Chulalongkorn University
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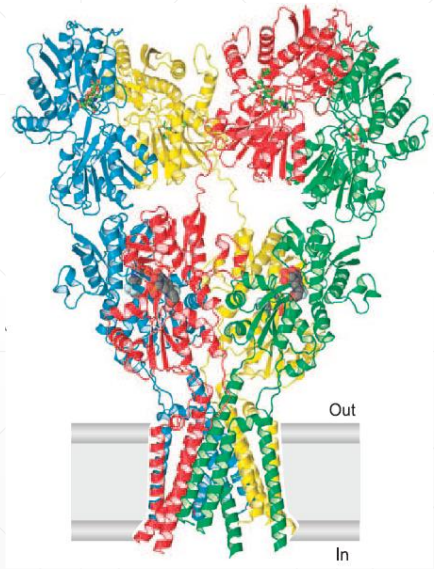
Chulalongkorn Comprehensive Epilepsy Centre

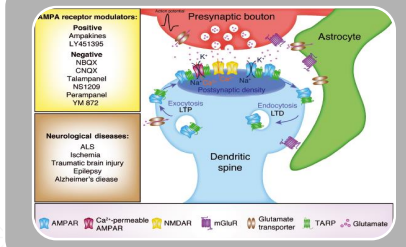


Glutamate receptor network in brain: what is the functions and its implications

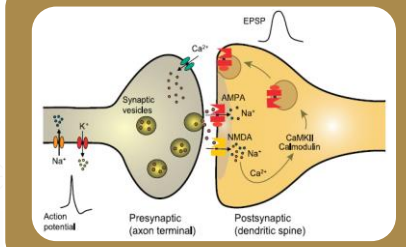
Assist. Prof. Chusak Limotai, MD., M.Sc., CSCN (C)

Chulalongkorn Comprehensive Epilepsy Center of Excellence (CCEC)
Division of Neurology, Faculty of Medicine, Chulalongkorn University

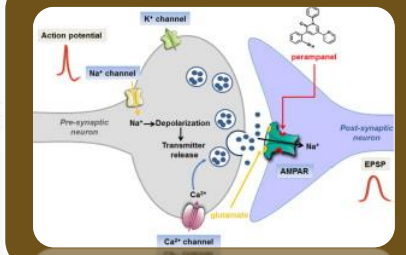
Talk overview



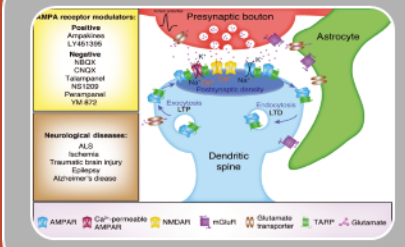
Roles of AMPA receptors in neurological and pyschiatric disorders



