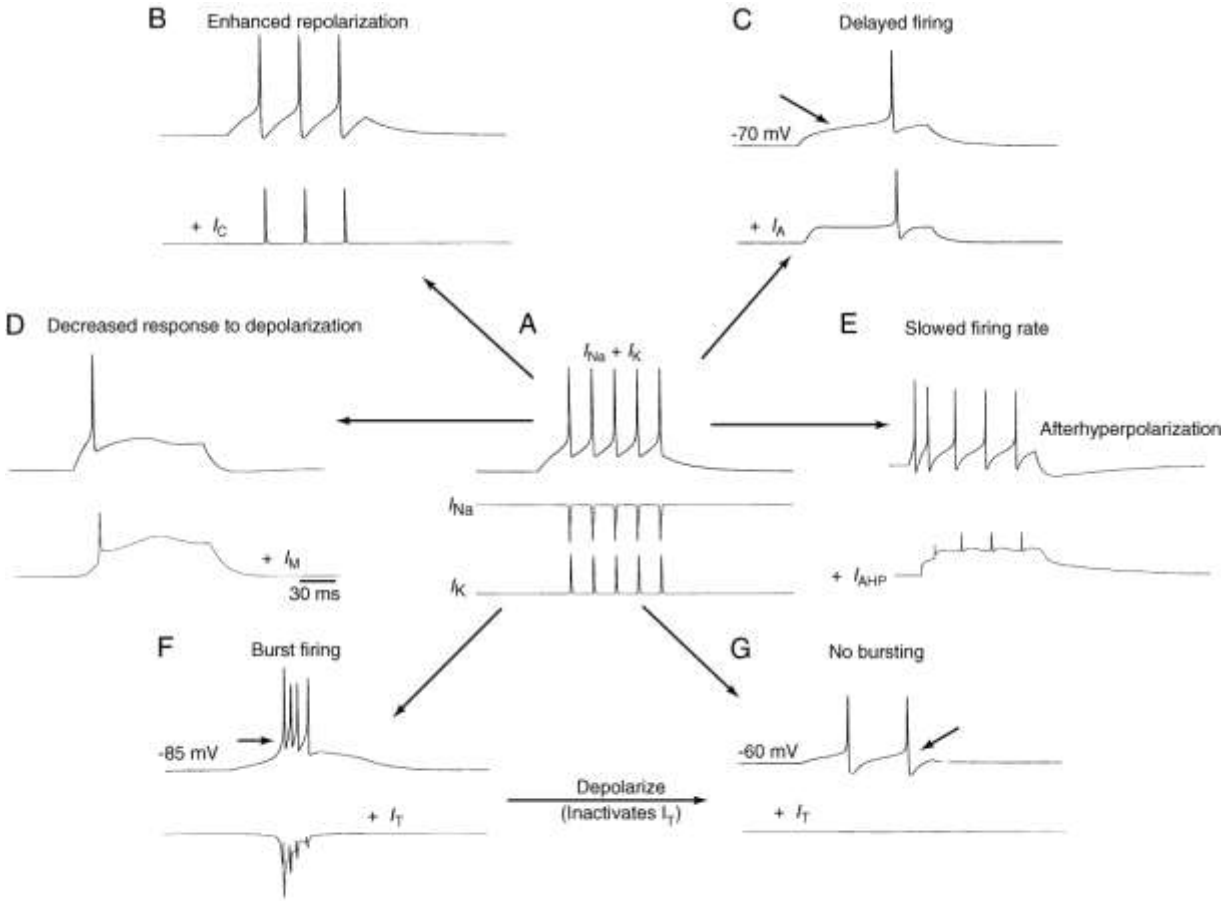
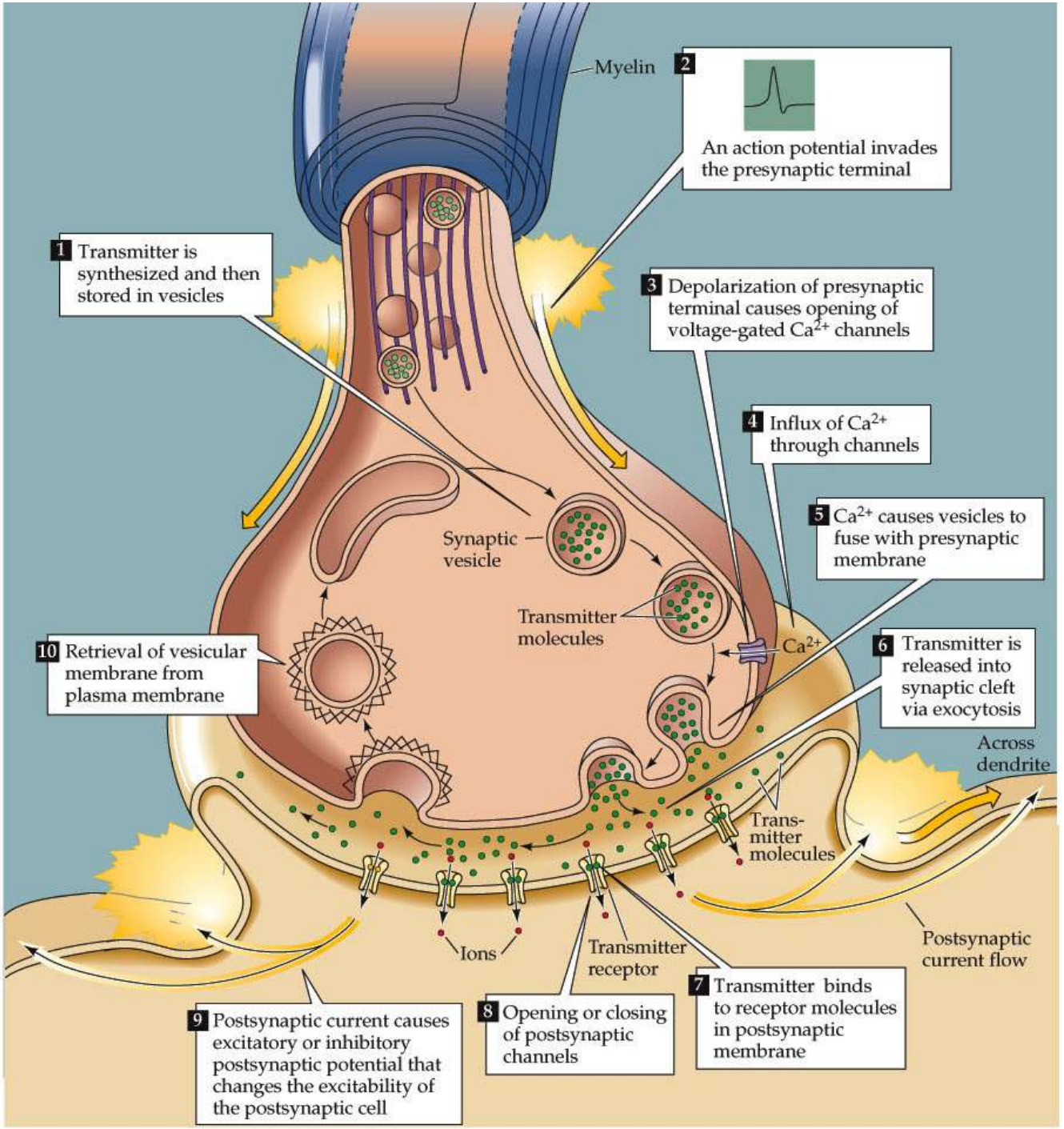


FIGURE 12 Simulation of the effects of the addition of various ionic currents to the pattern of activity generated by neurons in the mammalian CNS. (A) The repetitive impulse response of the classical Hodgkin–Huxley model (voltage recordings above, current traces below). With only *I*Na and *I*K, the neuron generates a train of five action potentials in response to depolarization. Addition of *I*C (B) enhances action potential repolarization. Addition of *I*A (C) delays the onset of action potential generation. Addition of *I*M (D) decreases the ability of the cell to generate a train of action potentials. Addition of *I*AHP (E) slows the firing rate and generates a slow afterhyperpolarization. Finally, addition of the transient Ca2+ current *I*T results in two states of action potential firing: (F) burst firing at -85 mV and (G) tonic firing at -60 mV. From Huguenard and McCormick (1994).

Sequence of events involved in transmission at a typical chemical synapse



There are three main types of ionotropic glutamate receptors

AMPA

Kainate

NMDA

AMPA and Kainate receptors are collectively also known as non-NMDA receptors. All three receptors are ligand-gated ion channels. AMPA and kainate receptors are permeable to Na^+ and K^+ ions, whereas NMDA receptors also have a high permeability to Ca^{2+} ions.

Each receptor type is a multimeric protein complex comprised of either 4 or 5 subunits.

Each subunit contains 3 transmembrane domains and a re-entrant loop.

NMDA receptors are unusual in that they also require a co-agonist in addition to glutamate for them to function properly. In addition they are blocked in a voltage dependent manner by Mg^{2+} ions. NMDA receptors are also modulated/blocked by a variety of endogenous and exogenous ligands.

AMPA and NMDA receptors are co-localised at glutamatergic synapses where they mediate ‘fast’ chemical synaptic transmission.

The precise role of kainate receptors is still a matter of much research.

Glutamatergic synaptic currents have a fast and slow component

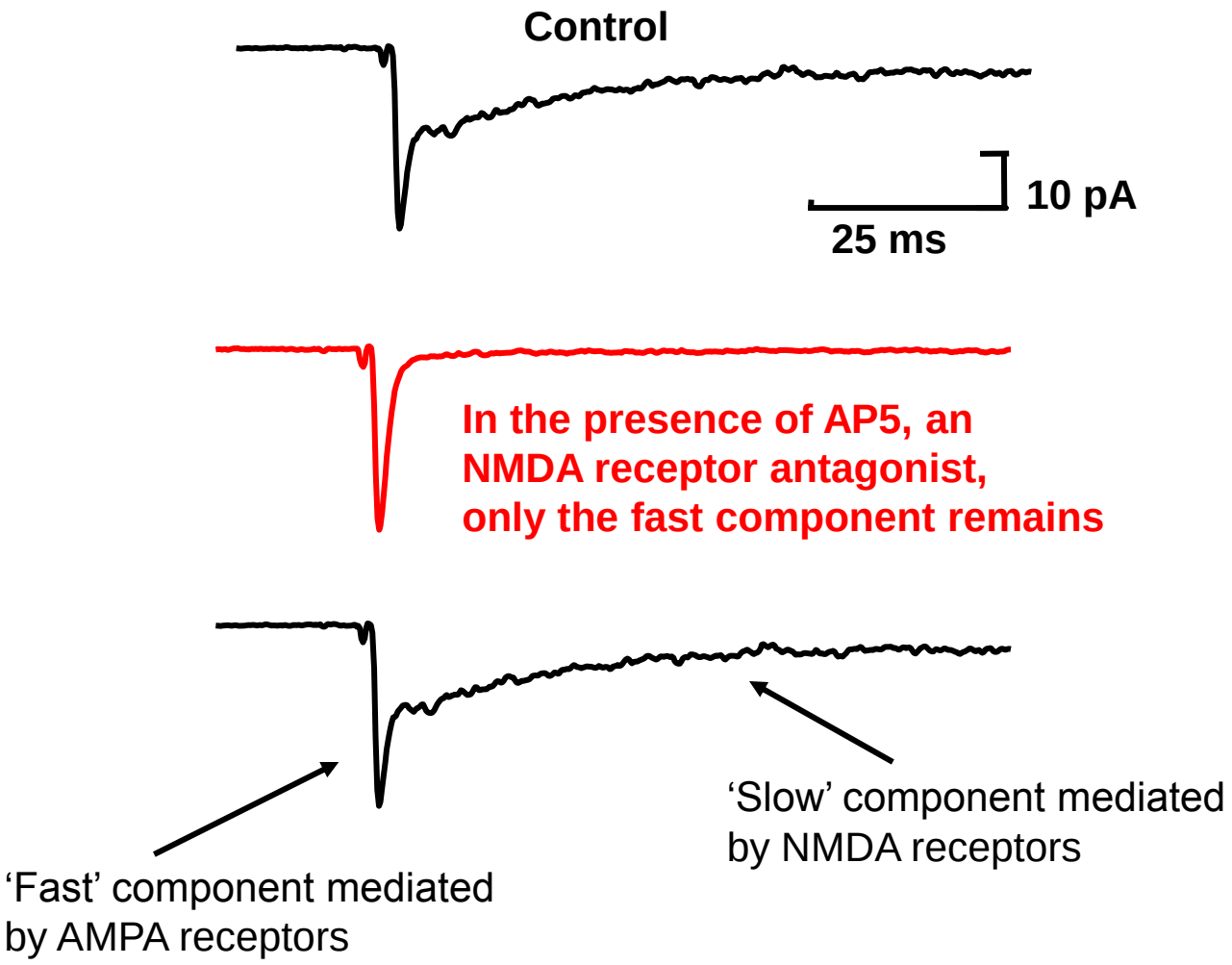
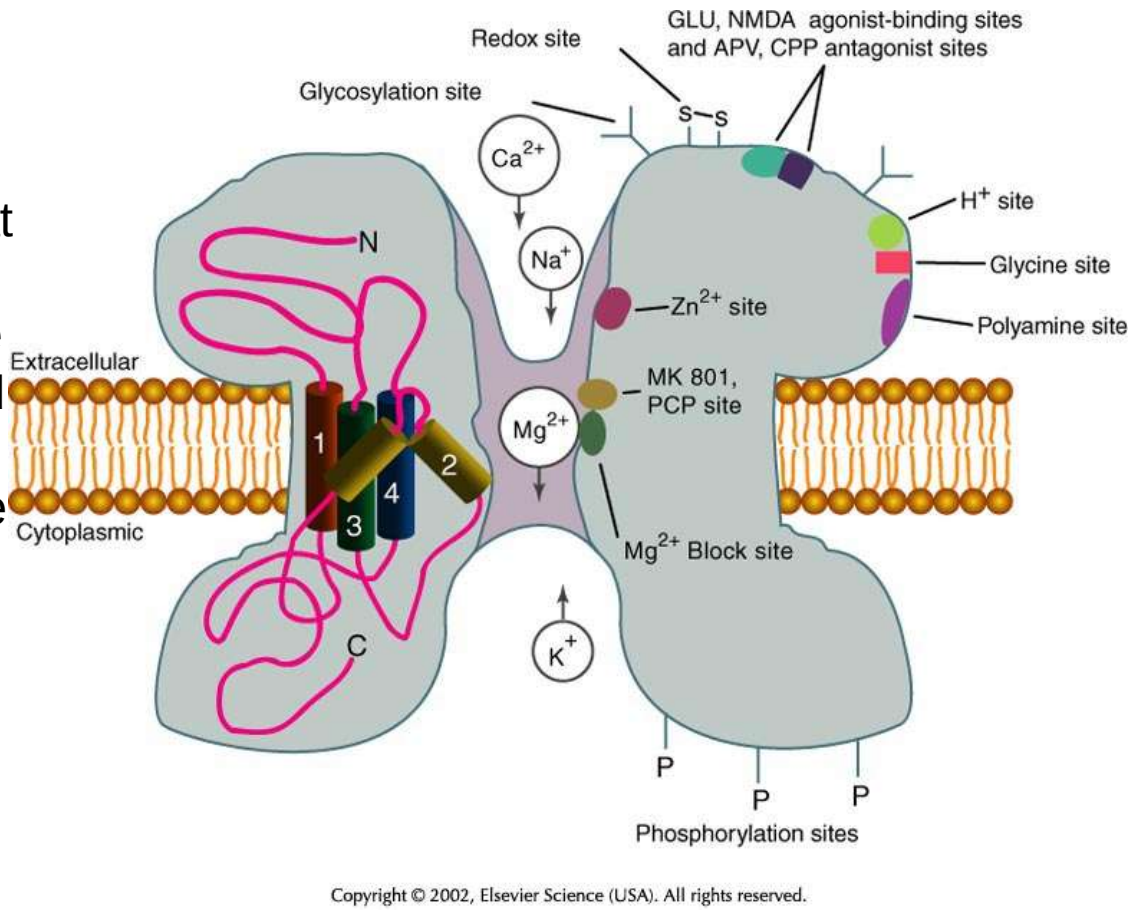


Figure from: Clark, Farrant & Cull-Candy (1997) *Journal of Neuroscience* 17, 107-116.

-- NMDA receptors (Glutamate), are ligand-gated ion channels that are also voltage dependent.
-- For the channel to be open, the receptor must bind glutamate and the membrane must be depolarized. This behavior is due to a magnesium-dependent block of the receptor at normal membrane resting potentials.



-- Second, the receptor permits a significant influx of Ca and increases in intracellular Ca activate a variety of processes that alter the properties of the neuron.

NMDA-receptors – physiological and pathophysiological roles

NMDA receptor-channels have been implicated in many CNS 'processes' ranging from synaptogenesis to excitotoxicity.

Blocking NMDA receptors prevents 'normal' synaptic connections to be made during development e.g. in barrel or visual cortex.

During stroke, overactivation of NMDA receptors results in cell death.

Models of learning and memory implicate a fundamental role of the NMDA receptor as a 'co-incidence' detector.

Drugs that block NMDA receptors may be of use in the treatment of stroke and Parkinson's disease, while those that potentiate receptor function may be of benefit in the treatment of Alzheimer's disease.

$GABA_A$ Receptor : Structure & Activation

Binding sites for:

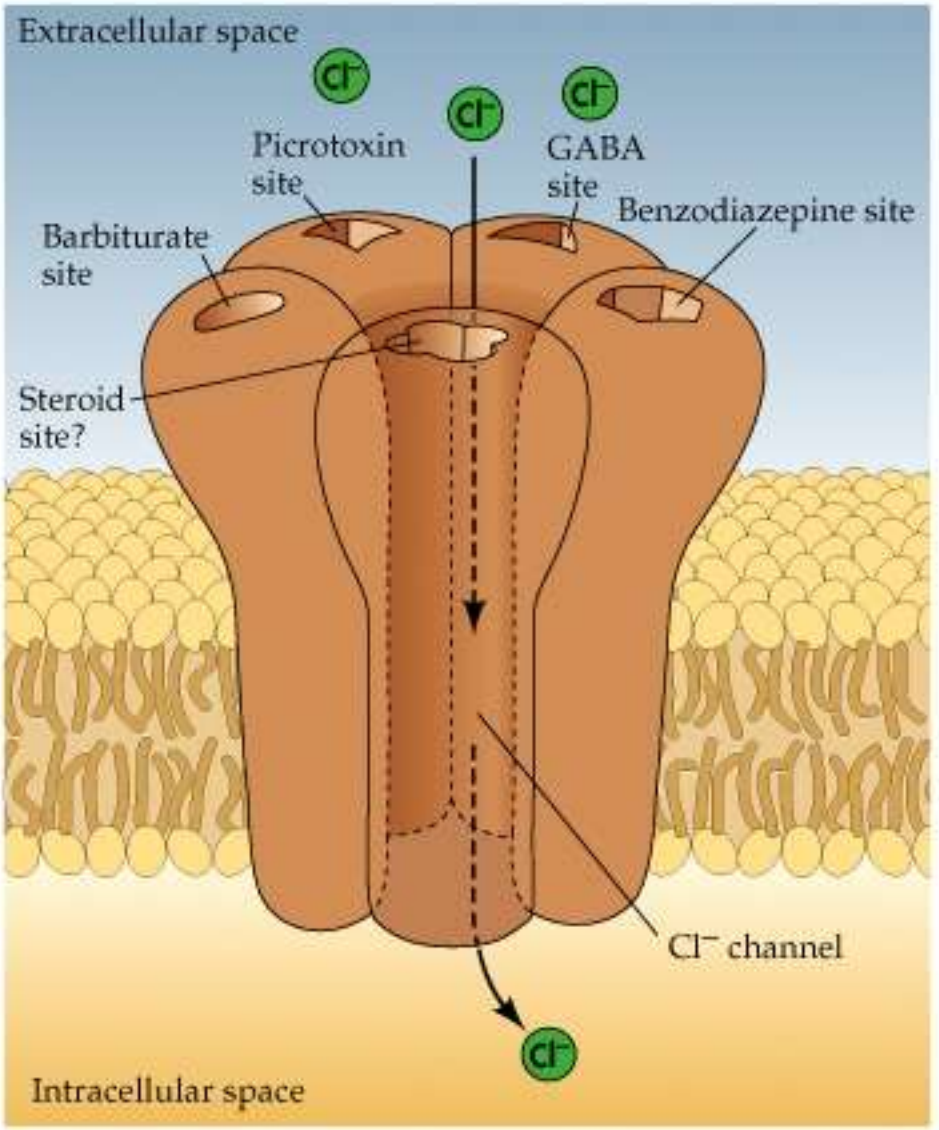
Benzodiazepines (BZ) (sedatives hypnotics)

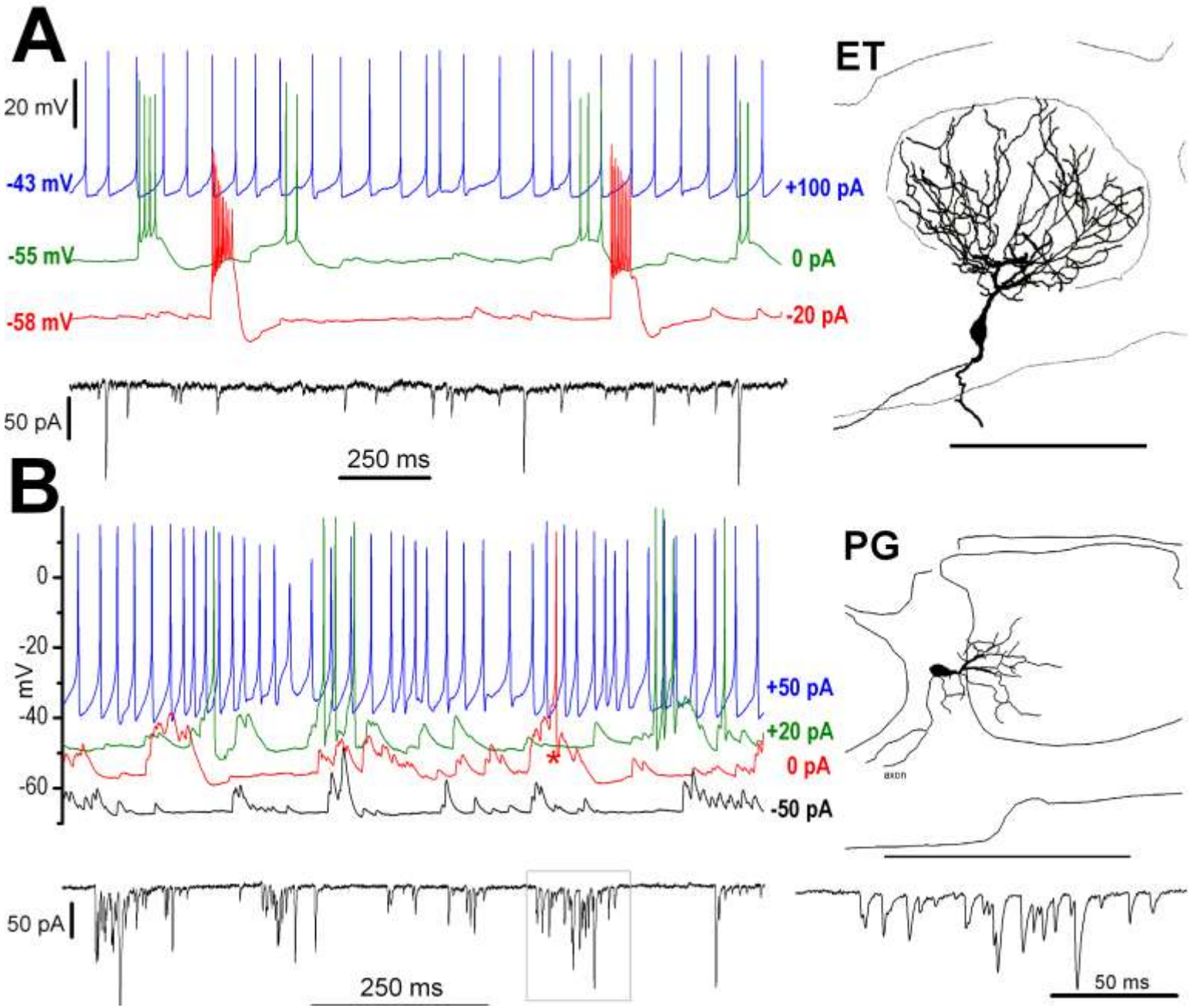
Steroids (New General Anaesthetics)

Barbiturates (Old Sedatives hypnotics)

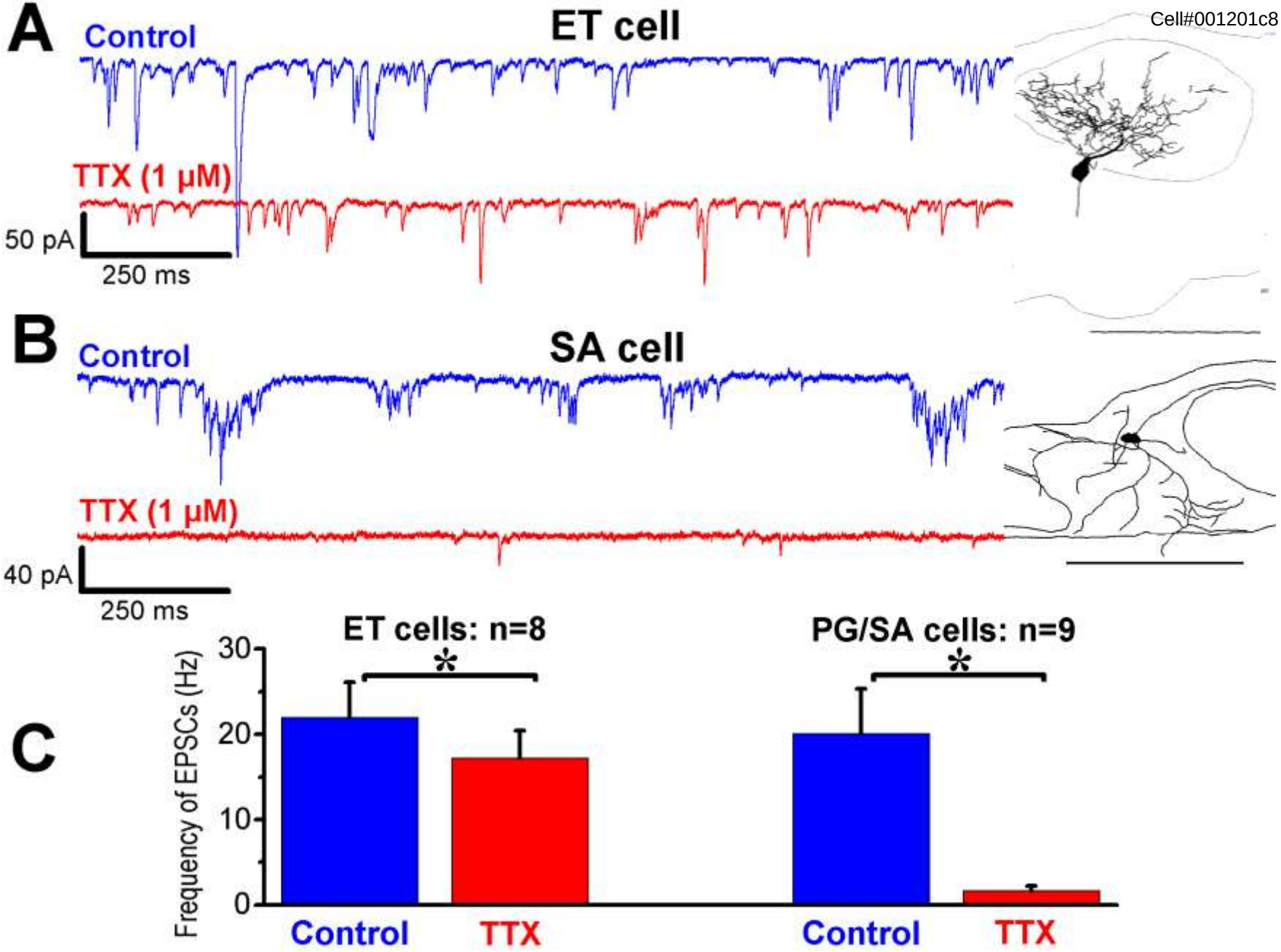
Ethanol (sedation)

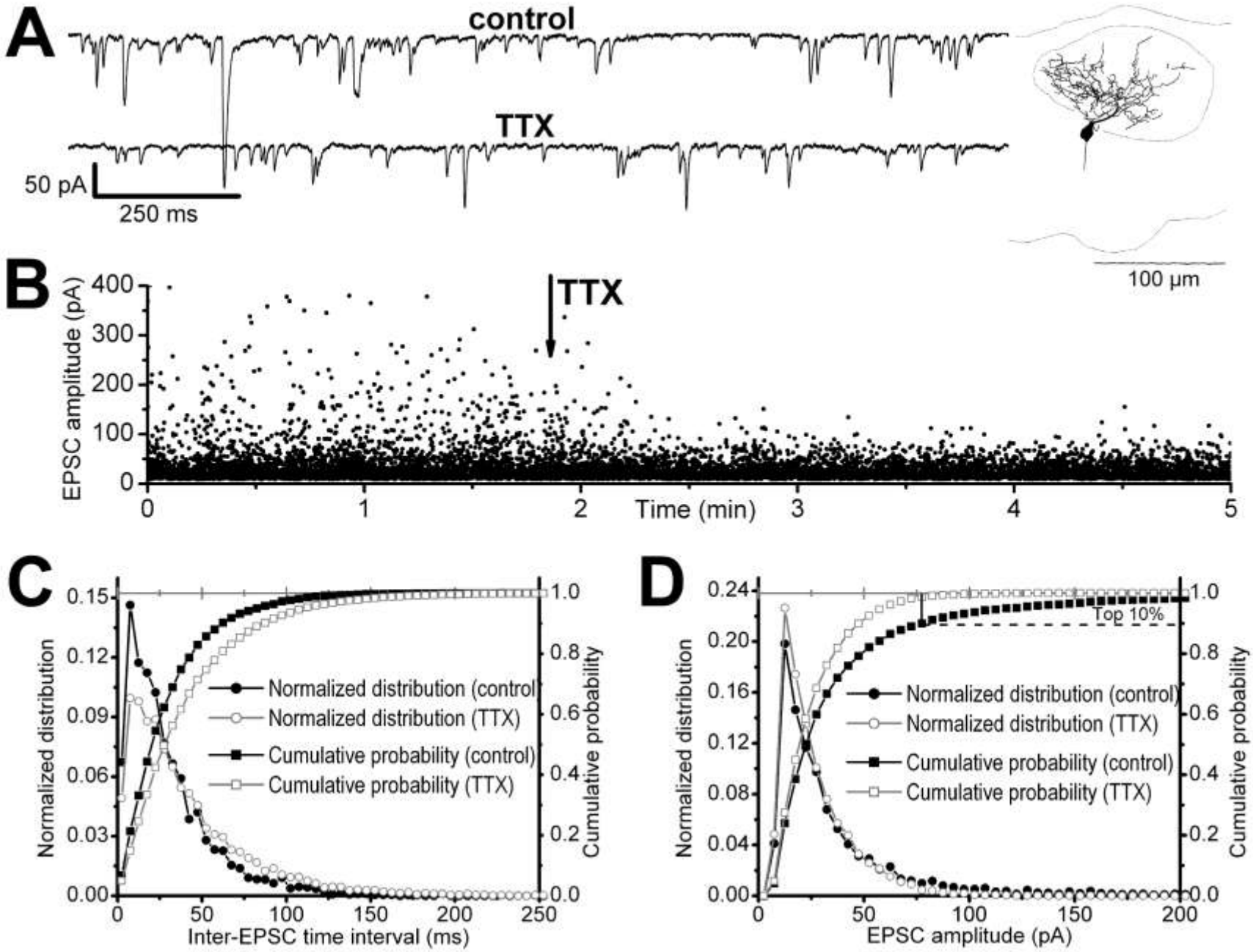
Anticonvulsants (Spasticity motor disorders)

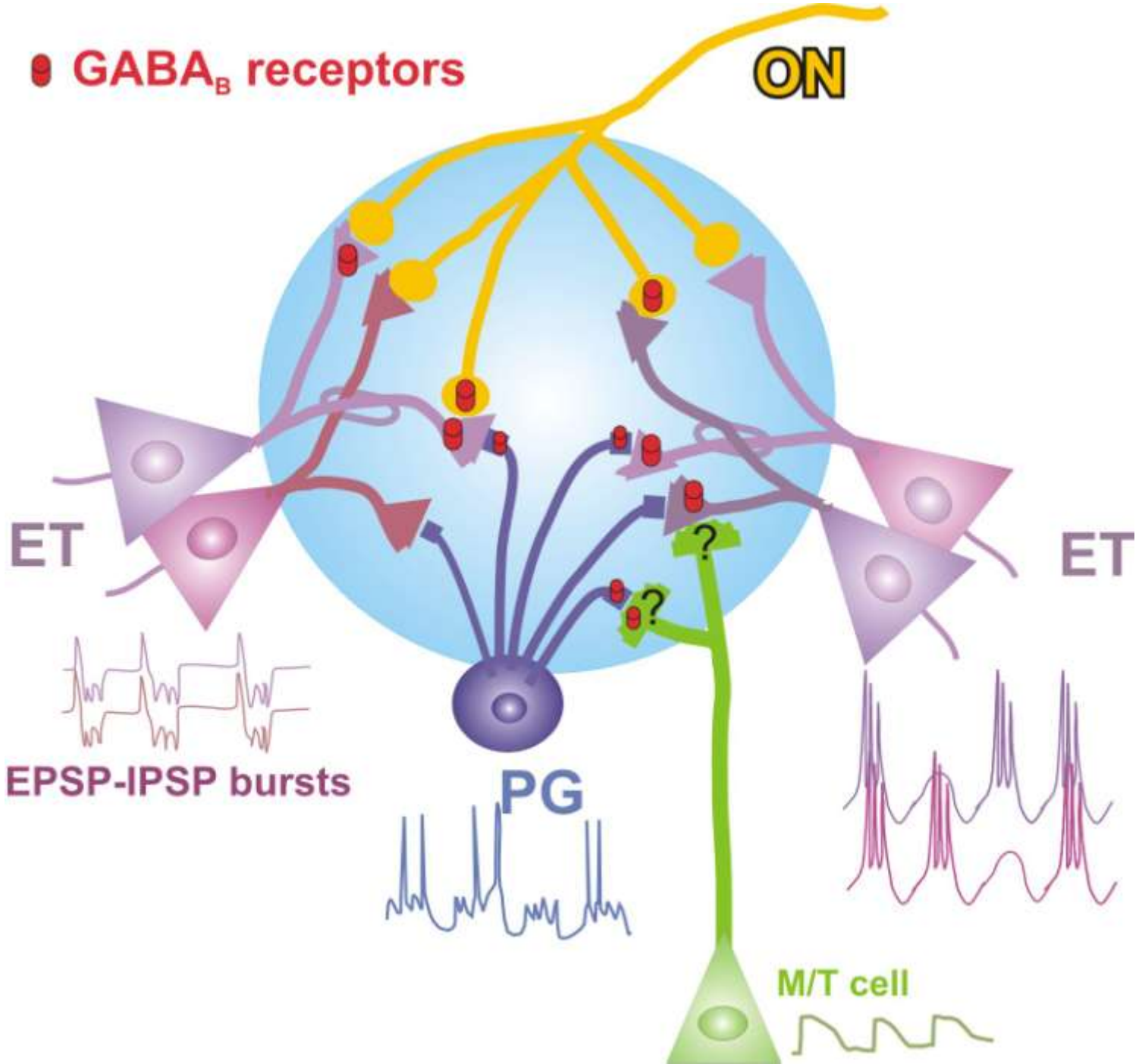


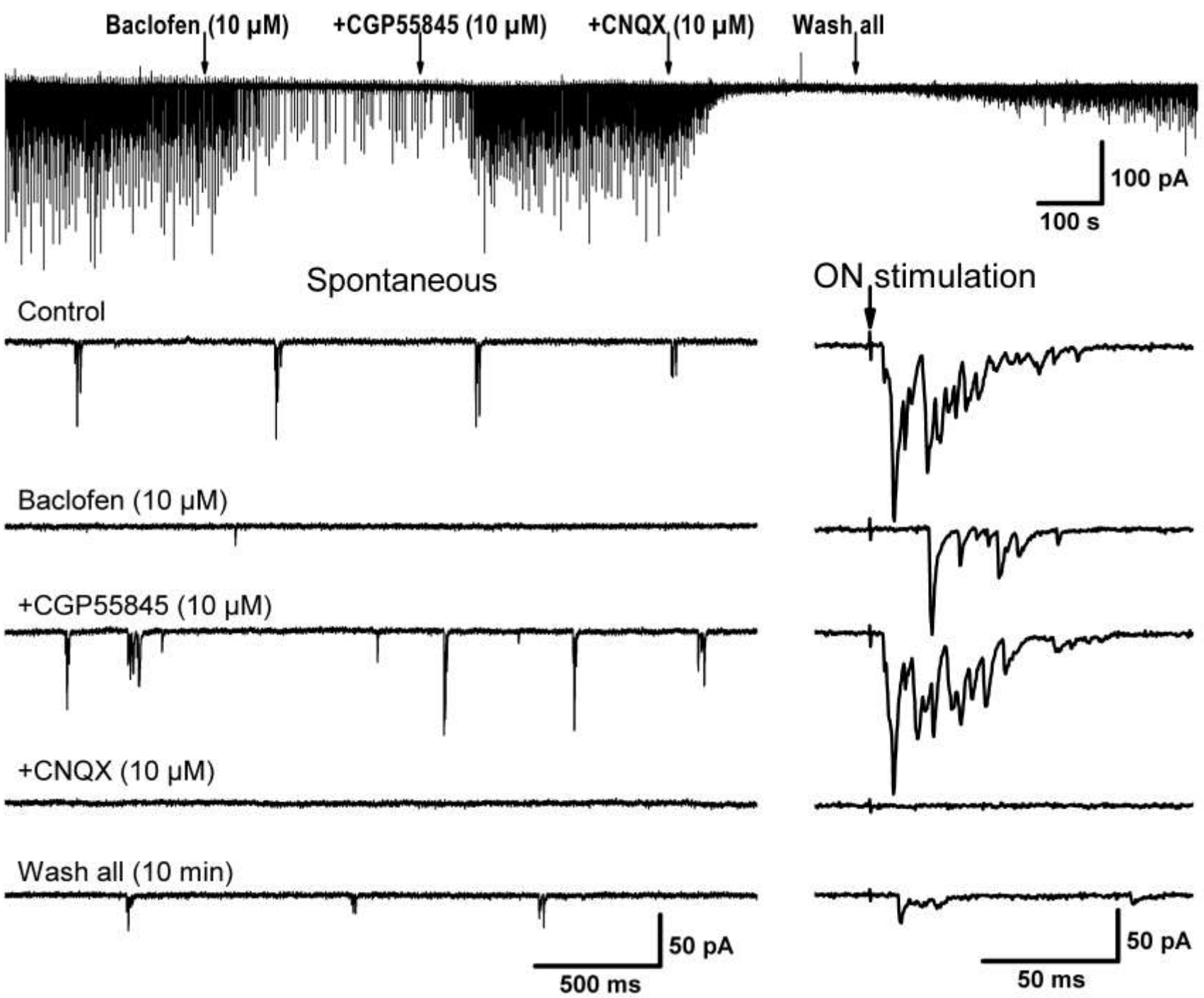

A
20 mV
-43 mV
-55 mV
-58 mV
+100 pA
0 pA
-20 pA
50 pA
250 ms
ET
B
0
-20
-40
-60
mV
+50 pA
+20 pA
0 pA
-50 pA
50 pA
250 ms
PG
axon
50 ms