AMPA receptors: a therapeutic target for epilepsy



Perampanel in selected epilepsies

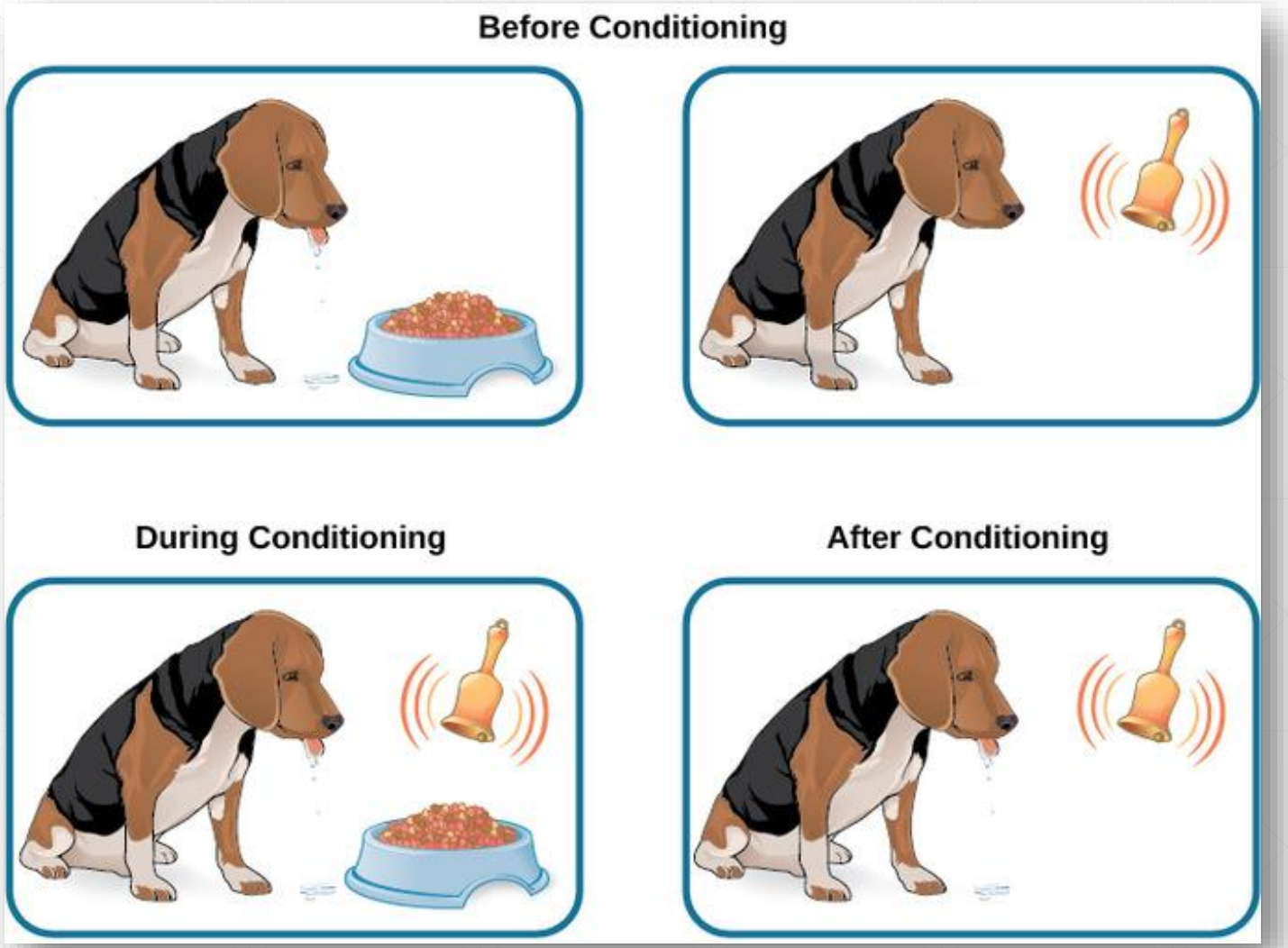


Roles of AMPA receptors in neurological and pyschiatric disorders

Synaptic plasticity

- The capacity of the neural activity **generated by an experience to modify neural circuit function** and thereby modify subsequent thoughts, feelings, and behavior
- Common cellular mechanism for information storage (e.g. memory)
- Play a central role in the capacity of the brain to **incorporate transient experiences into persistent memory traces**
- **Maladaptive synaptic plasticity** may contribute to neurological diseases and neuropsychiatric disorders