The bursts of EPSP/Cs are action-potential dependent

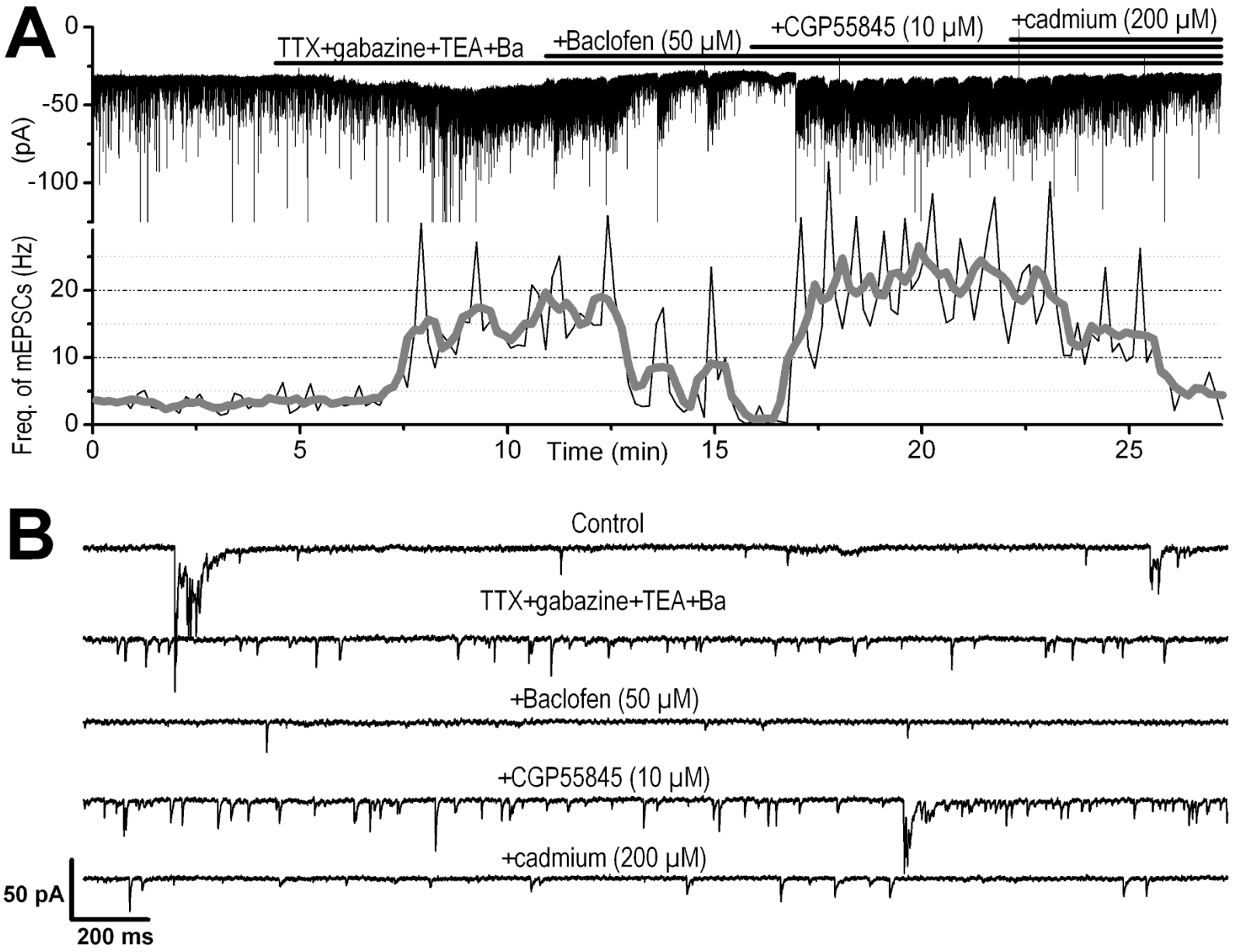


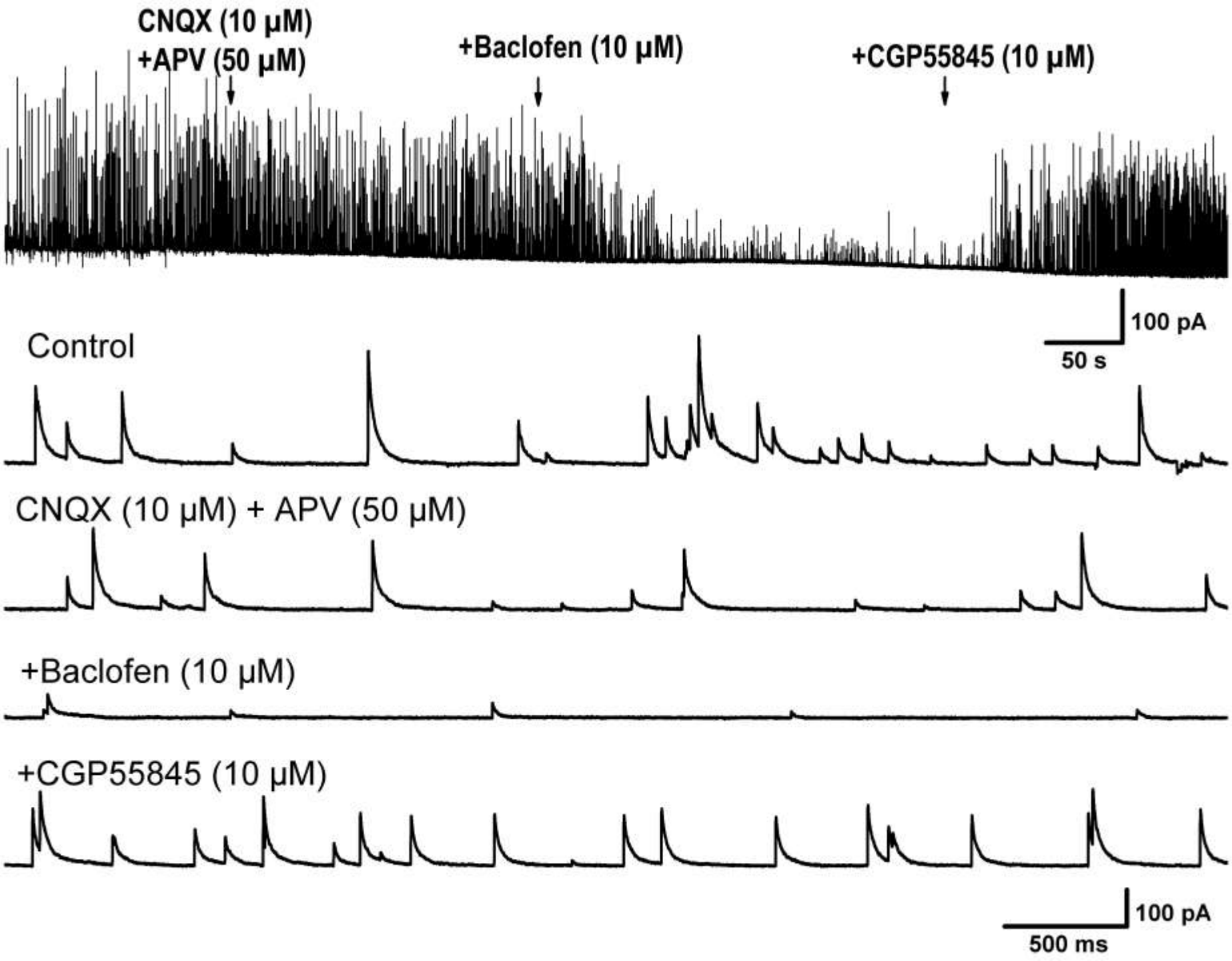

A
control
TTX
50 pA
250 ms
100 μm
B
TTX
EPSC amplitude (pA)
Time (min)
C
Normalized distribution
Cumulative probability
Normalized distribution (control)
Normalized distribution (TTX)
Cumulative probability (control)
Cumulative probability (TTX)
Inter-EPSC time interval (ms)
D
Top 10%
EPSC amplitude (pA)

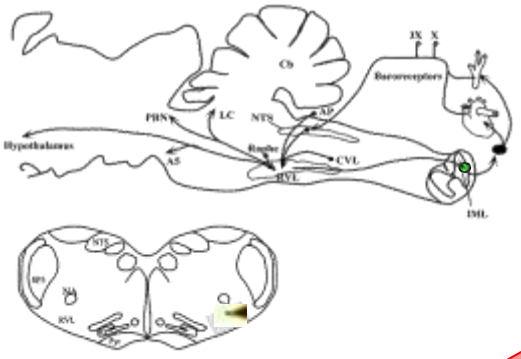

GABA$_B$ receptors
ON
ET
ET
?
?
EPSP-IPSP bursts
PG
M/T cell


Baclofen (10 µM)
+CGP55845 (10 µM)
+CNQX (10 µM)
Wash all
100 pA
100 s
Spontaneous
ON stimulation
Control
Baclofen (10 µM)
+CGP55845 (10 µM)
+CNQX (10 µM)
Wash all (10 min)
50 pA
500 ms
50 pA
50 ms

Baclofen reduces the frequency of action potential-independent EPSCs (mEPSCs)

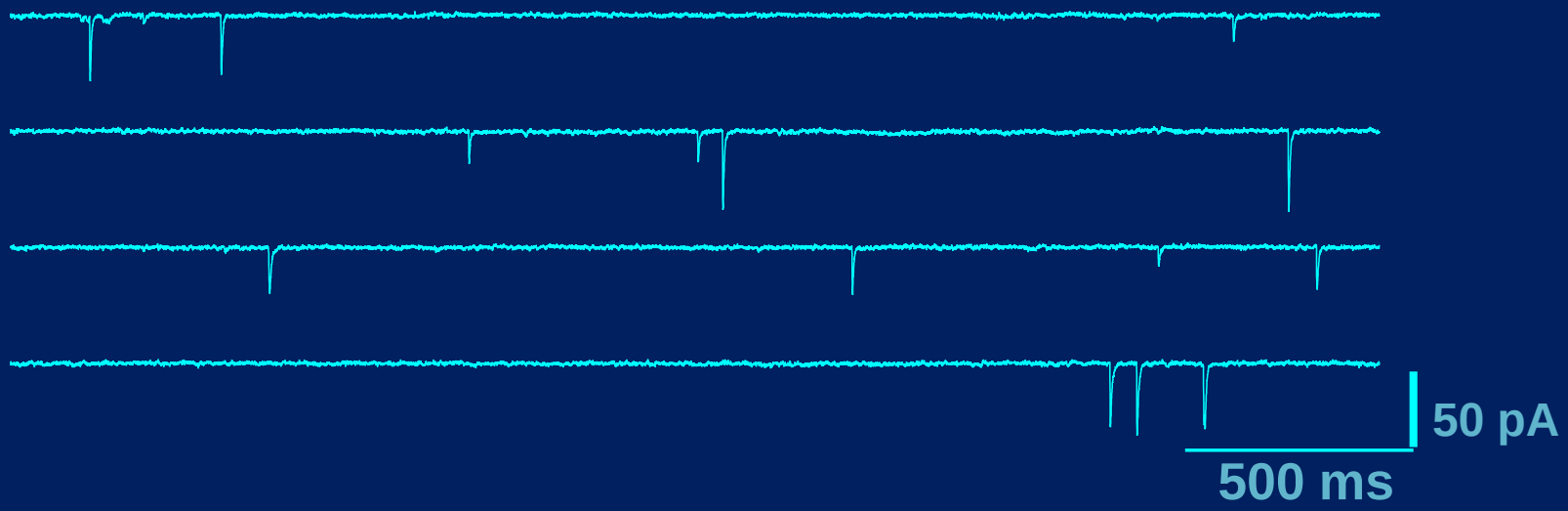



CNQX (10 µM)
+APV (50 µM)
+Baclofen (10 µM)
+CGP55845 (10 µM)
100 pA
50 s
Control
CNQX (10 µM) + APV (50 µM)
+Baclofen (10 µM)
+CGP55845 (10 µM)
100 pA
500 ms

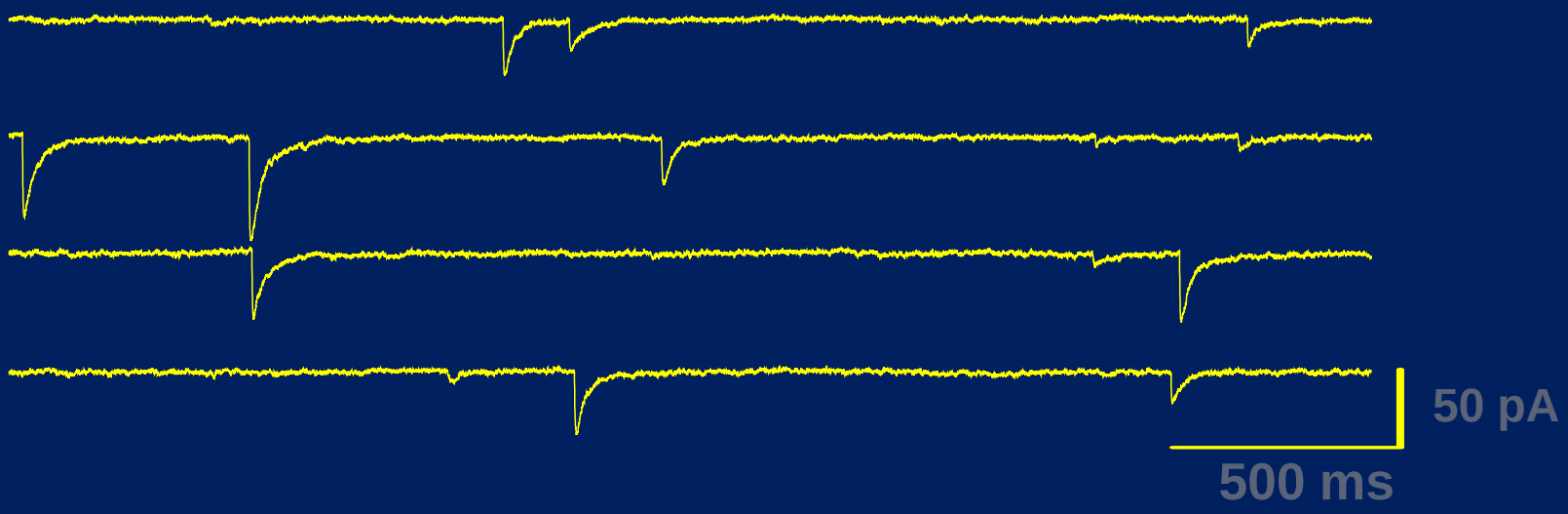

Glutamate
GABA
μ-opioid receptor
α2-adrenoceptor
RVL Bulbospinal neuron
Blood vessels
Heart
Spinal cord

Excitatory and inhibitory miniature postsynaptic currents

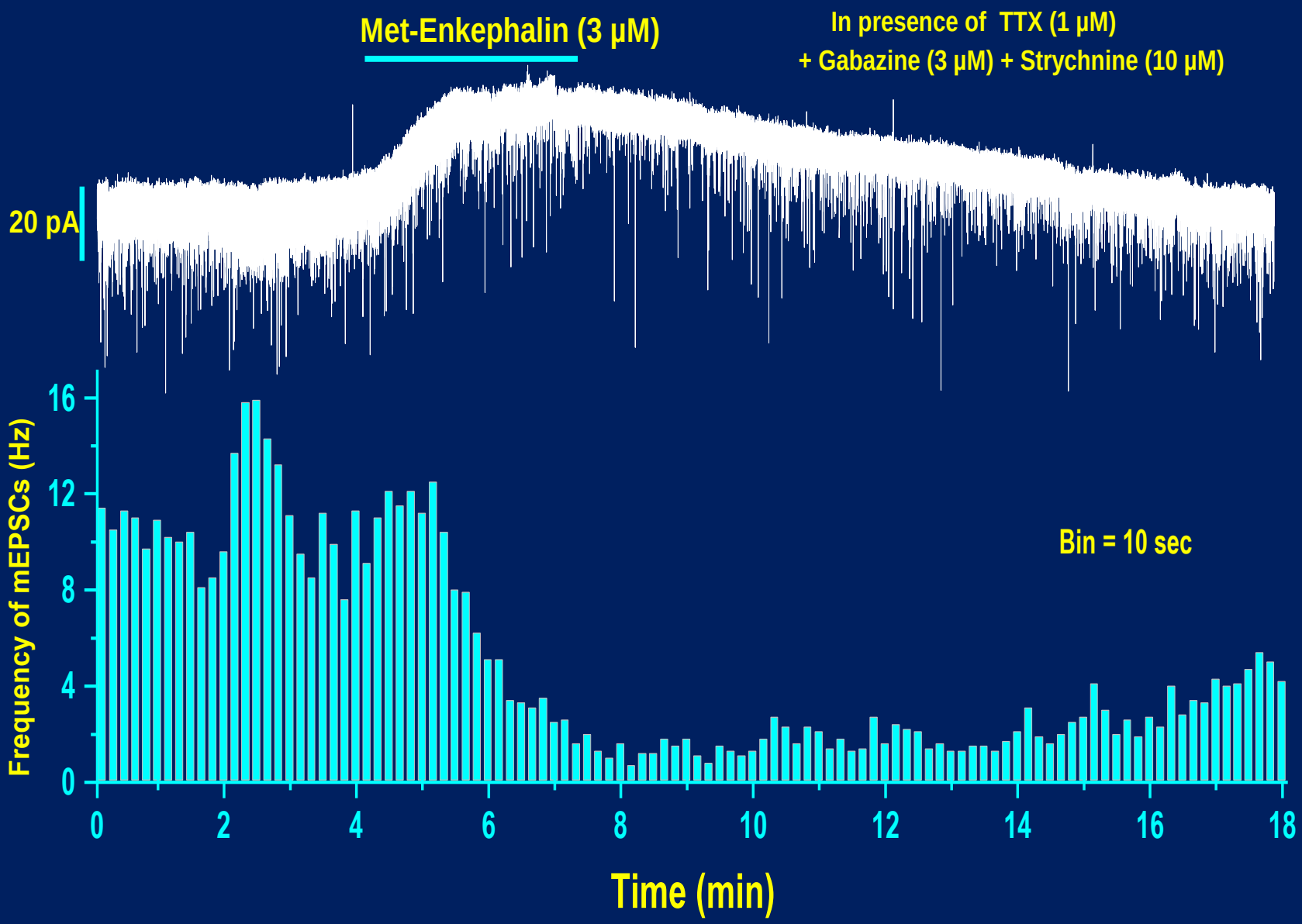
mEPSCS (in presence of TTX + Gabazine)