Pavlovian classical conditioning

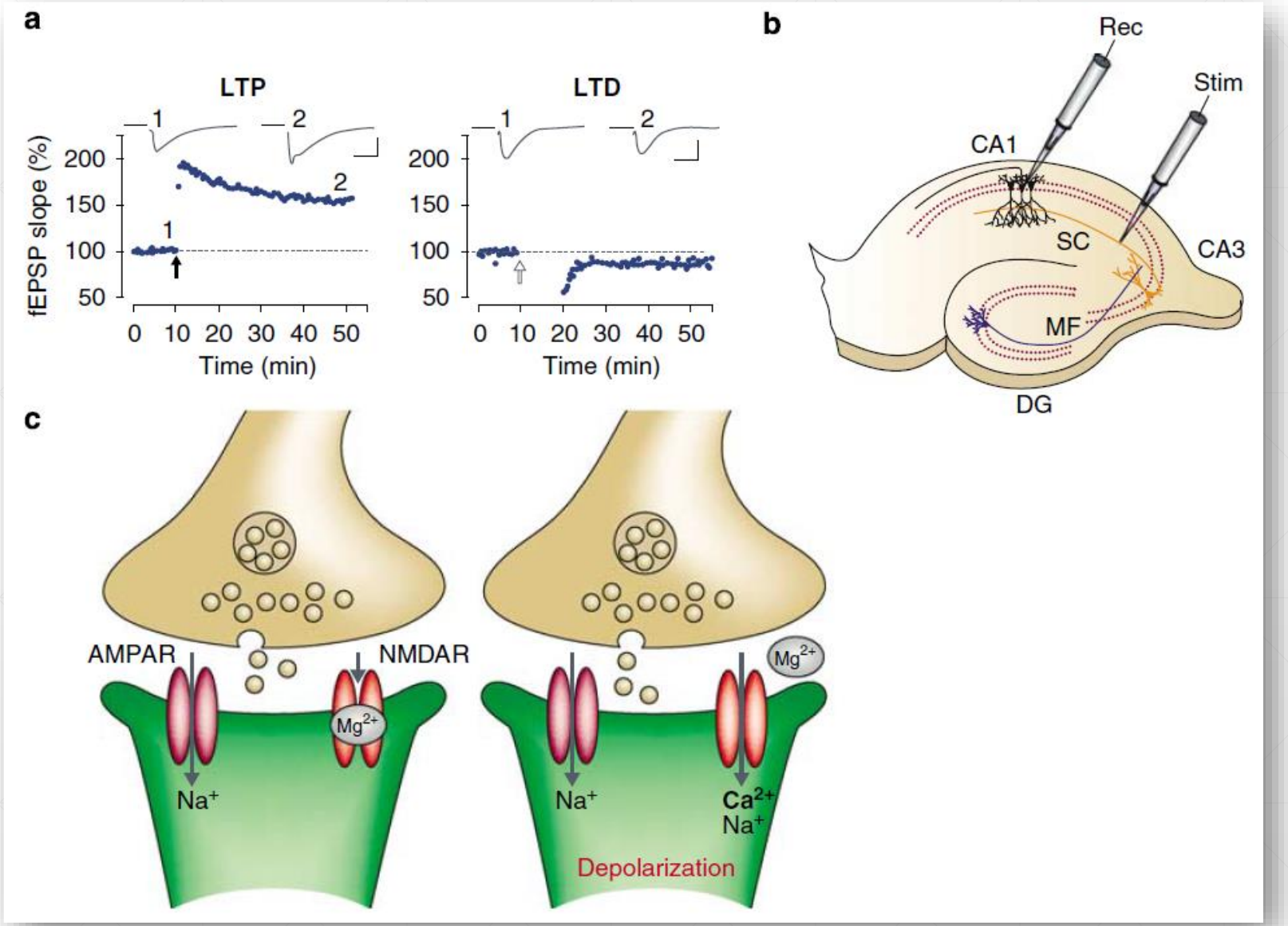


Major forms of synaptic plasticity at excitatory synapses in the mammalian brain

- **Long-term potentiation (LTP)**
- **Long-term depression (LTD)**

NMDAR-dependent LTP and LTD at hippocampal CA1 synapses

Mechanism for learning and memory

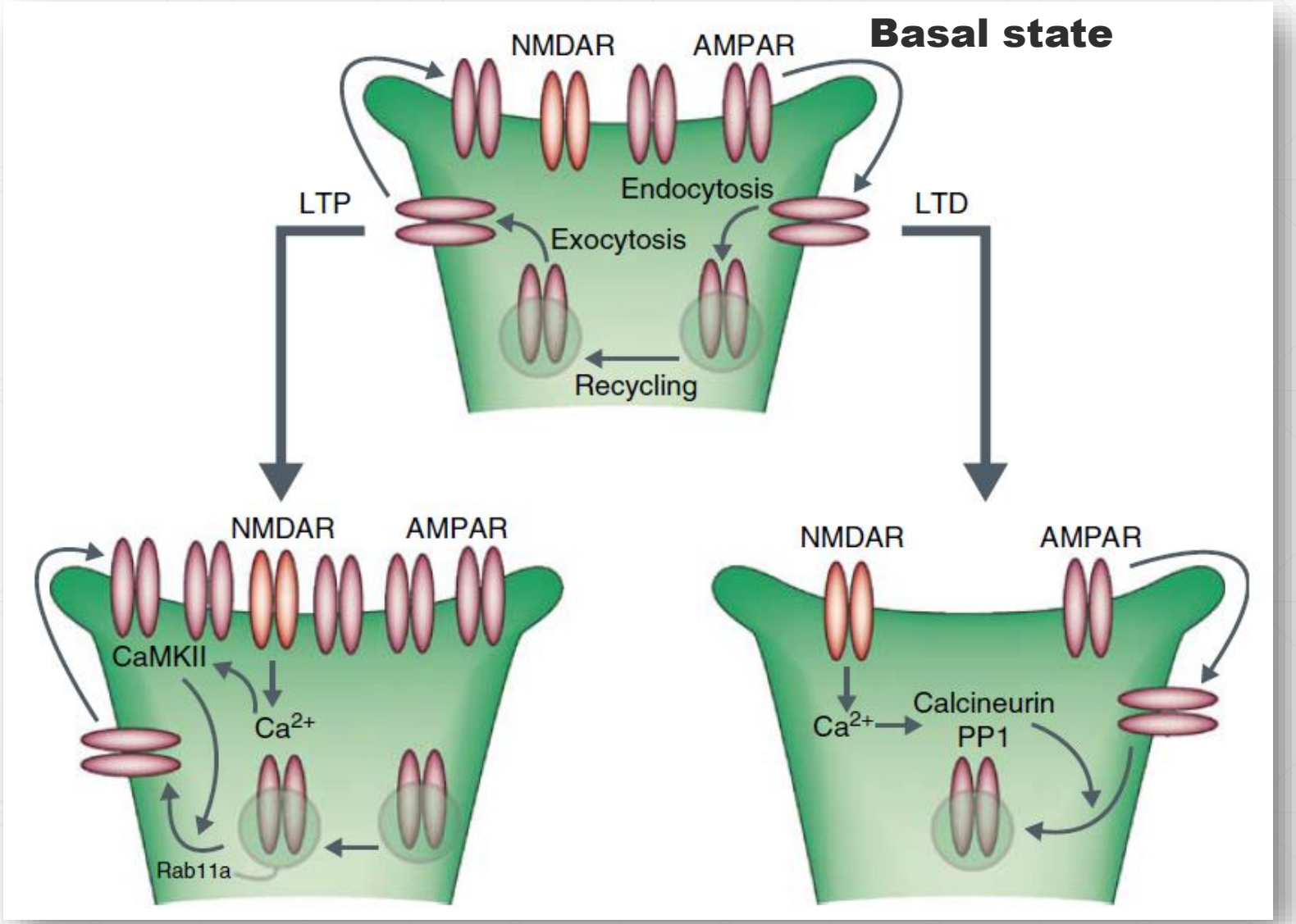


Citri A and Malenka RC.; Neuropsychopharmacology 2008

Model of "AMPAR trafficking" during LTP and LTD

LTP: enhanced exocytosis
LTD: enhanced endocytosis

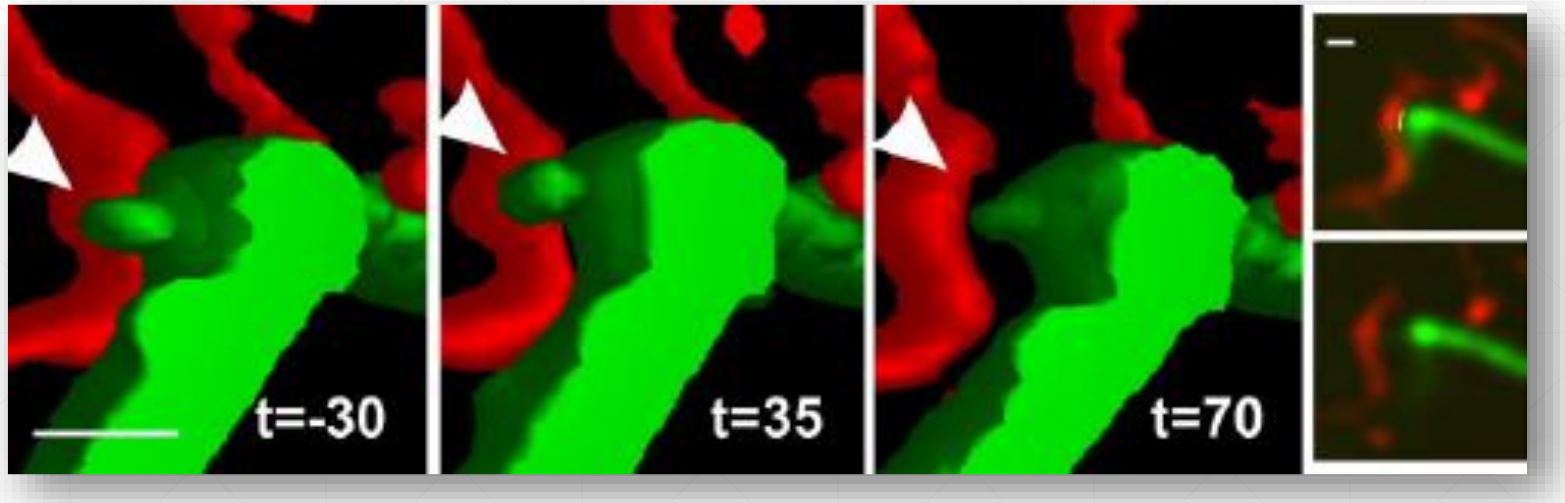
- **CaMKII** promotes the incorporation of additional AMPARs into the postsynaptic density (PSD)
- **Calcineurin and protein phosphatases 1 (PP1)** promote endocytosis



Citri A and Malenka RC.; Neuropsychopharmacology 2008

Morphological growth or shrinkage of synapses

- LTP: drives an increase in the size of the presynaptic active zone such that the potentiated synapses are ‘permanently’ enlarged.
- LTD is accompanied by a shrinkage in the size of dendritic spines and that this may be due to the loss of AMPARs



Persistent synapse loss after LTD with a reduction in spine volume

Nagerl et al, 2004; Zhou et al, 2004; Hsieh et.al 2006
Bastrikova N et.al.; PNAS 2008

Morphological growth or shrinkage of synapses

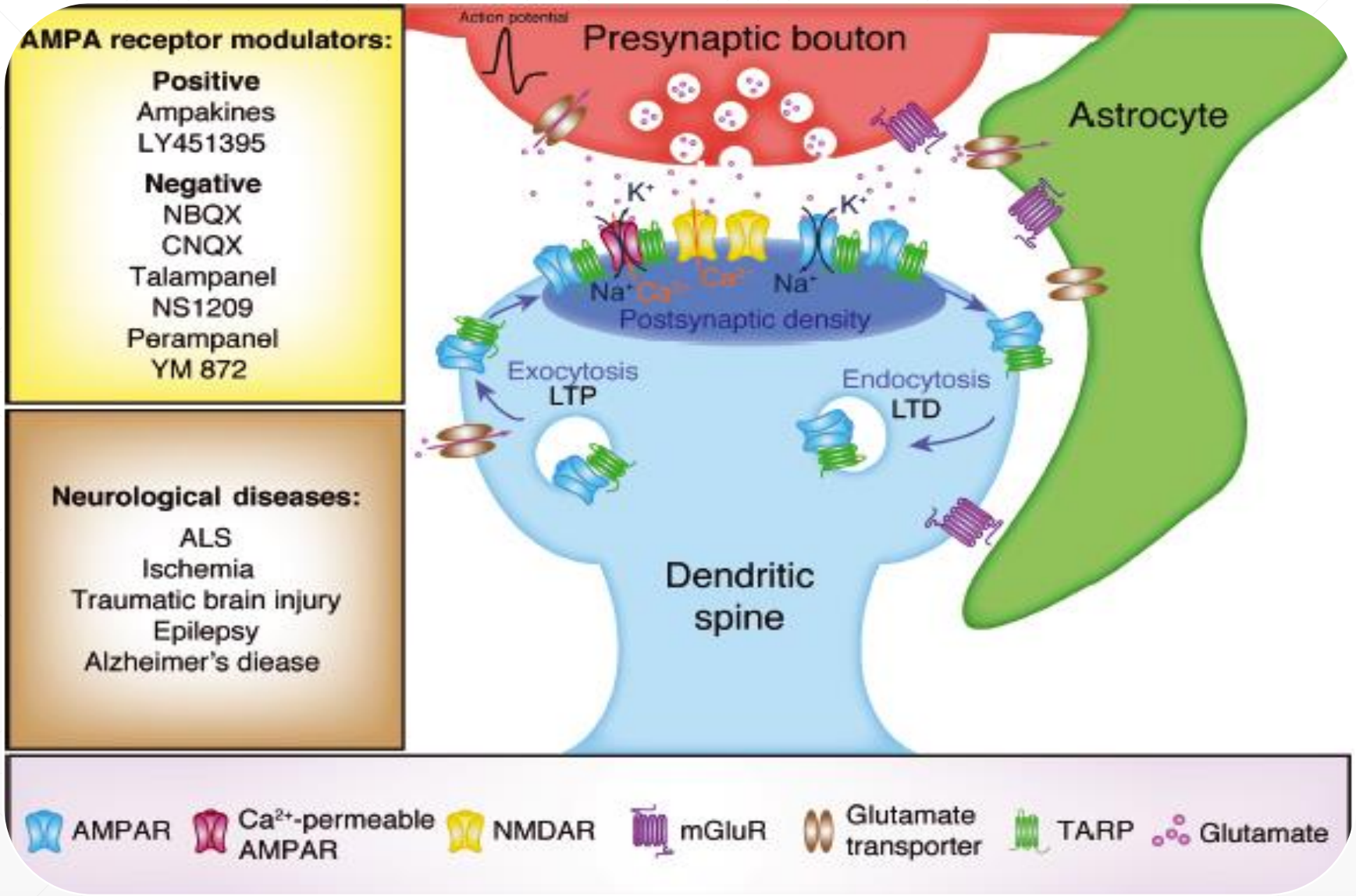
- Dendritic spine volume seems to correlate with AMPA receptor content, so bigger spines have more AMPA receptors and represent **stronger synapses**
- AMPA receptor activation is known to be required for maintaining dendritic spines

Harris et al., 1992; Matsuzaki et al., 2001; Kasai et al., 2010

McKinney et al., 1999

Any dysregulation of glutamate receptor trafficking will play a role in many neurological disorders