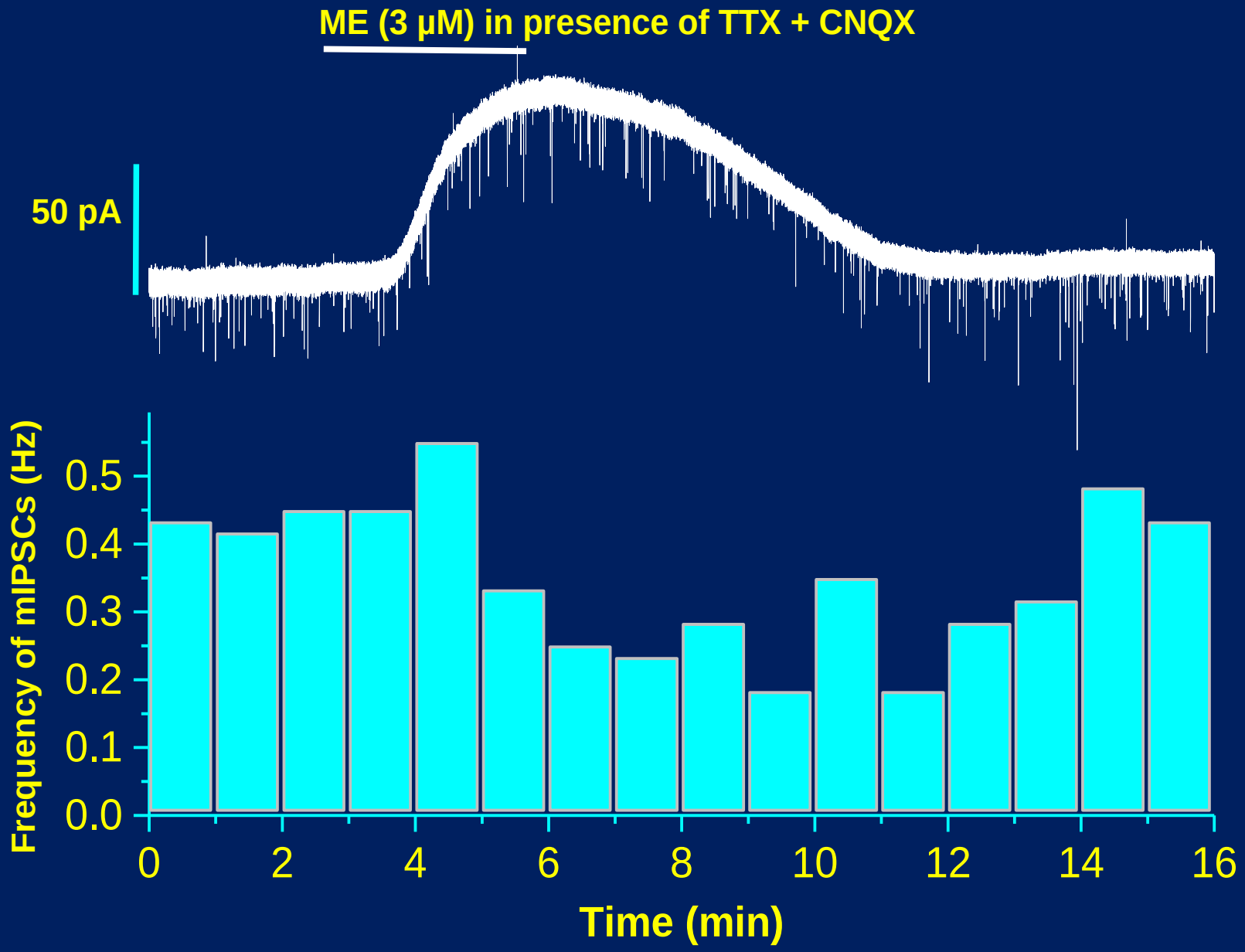
mIPSCS (in presence of TTX + CNQX)



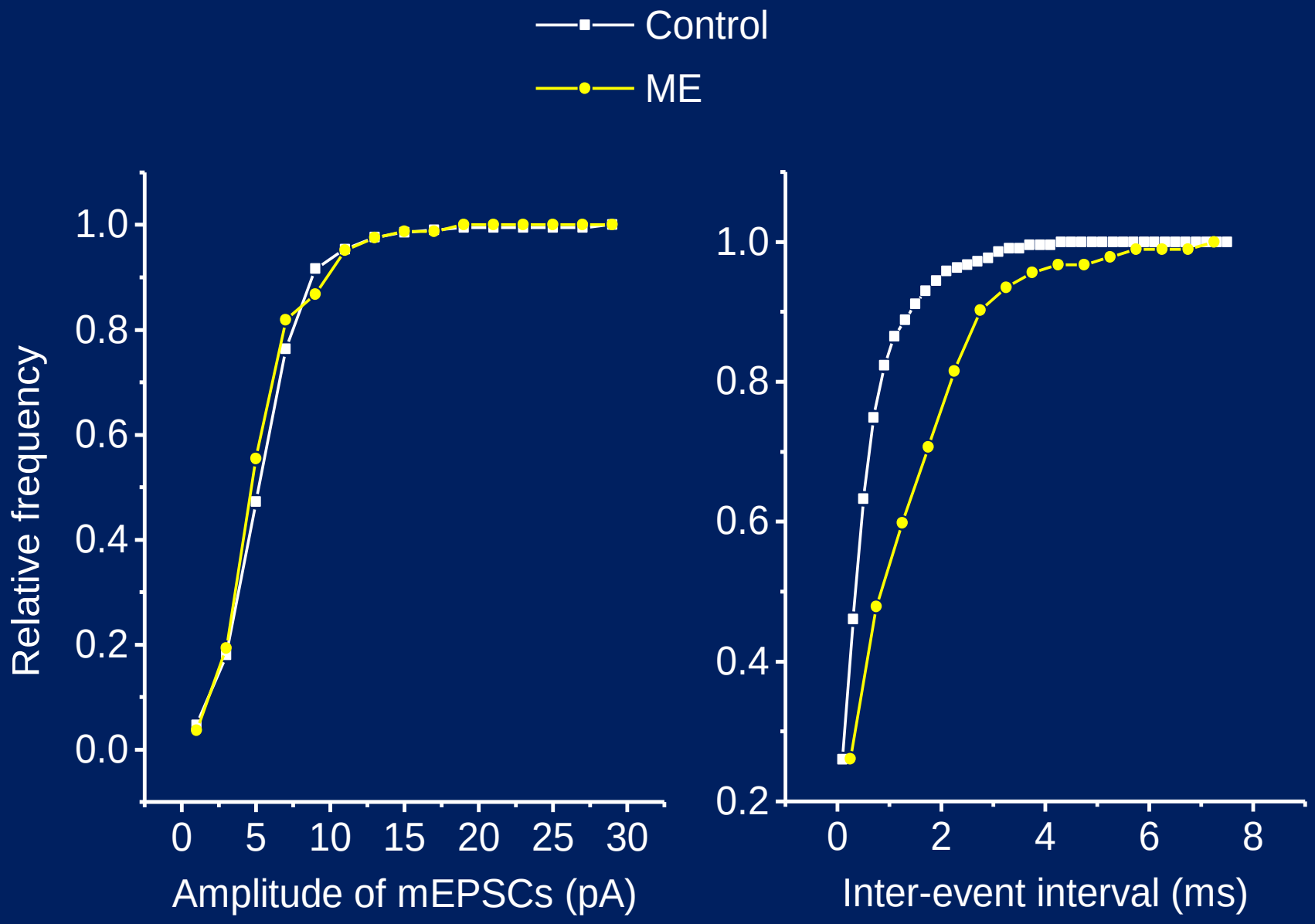
ME decreases the frequency of miniEPSCs



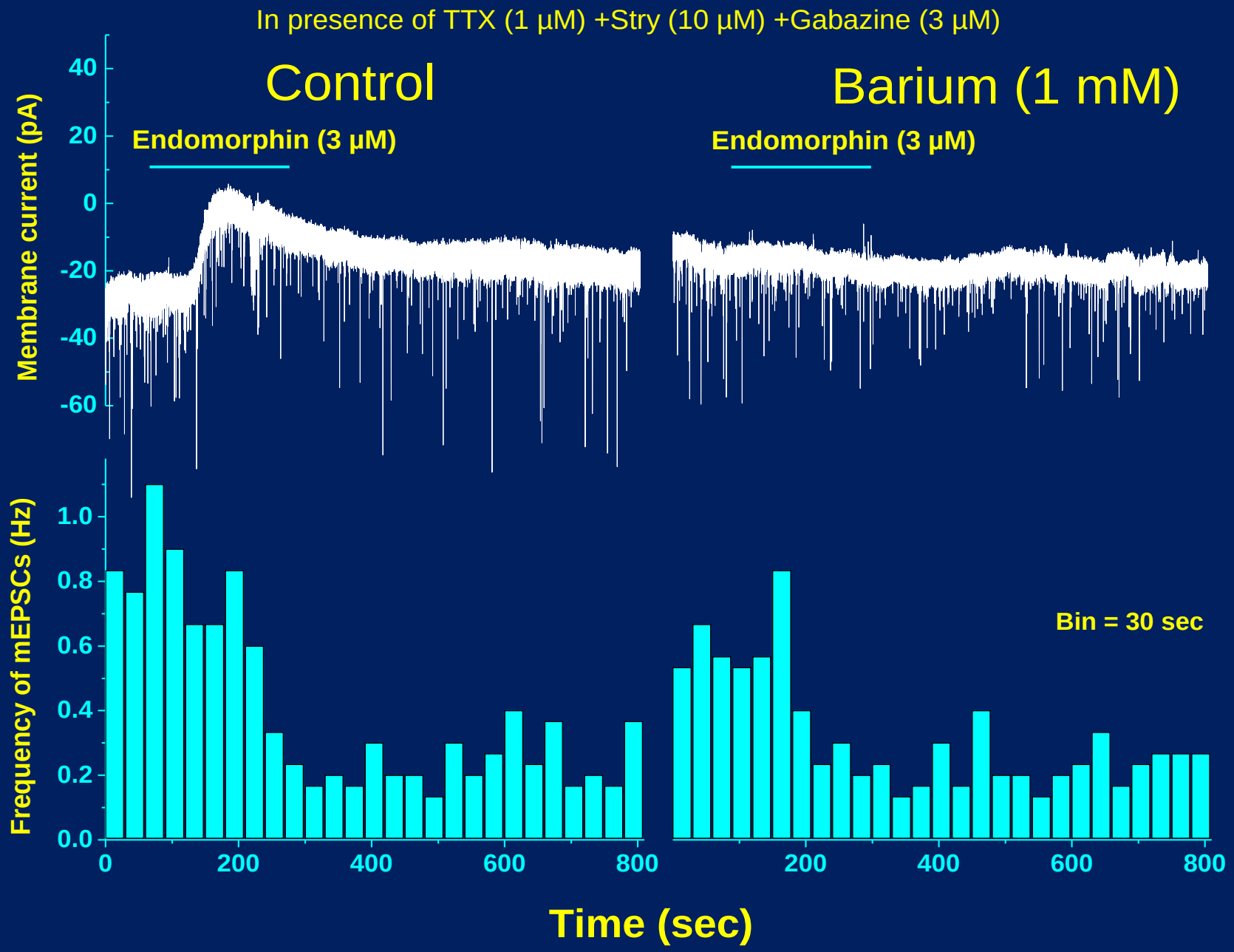
ME decreases the frequency of miniIPSCs



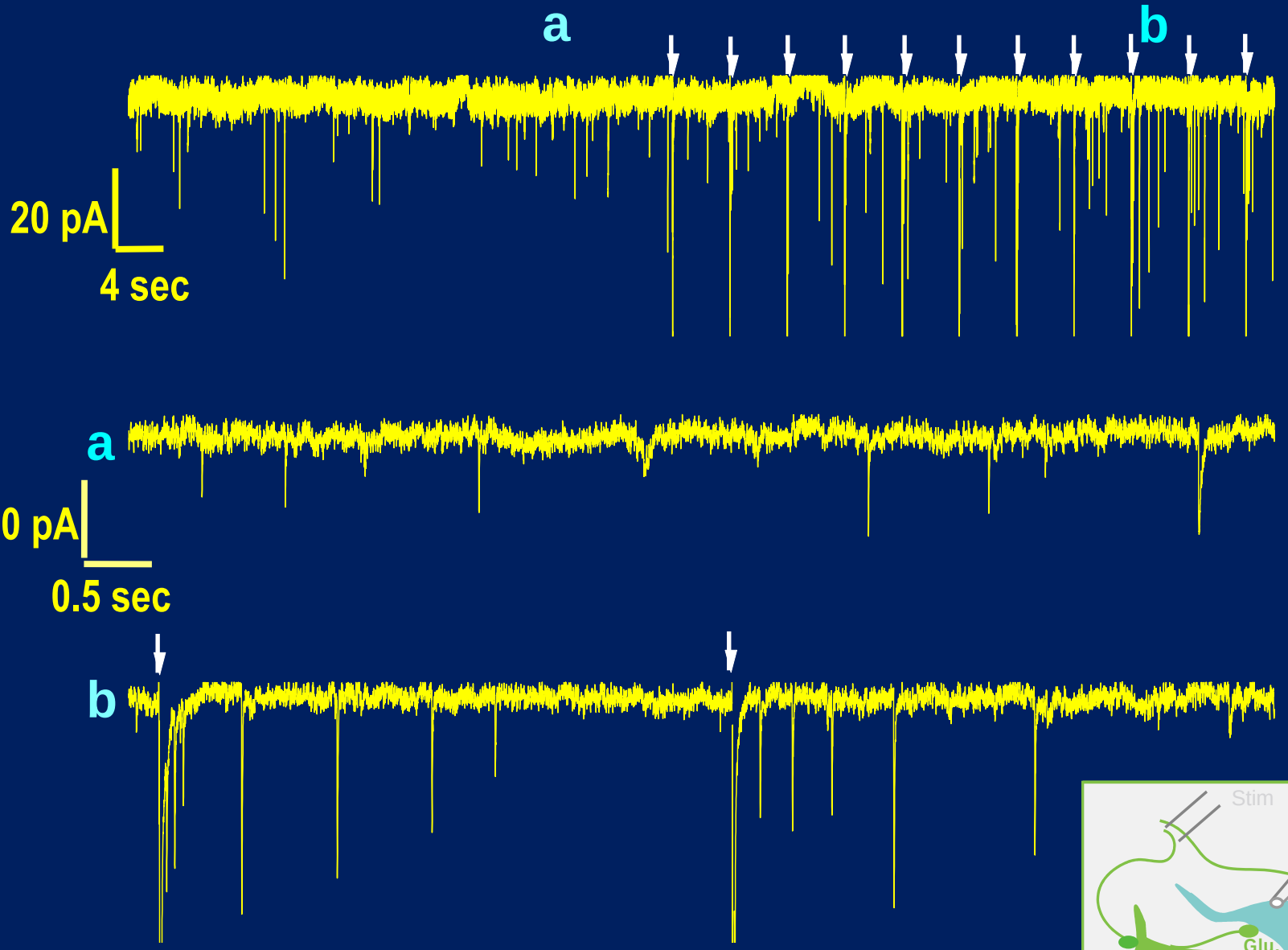
ME reduces the frequency but not the amplitude of miniEPSCs



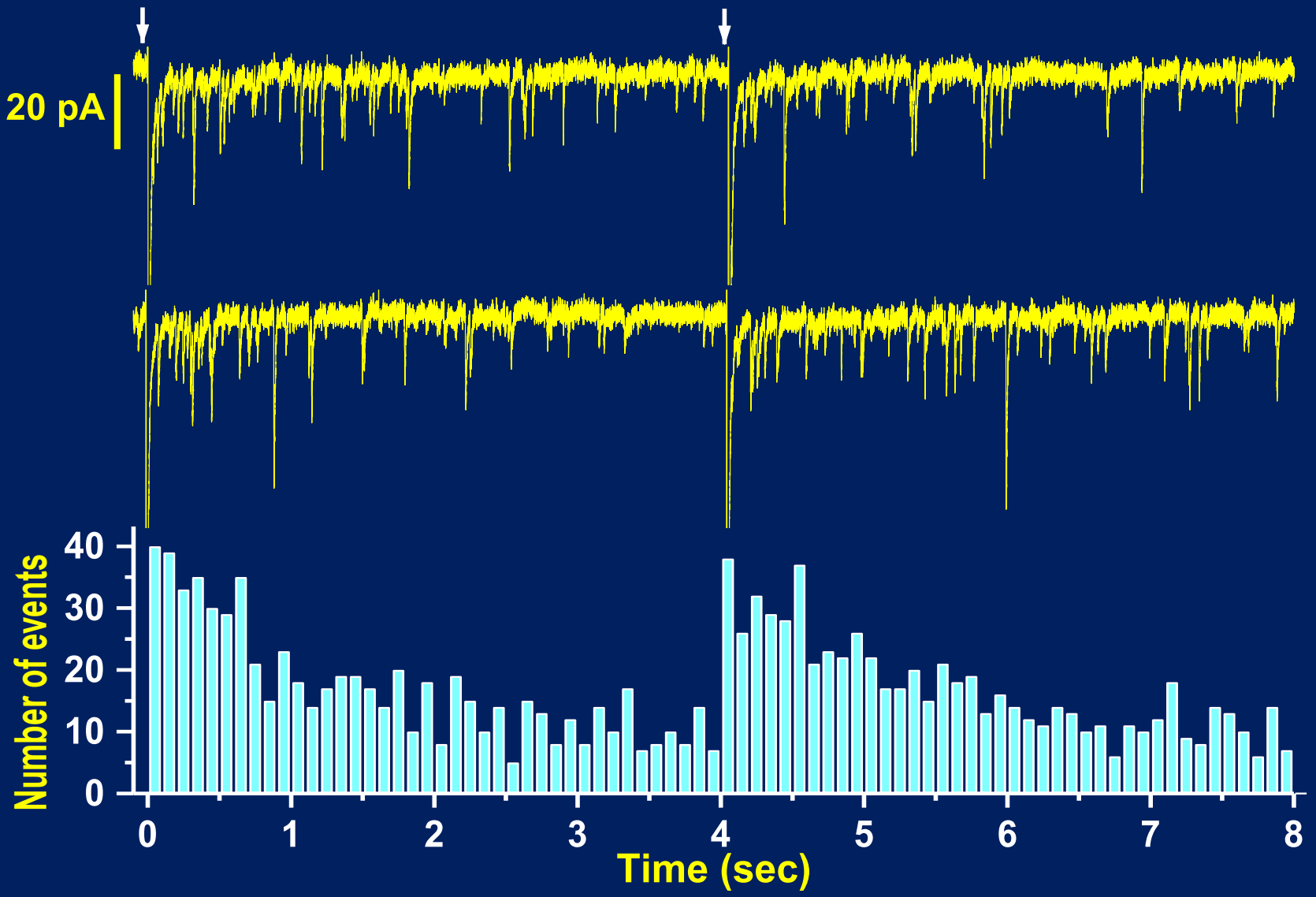
The decrease in miniEPSC frequency by EM persisted in barium

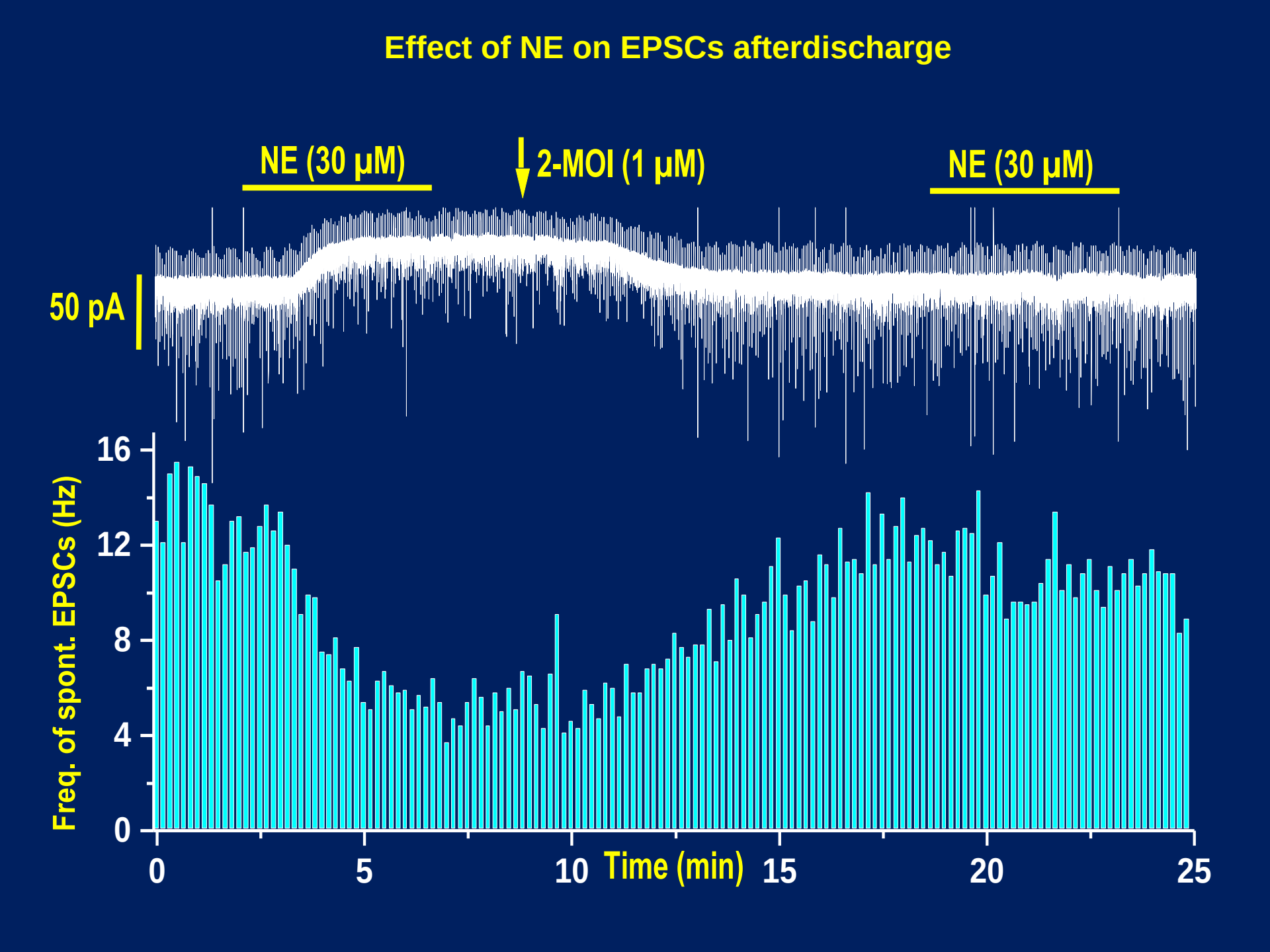


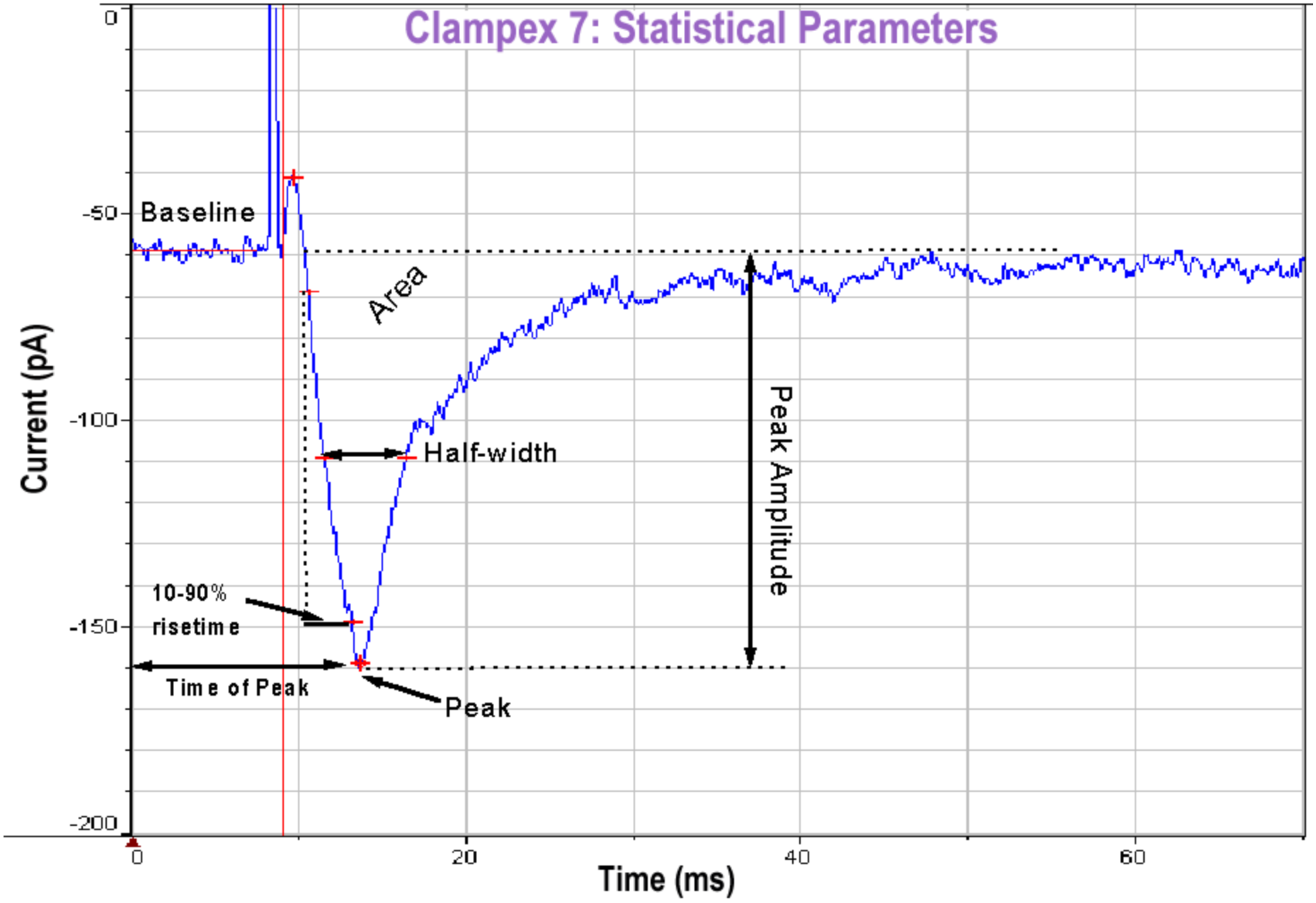
Stimulation paradigm



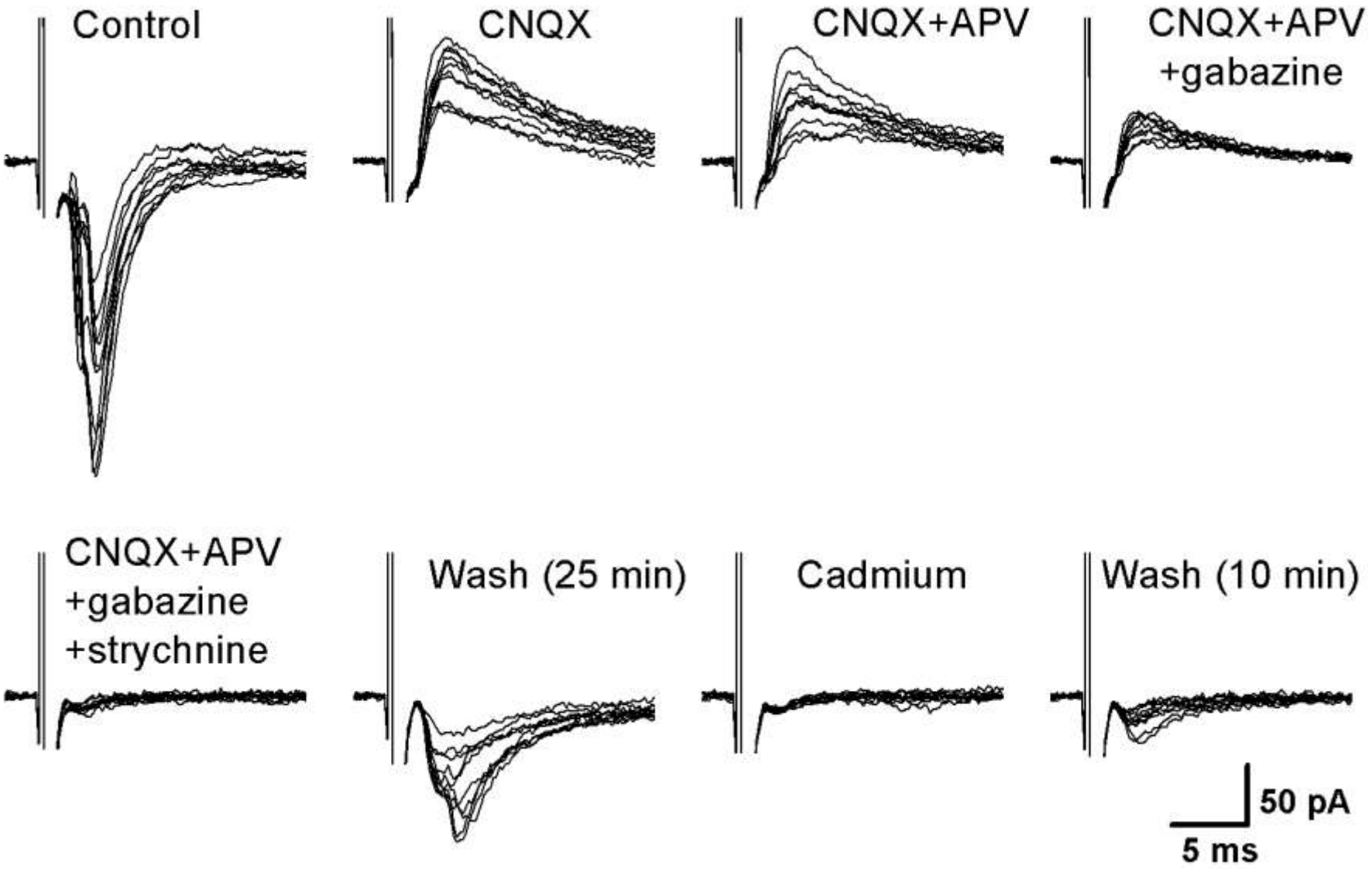
EPSCs afterdischarge




Effect of NE on EPSCs afterdischarge
NE (30 μM)
2-MOI (1 μM)
NE (30 μM)
50 pA
Freq. of spont. EPSCs (Hz)
16
12
8
4
0
0
5
10
Time (min)
15
20
25

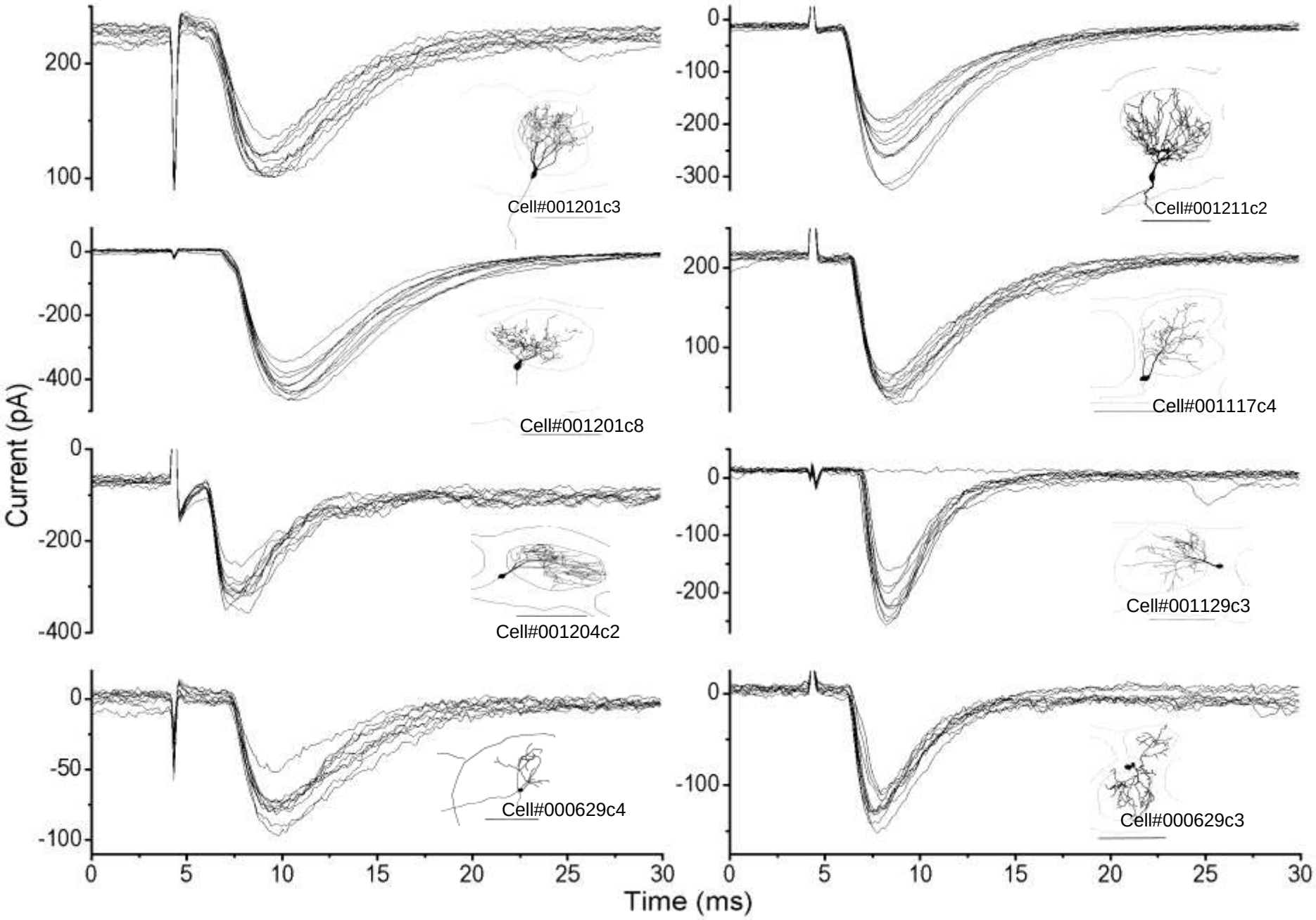

Clampex 7: Statistical Parameters
0
-50
-100
-150
-200
Current (pA)
Baseline
Area
Half-width
Peak Amplitude
10-90%
risetime
Time of Peak
Peak
0
20
40
60
Time (ms)

Pharmacological characterization of evoked PSCs in the SubCD



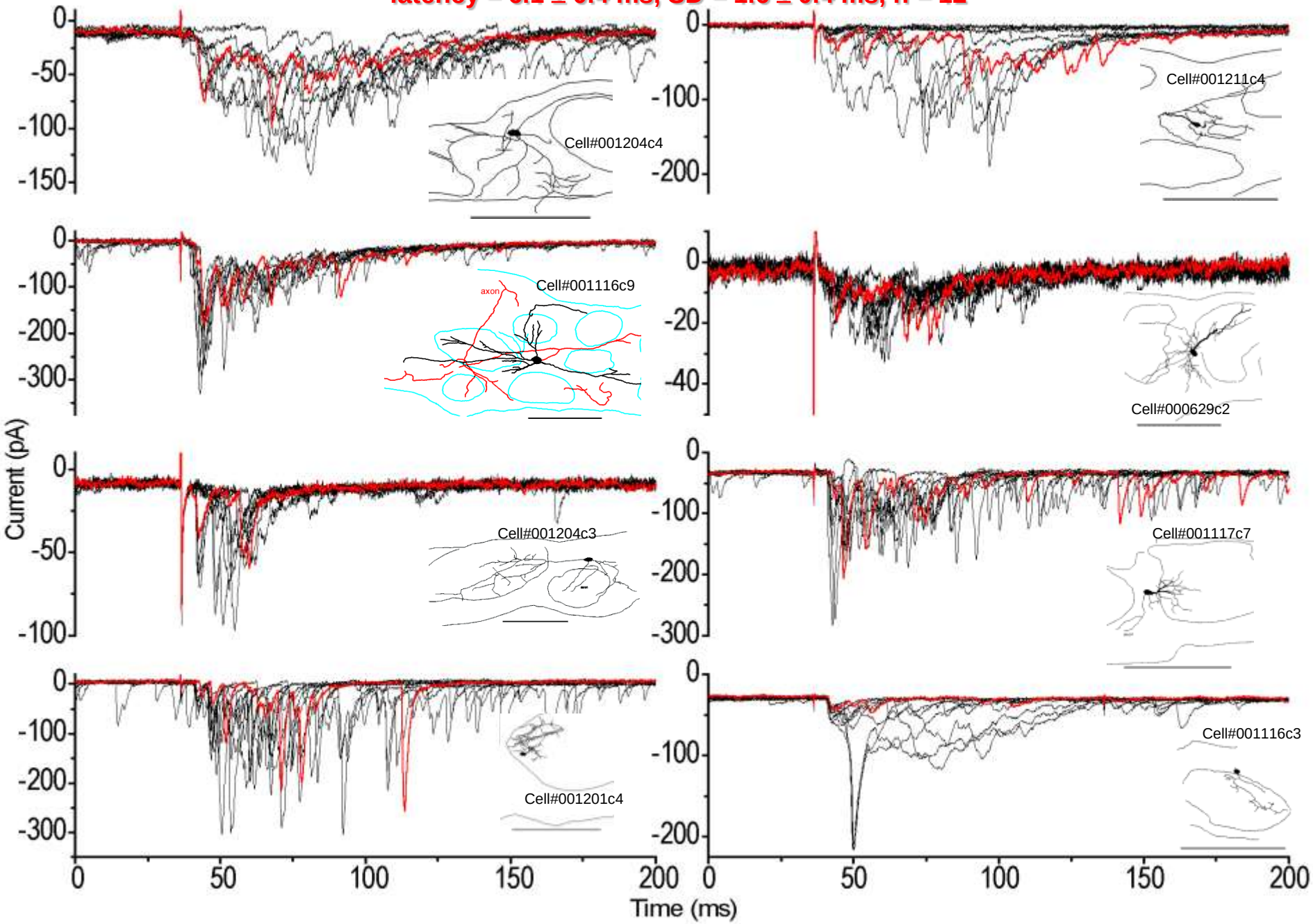
ON stimulation evokes constant latency single EPSCs in ET cells:

latency = 2.1 ± 0.1 ms, SD = 87 ± 9 µs, n = 8

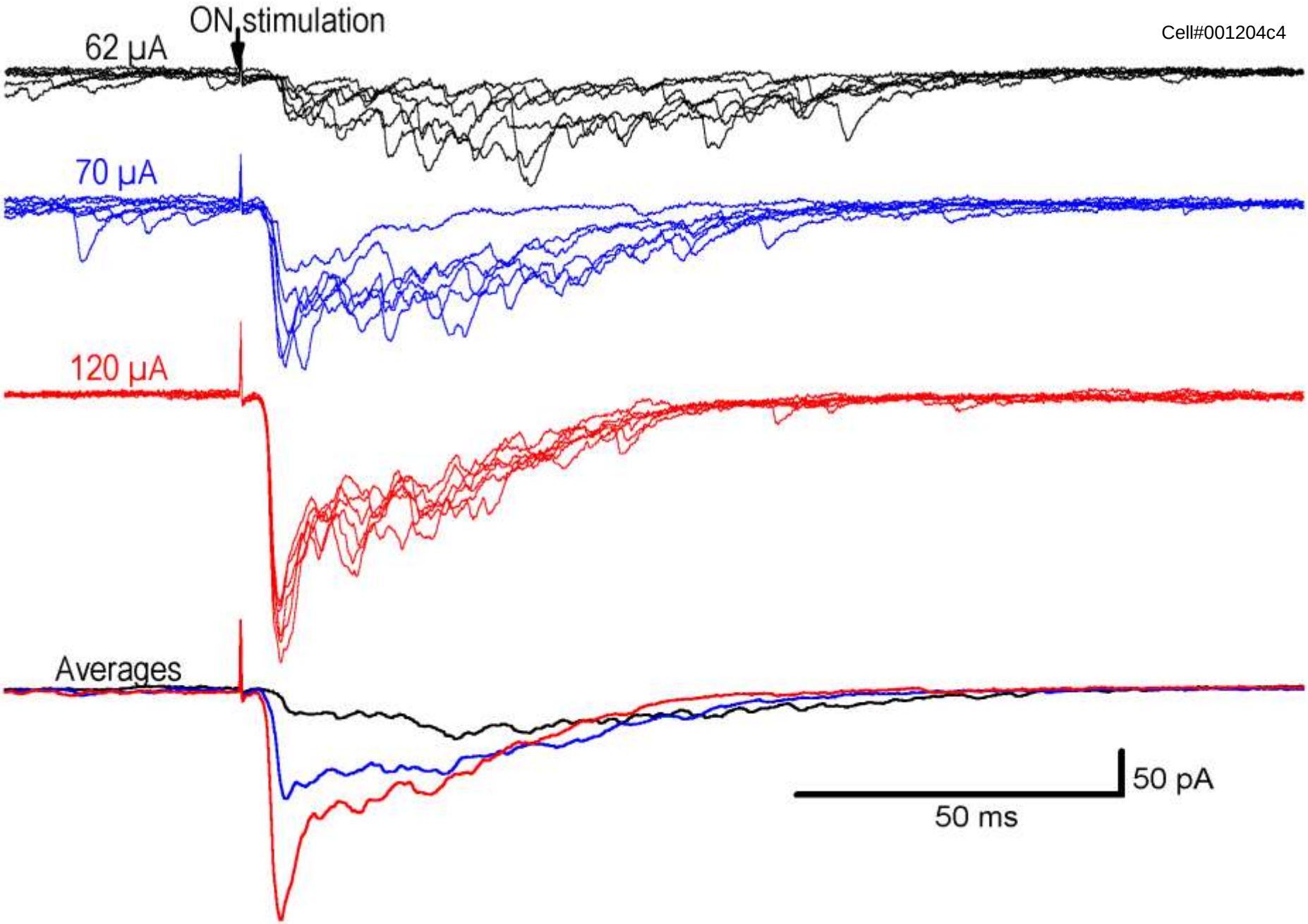


ON stimulation evokes bursts of EPSCs in SA and PG cells

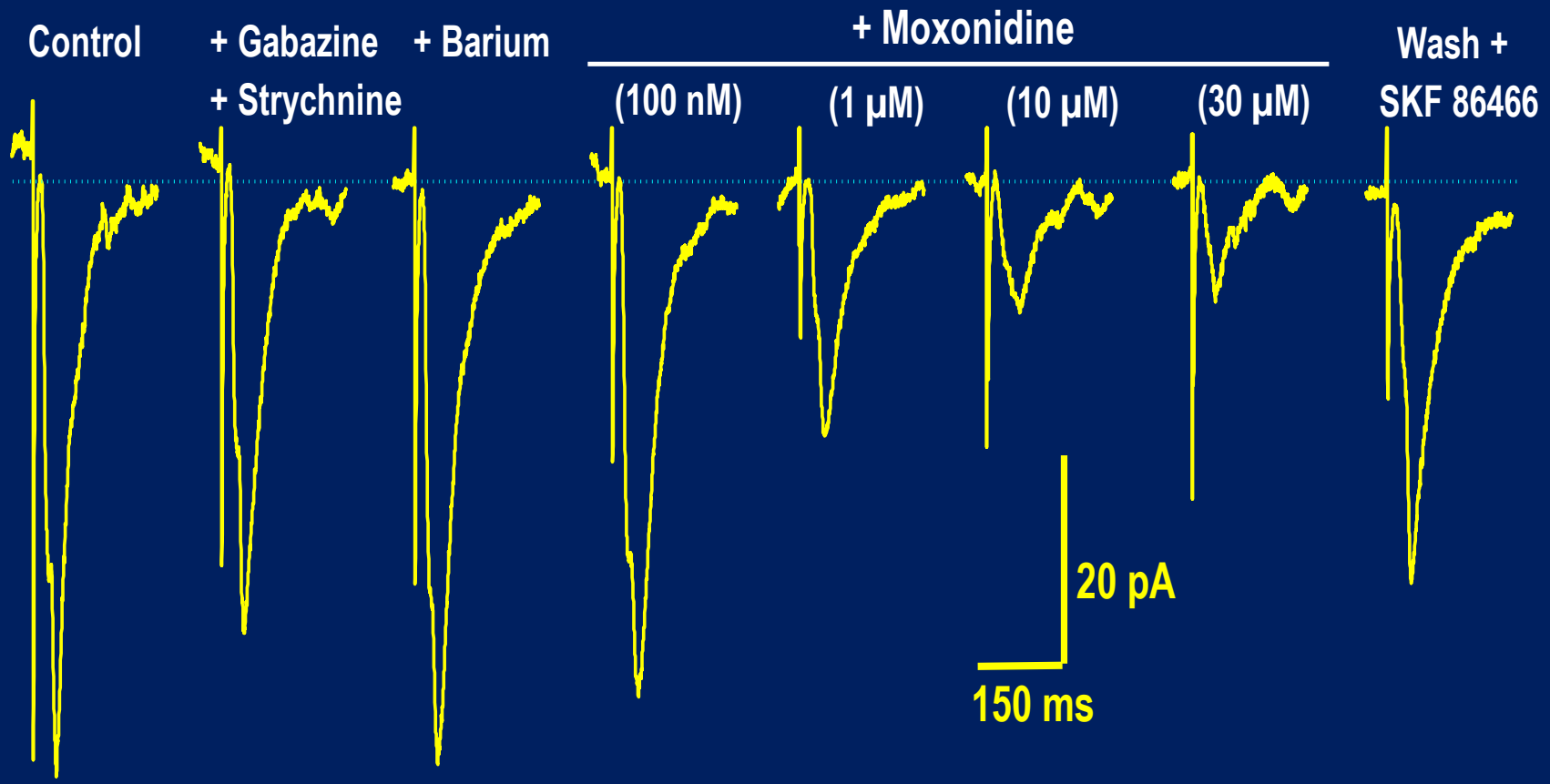
latency = 6.1 ± 0.4 ms, SD = 1.6 ± 0.4 ms, n = 12



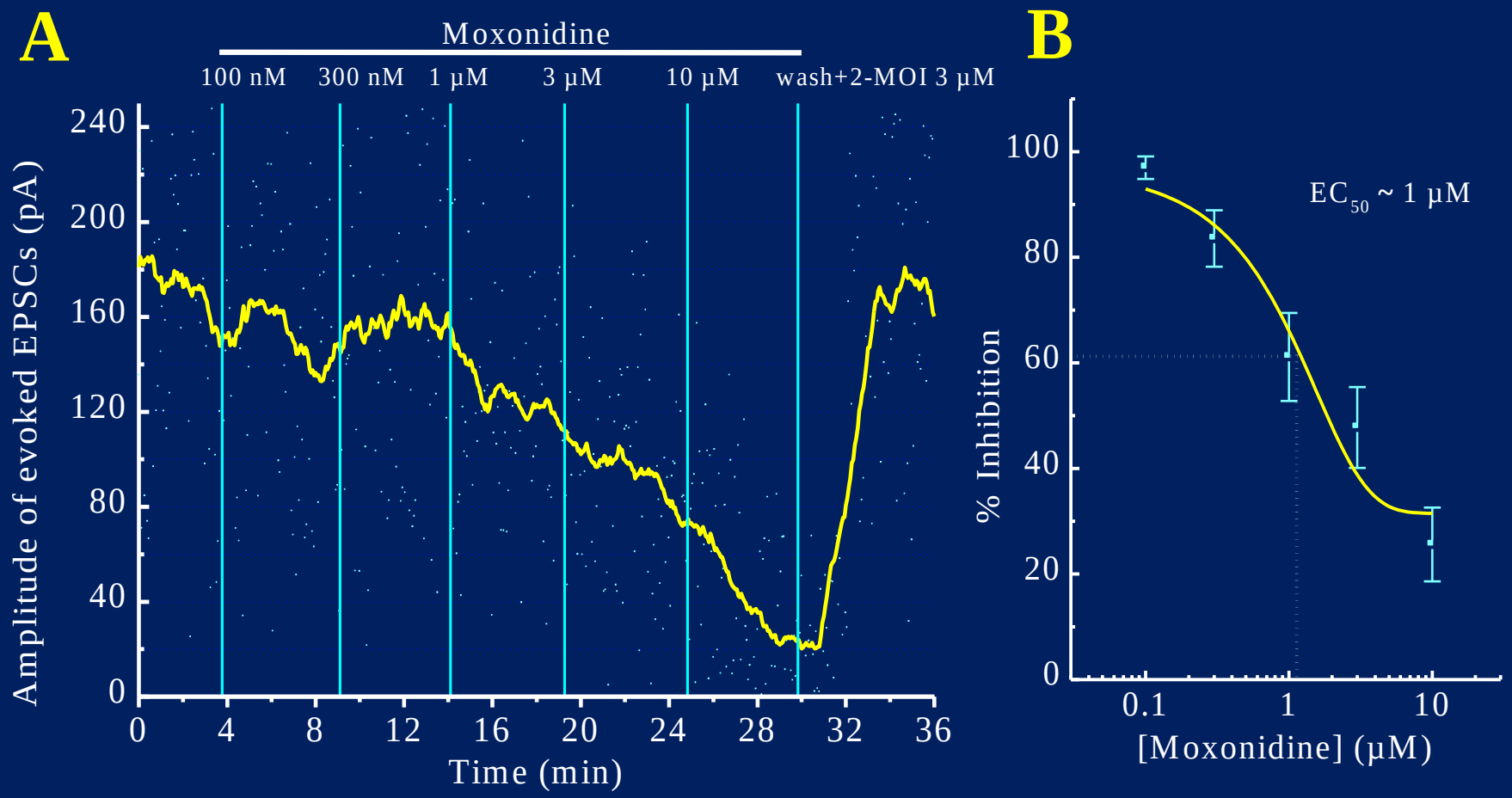
Effect of increasing intensity of stimulation on evoked bursts of EPSCs



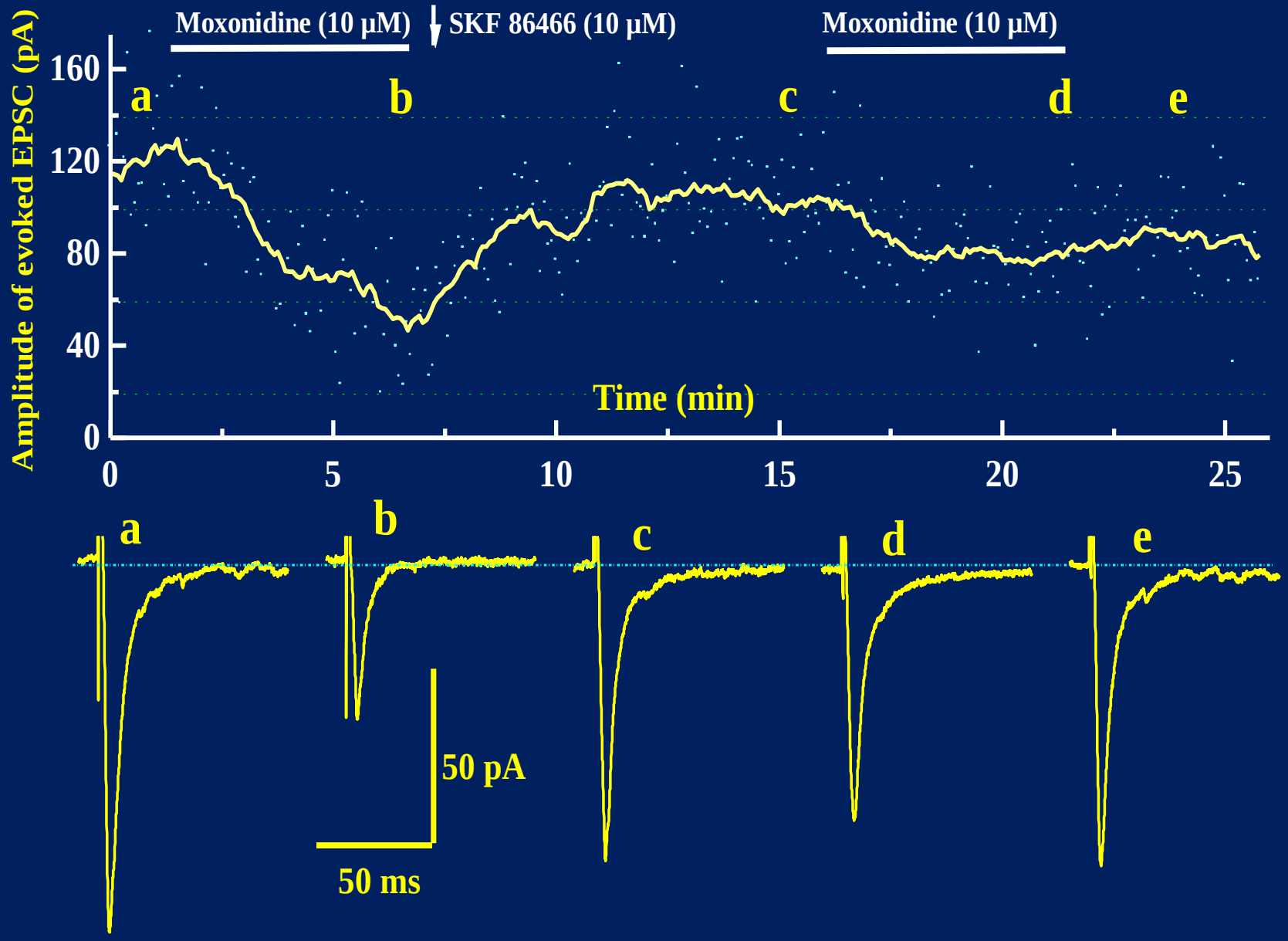
Effect of moxonidine on evoked EPSCs in barium



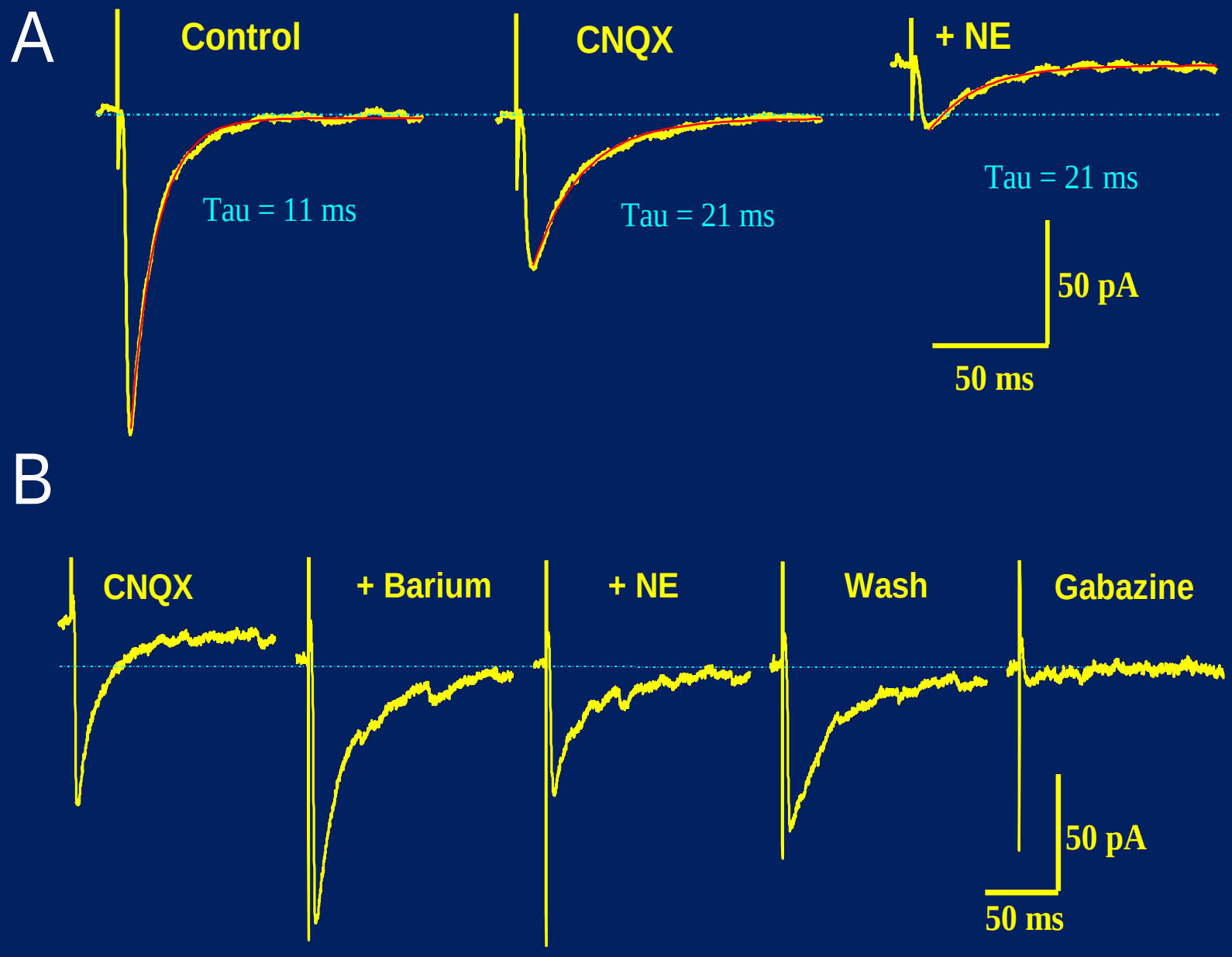
Concentration-dependent inhibition of the evoked EPSCs by moxonidine