Neurological and psychological and psychiatric disorders



Chang PK et.al.; Eur J Neurosci

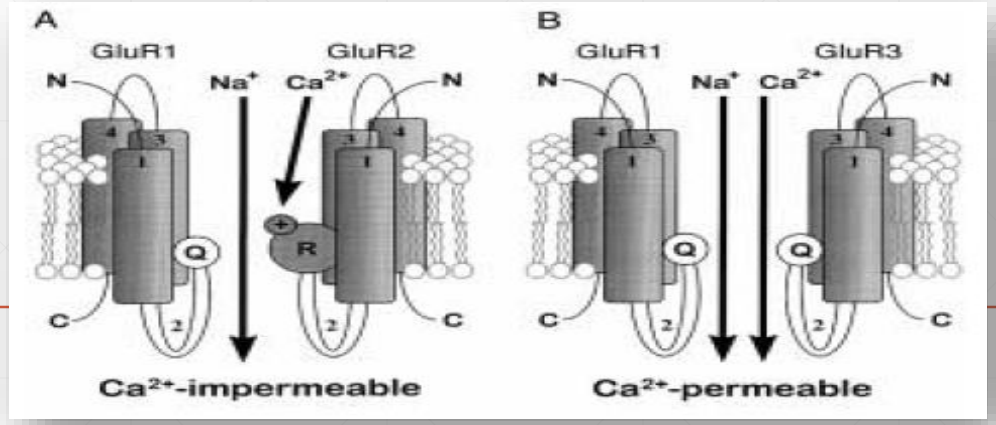
AMPA receptor

- Tetramers from combination of the protein **subunits GluA1-4 (formerly GluR1-4)**
- At most excitatory synapses onto principal neurons-which are localized to dendritic spines-AMPA receptors contain the **GluA2 subunit.** Most AMPA receptors are permeable to **sodium and potassium** but **NOT CALCIUM**

  - Presence of the GluR2 subunit restricts calcium entry and renders the receptor calcium-impermeable

  - A fall in GluR2 subunit levels render AMPA receptor calcium-permeable causing excitotoxicity which is associated with a number of neurologic diseases

- **GluR2-lacking AMPA receptor (made up of GluR1, GluR3, and GluR4) are calcium permeable**

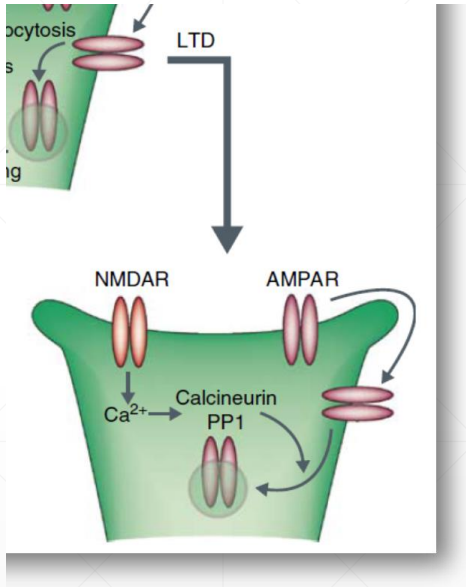


Pellegrini-Giampietro DE et.al; TINS 1997
Tanaka H et.al; Brain Res 2000
Jayakar SS and Dikshit M; Intern J Neuroscience 2004

Alzheimer's disease (AD)

- AD-inducing oligomeric **amyloid-b protein (Ab) enhances LTD** at CA3–CA1 hippocampal synapses, perhaps accounting for the **decreased spine density on CA1 neurons** that may contribute to the cognitive decline seen in AD patients.

- LTP is impaired but LTD is facilitated in the presence of soluble Ab



Shankar et al., 2008; Shankar et.al. 2007; Wei et al. 2010

Kim et al., 2001; Selkoe, 2002

ALS

- Any disruption that allows excessive Ca2+ to be present inside neurons can trigger a cascade leading to neuronal death, known as **“excitotoxicity”**
- Notably, spinal neurons contain relatively **low amounts of GluA2** mRNA, making them **Ca2+- permeable**
- This presumably increases the number of Ca2+-permeable AMPA receptors, converting normal AMPA-mediated activity into AMPA-mediated excitotoxicity

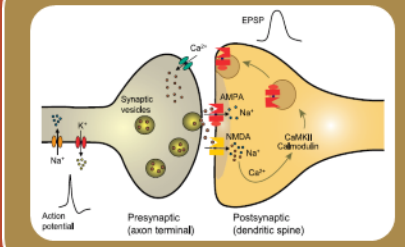
Lau & Tymianski, 2010
Kawahara et al., 2003
Kwak et al., 2010