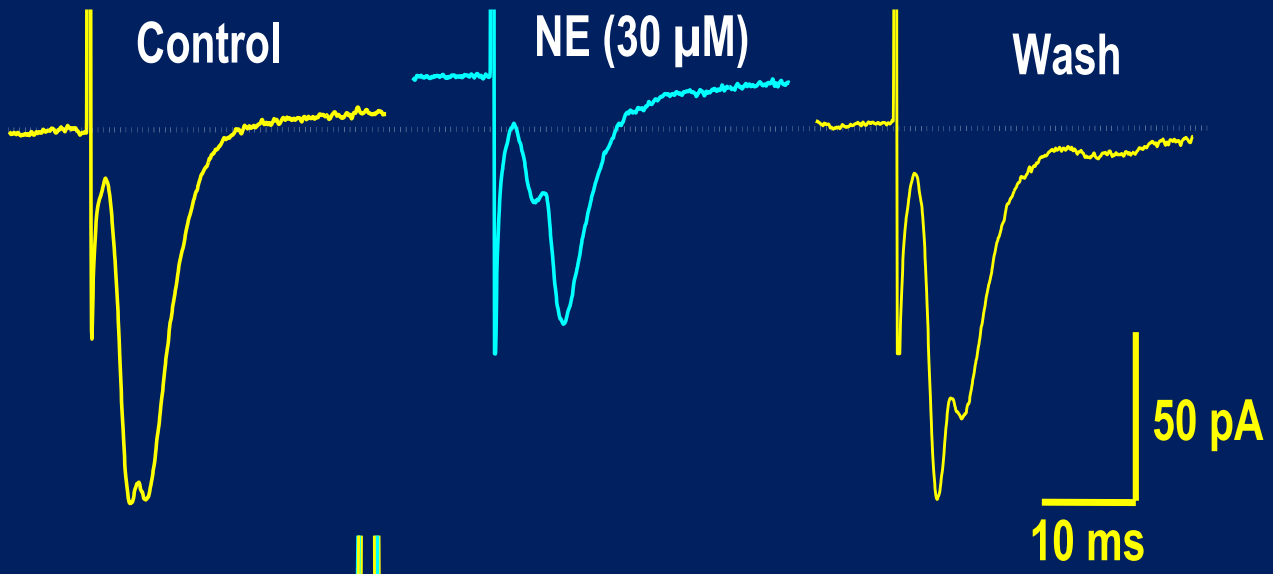
Inhibition of the evoked EPSCs by moxonidine

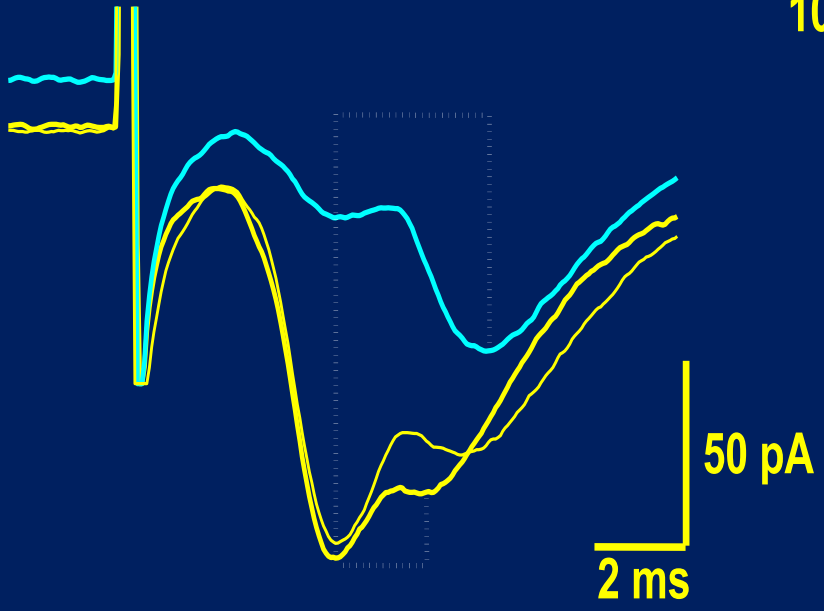


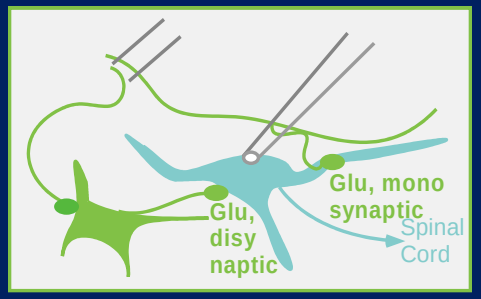
NE inhibits the evoked IPSCs in barium



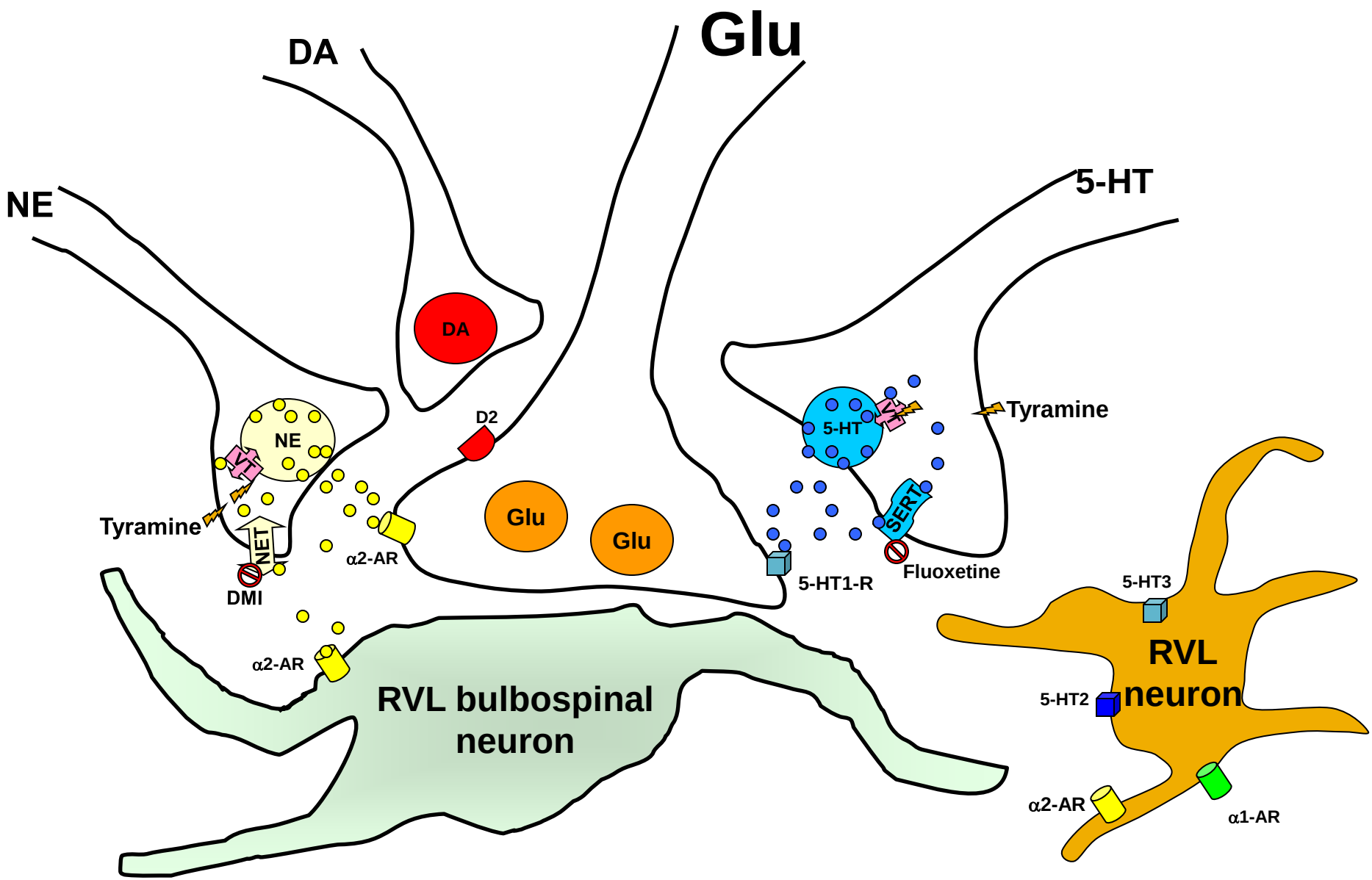
Effect of NE on mono- and disynaptic evoked EPSCs

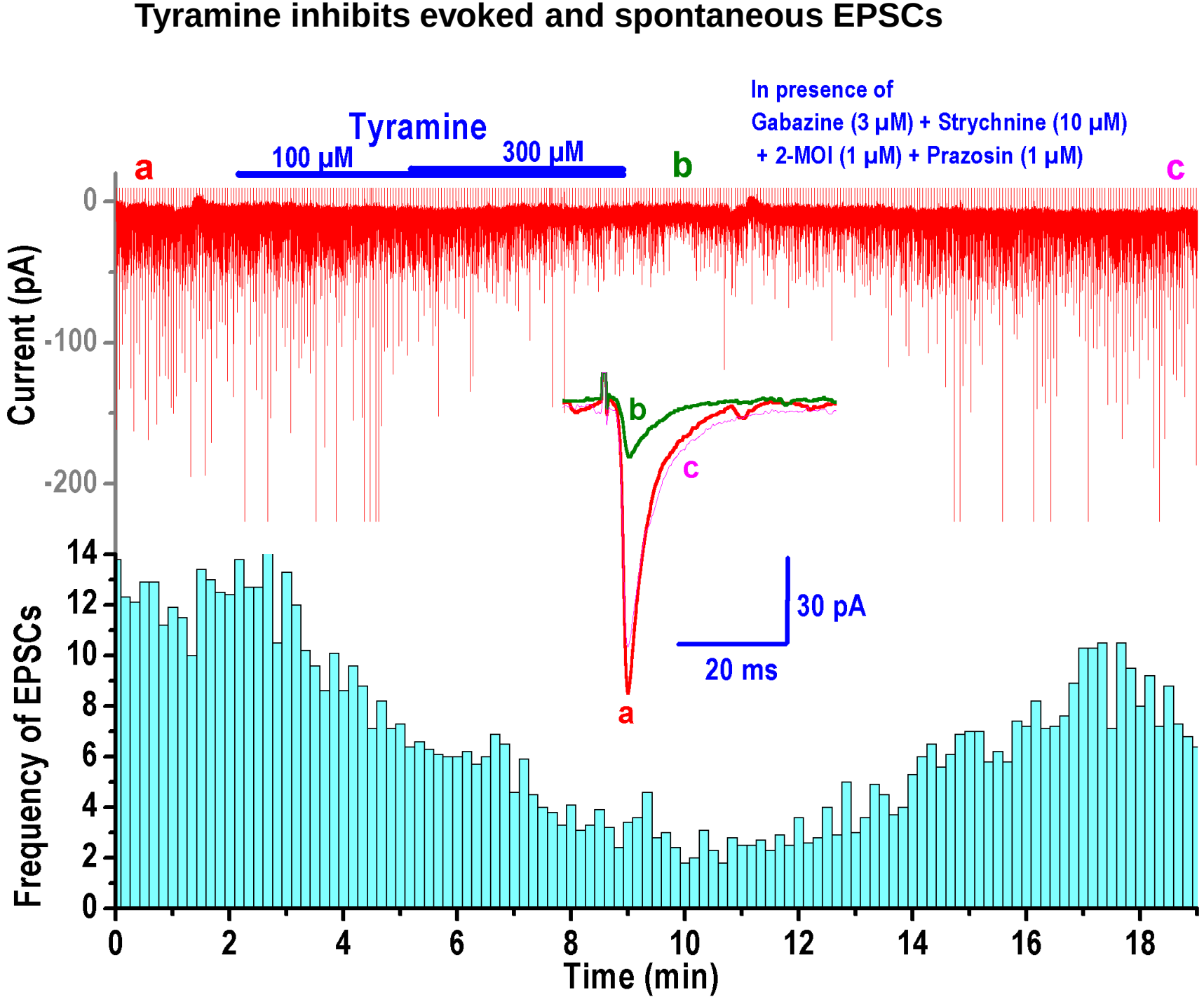




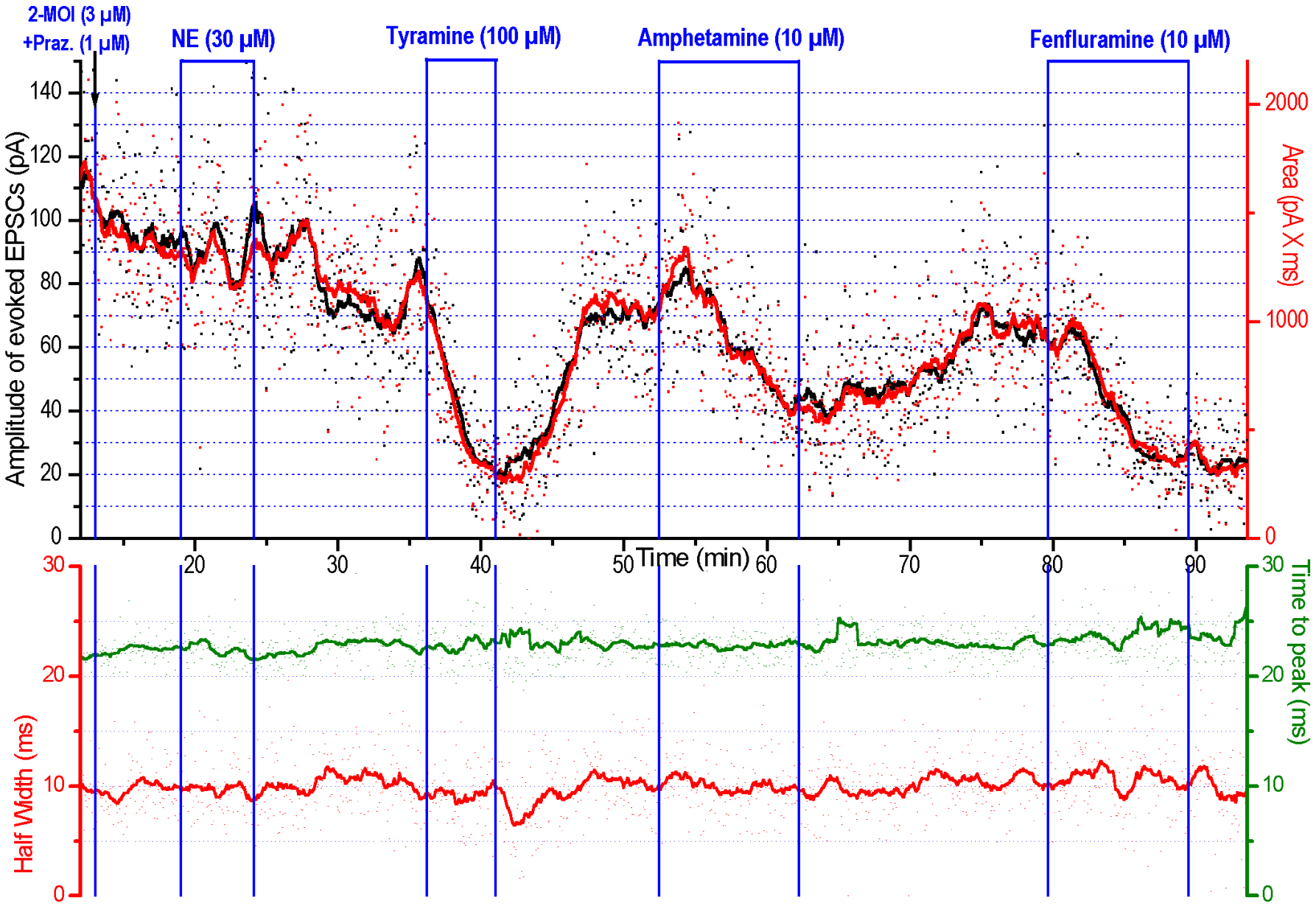


Presynaptic monoaminergic modulation in RVL

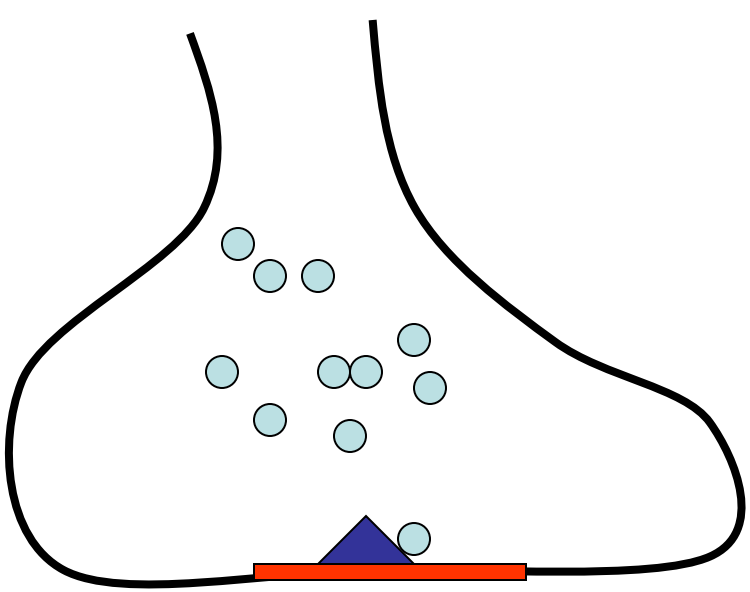


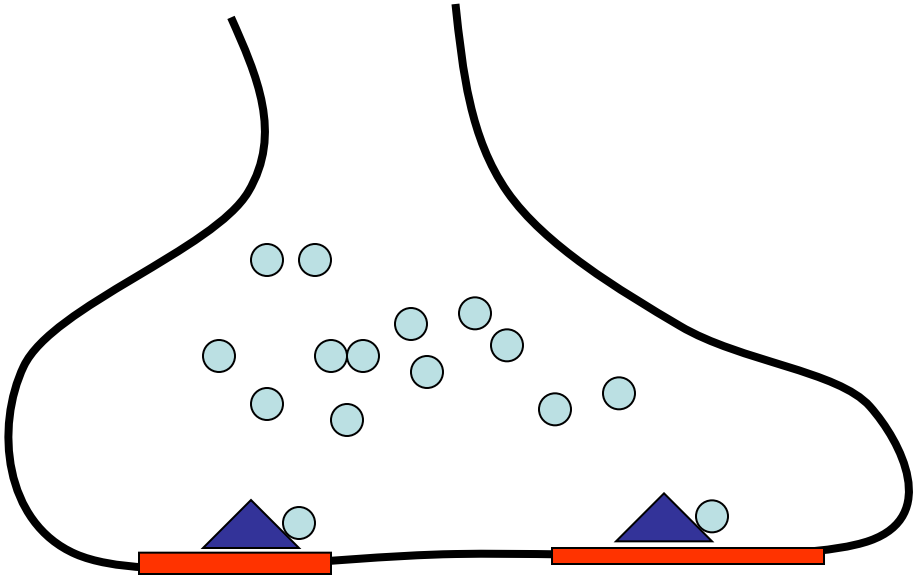

Tyramine inhibits evoked and spontaneous EPSCs
Tyramine
100 µM
300 µM
a
b
c
In presence of
Gabazine (3 µM) + Strychnine (10 µM)
+ 2-MOI (1 µM) + Prazosin (1 µM)
Current (pA)
0
-100
-200
30 pA
20 ms
Frequency of EPSCs
0
2
4
6
8
10
12
14
16
18
Time (min)

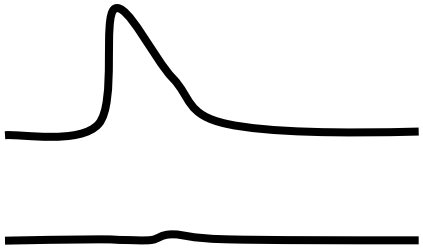
The effect of tyramine is mimicked by other monoamine releasing agents

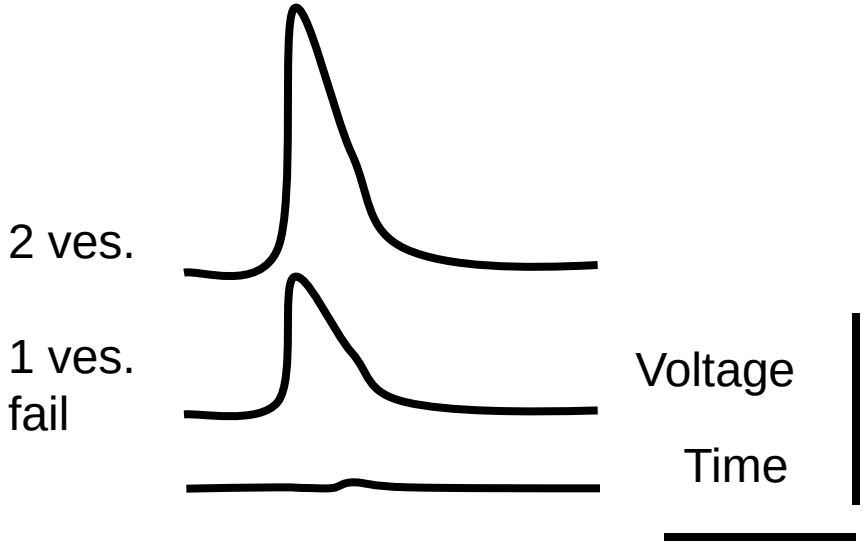


Response types at single CNS synapses with different #s of release sites.