Schizophrenia

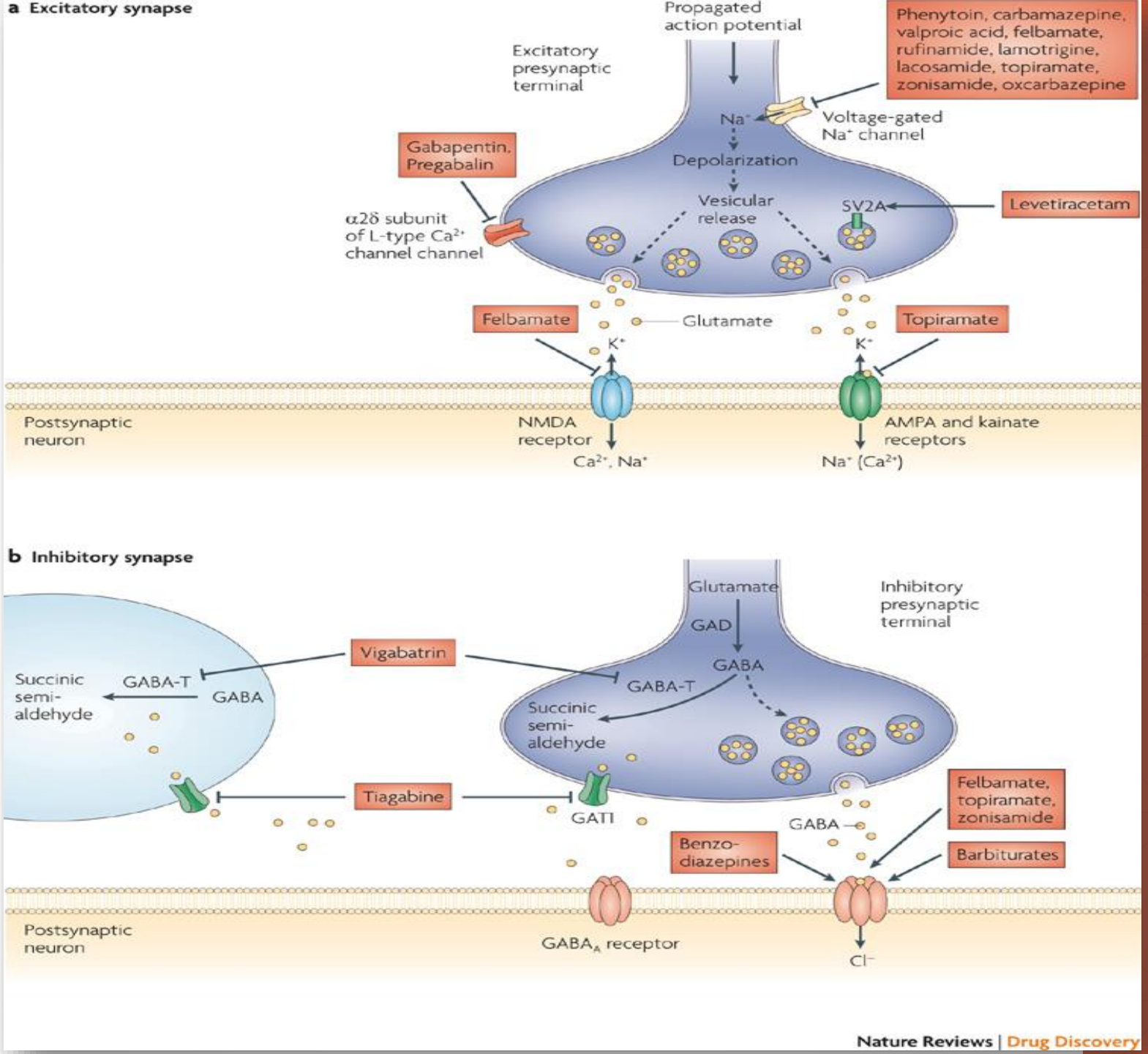
- **NMDA receptor dysfunction** hypothesis of schizophrenia has subsequently been refined to include **abnormalities of other glutamate receptor subtypes** (AMPA, kainate, and metabotropic receptors), glutamate transporters, and glutamatergic enzymes
- Clinical studies have suggested that **ampakines, AMPA receptor positive modulators**, can **improve cognitive function in schizophrenia**, while enhancement of AMPA receptor-mediated currents by these compounds may **potentiate the efficacy of antipsychotics**
- Evidence of decreased GluR2 subunit expression and increased expression of stargazing (TARP) in DLPFC in schizophrenia

Coyle, 1996

Beneyto M & Meador-Woodruff JH, Synapse 2006



AMPA receptors: a therapeutic target for epilepsy



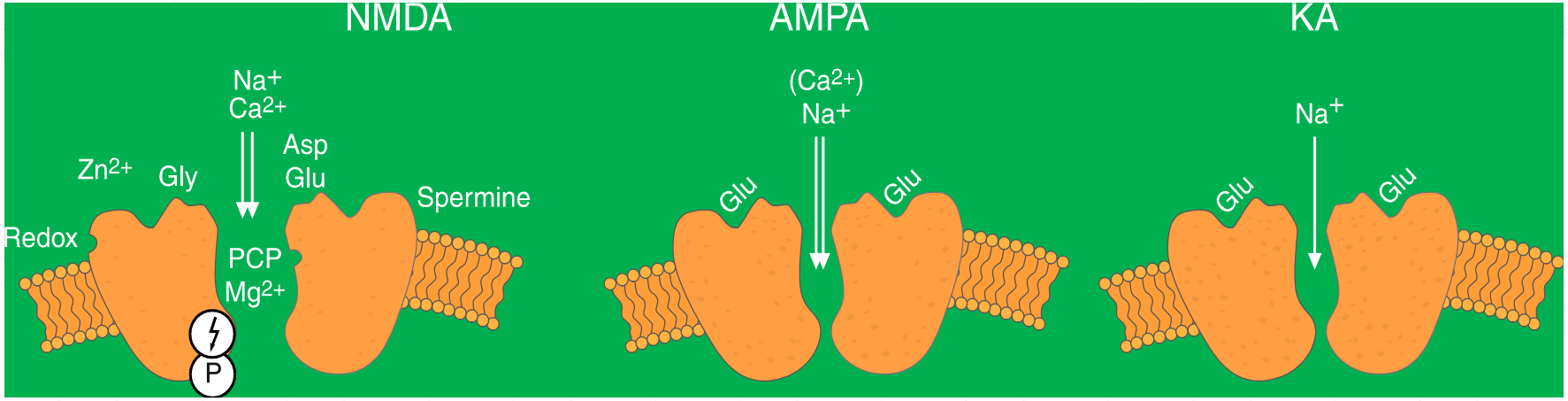
Mechanism of action of AEDs

Target and mechanism	AEDs
Sodium channel actions	
- Blockade by stabilizing fast-inactivated state	PHT, CBZ, LTG, OXC, RFM, ESL
- Blockade by stabilizing slow-inactivated state	LCM
Calcium channel actions	
- Blockade of high voltage-activated channel (P/Q type)	GBP, PGN
- Blockade of low voltage-activated channel (T-type)	ETX
GABA-related actions	
- Activation of $GABA_A$ receptor	PB, BZD, STP
- Blockade of GABA transporter (GAT1 selective)	TGB
- Inhibition of GABA transaminase	VGB
Glutamate-related actions	
- AMPA and Kainate-type	TPM
- AMPA-type glutamate receptor antagonist	PER
SV2A actions	
- Modulation of SV2A	LVT
Multiple actions (various actions on multiple targets)	VPA, FBM, TPM, ZNM
Potassium channel activity	
- Open Kv7 potassium channels	RTG