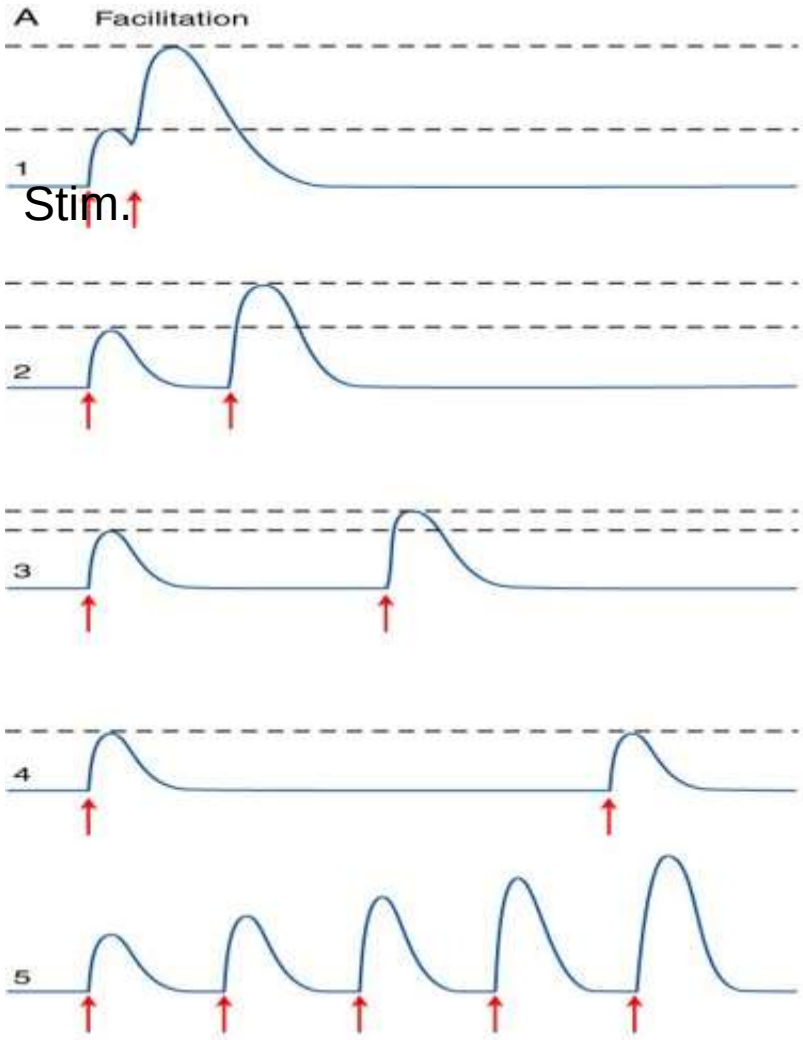


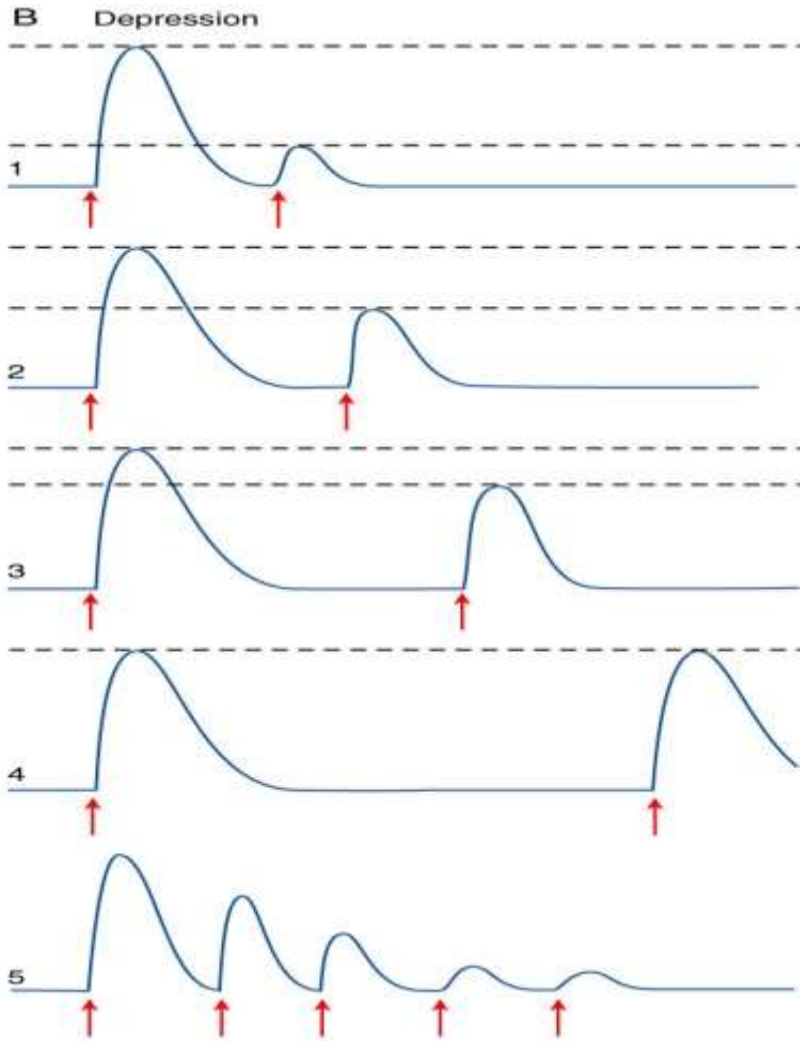






Short term plasticity, history dependent changes in responsiveness

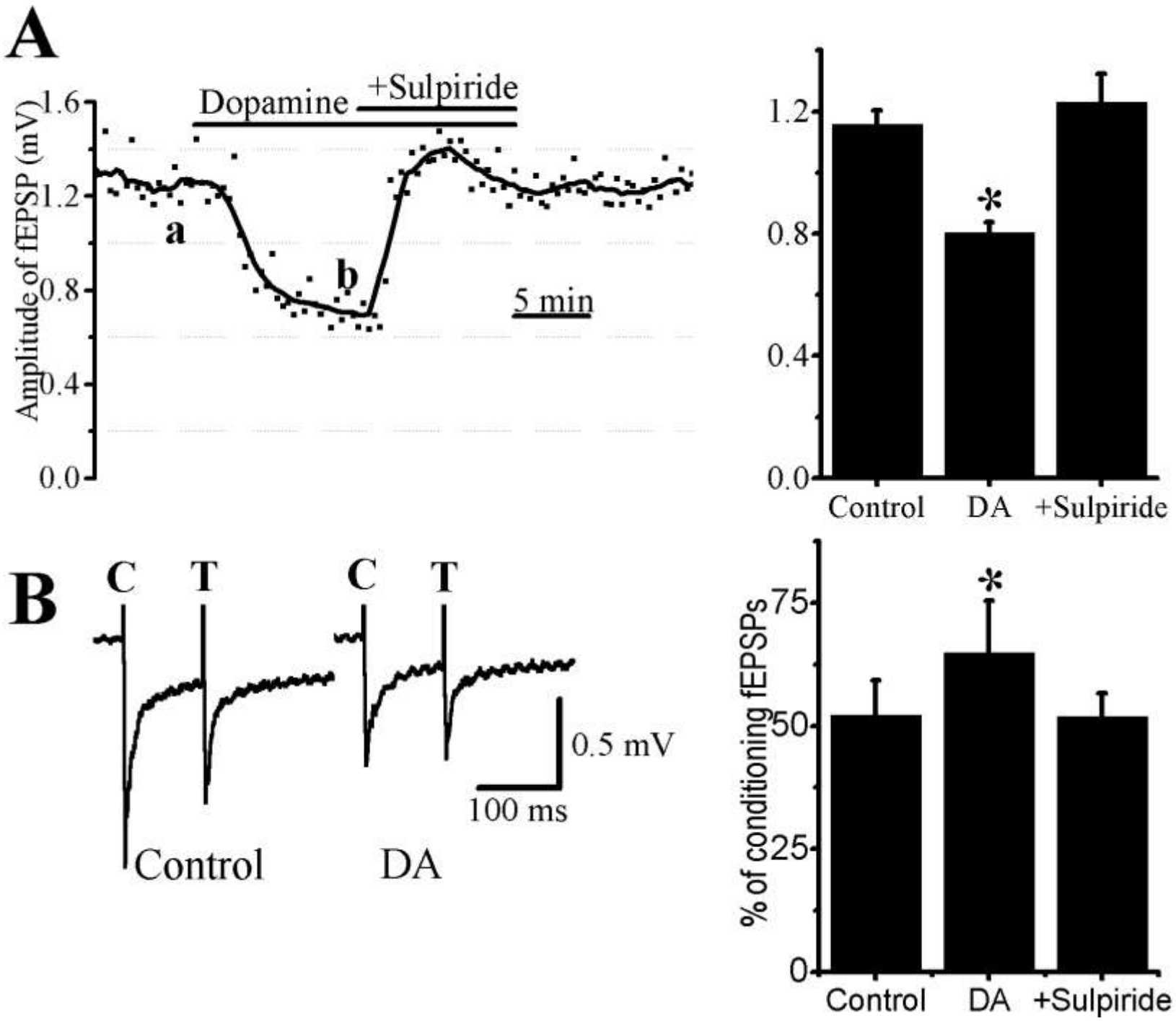




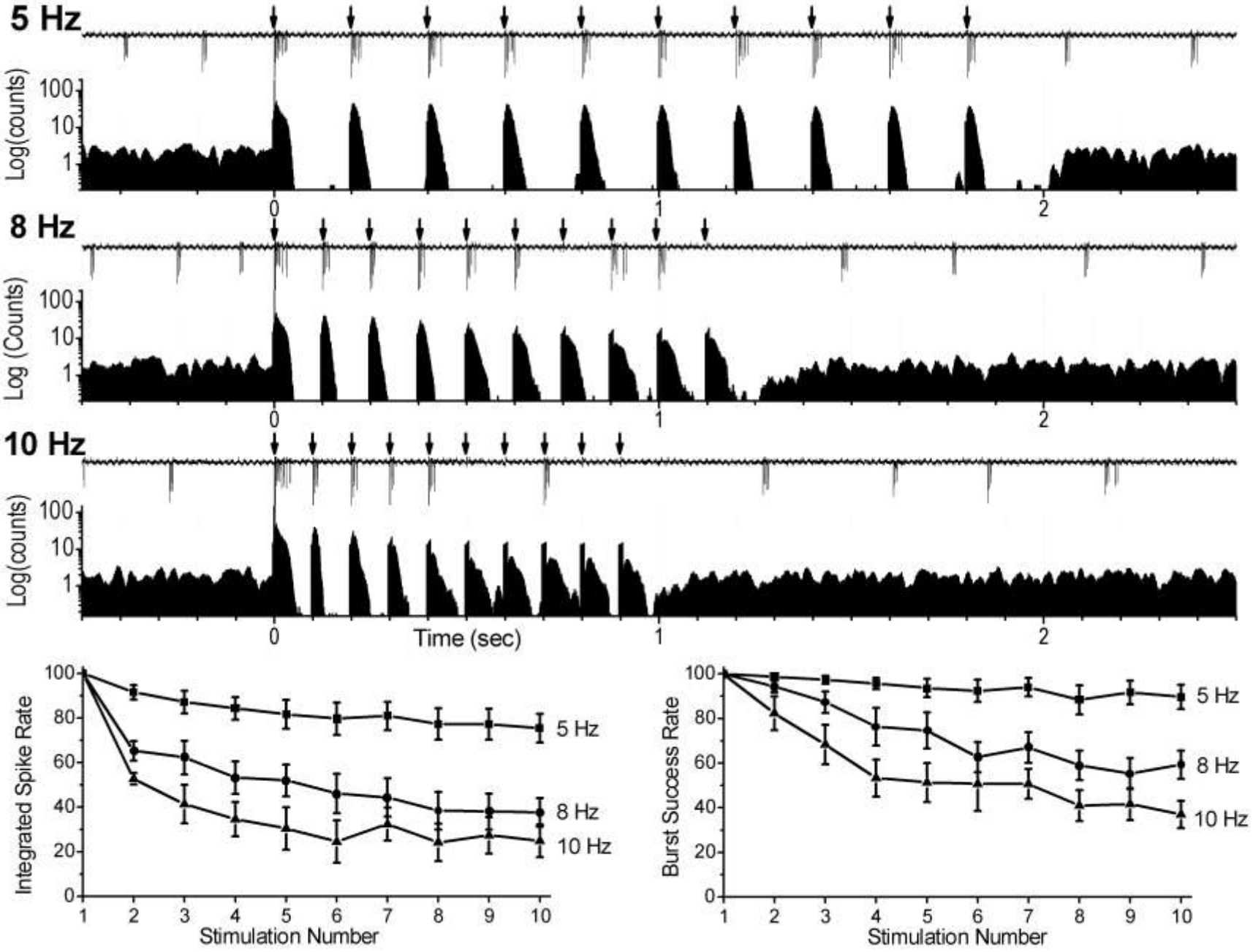
Residual Ca can facilitate transmission if not all quanta are released on the first stimulus.

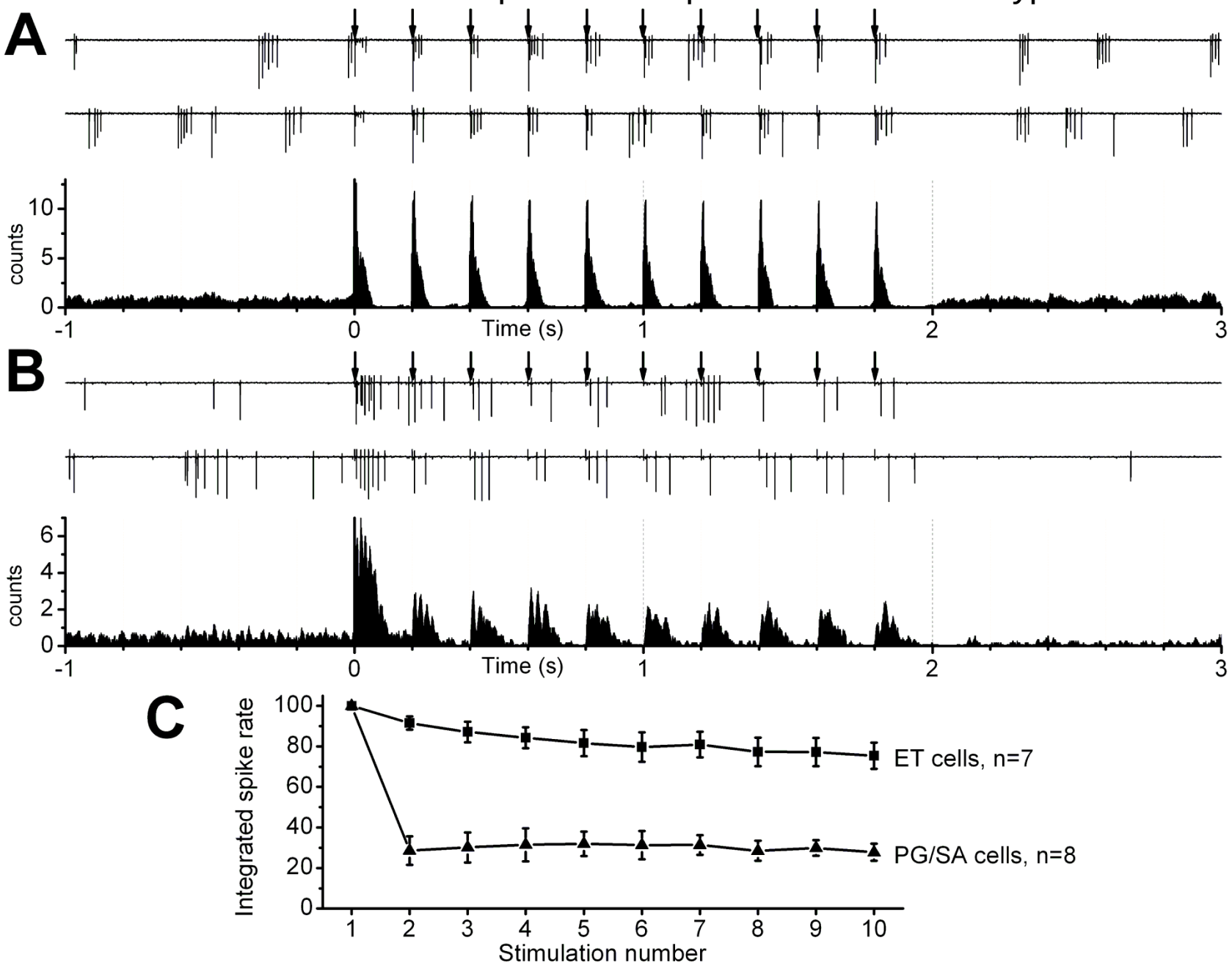
If transmission is robust on the first stimulus most readily releasable vesicles will be gone and depression results.

Squire Fundamental Neurosci. 2002


A
Dopamine
+Sulpiride
Amplitude of fEPSP (mV)
1.6
1.2
0.8
0.4
0.0
a
b
5 min
1.2
0.8
0.4
0.0
*
Control
DA
+Sulpiride
B
C
T
C
T
0.5 mV
100 ms
Control
DA
% of conditioning fEPSPs
75
50
25
0
*
Control
DA
+Sulpiride

ET cells are entrained by ON input at physiological theta frequencies




Paired-Pulse Depression Depends on Neuronal Type
A
counts
10
5
0
-1
0
Time (s)
1
2
3
B
counts
6
4
2
0
-1
0
Time (s)
1
2
3
C
Integrated spike rate
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
Stimulation number
ET cells, n=7
PG/SA cells, n=8