Brodie MJ et.al; Epilepsy & Behavior 2011

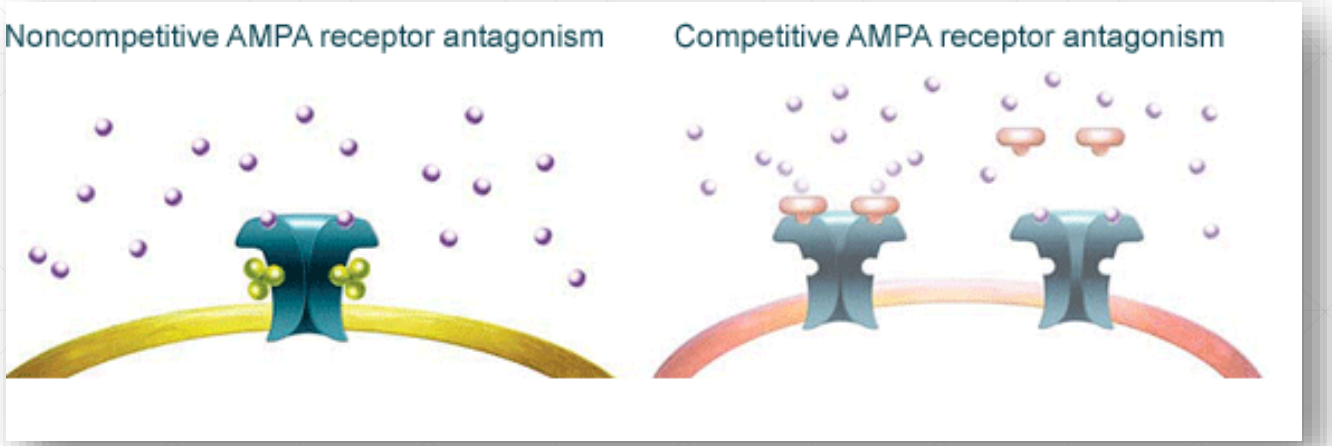
AMPA receptor antagonist AEDs

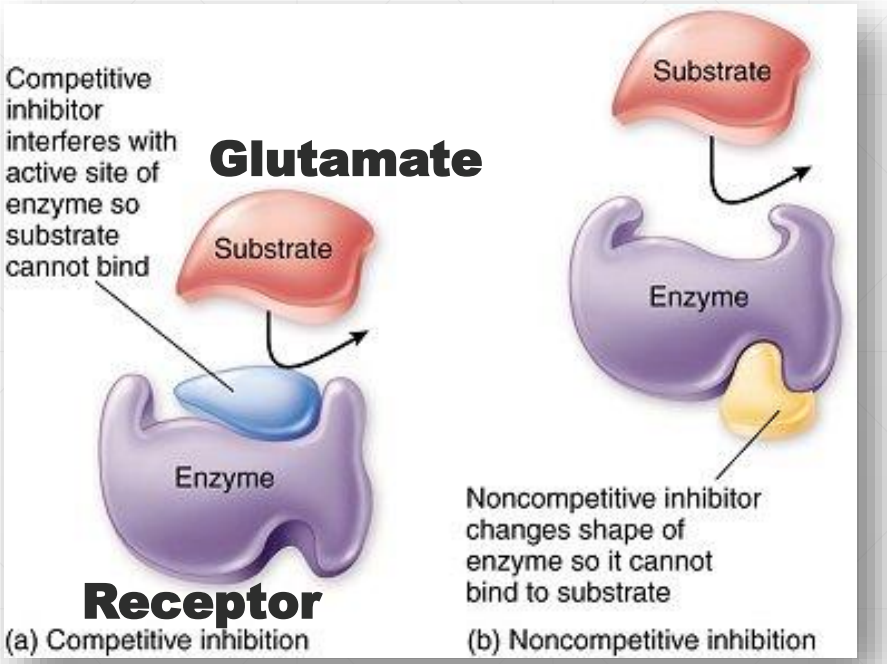
Putative Effects of Anticonvulsants on Ionotropic Glutamate Receptors



Competitive antagonist		
CPP-ene (Glu site)	NBQX	Topiramate
Felbamate (Gly site)	Topiramate	
Zonisamide		
Noncompetitive antagonists		
MK-801	Phenobarbital (?)	Phenobarbital (?)
Felbamate		
Remacemide		
Carbamazepine		
Phenytoin		

Conformational Changes and Mechanism of AMPA Receptor Noncompetitive Inhibition





Dose-response curve

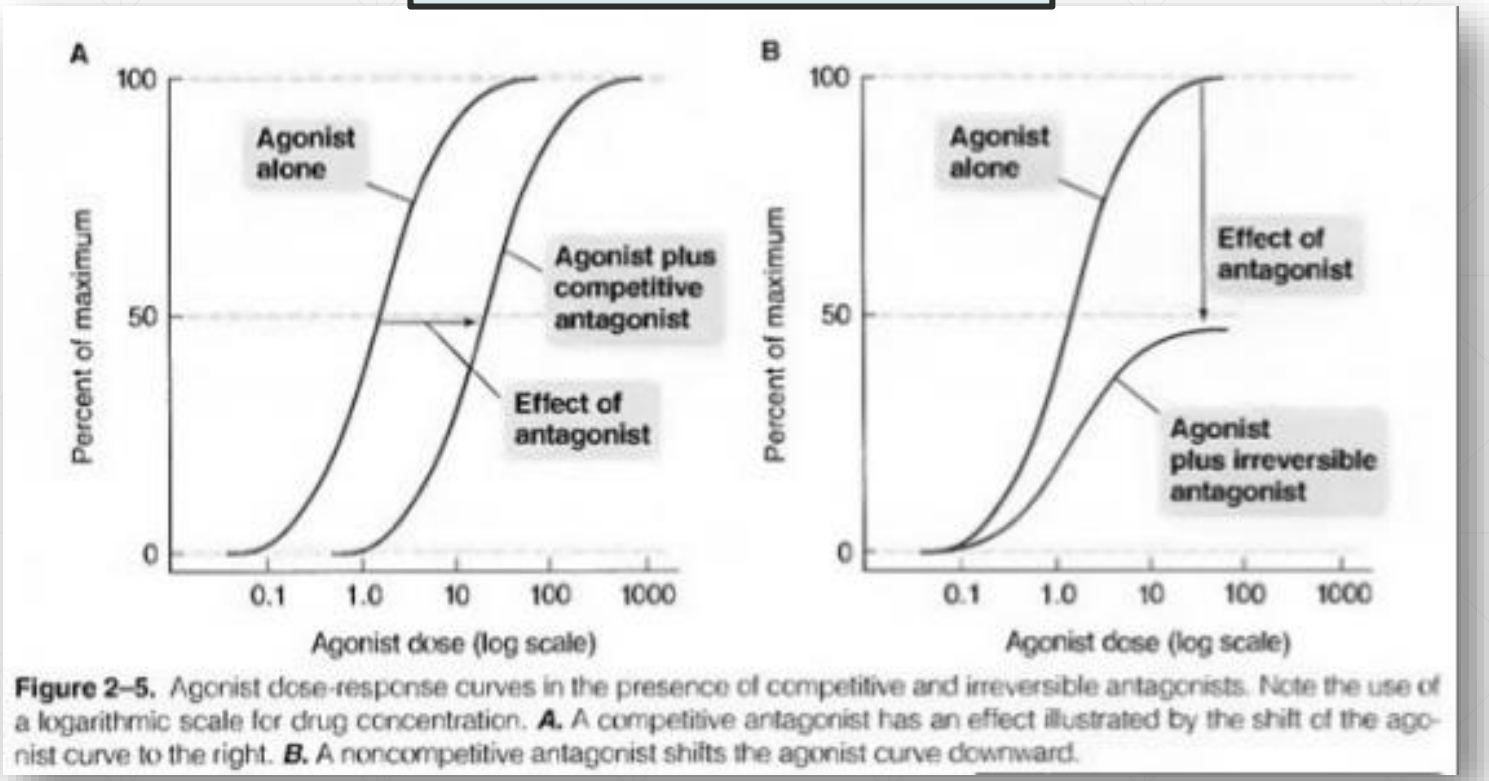
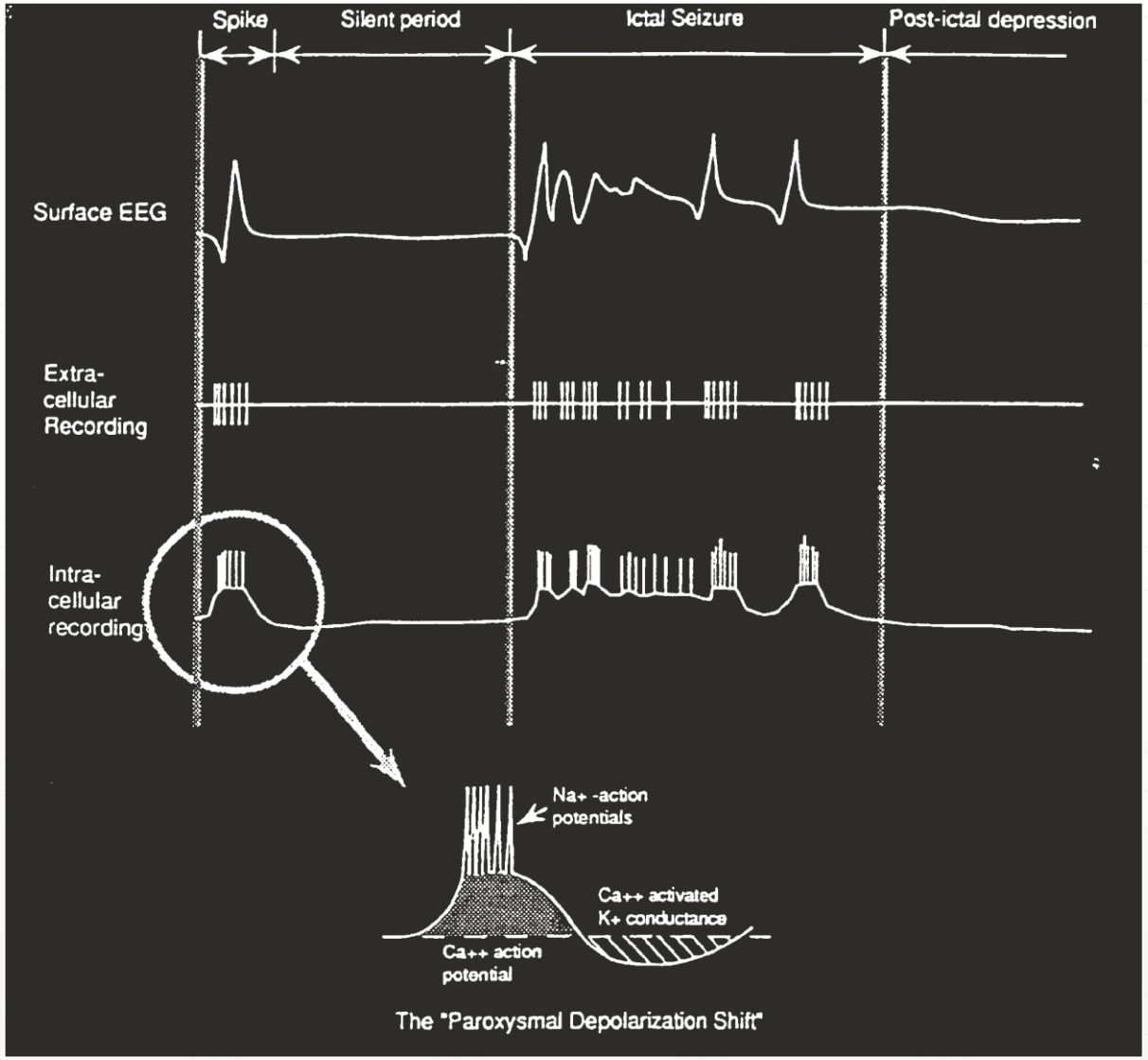


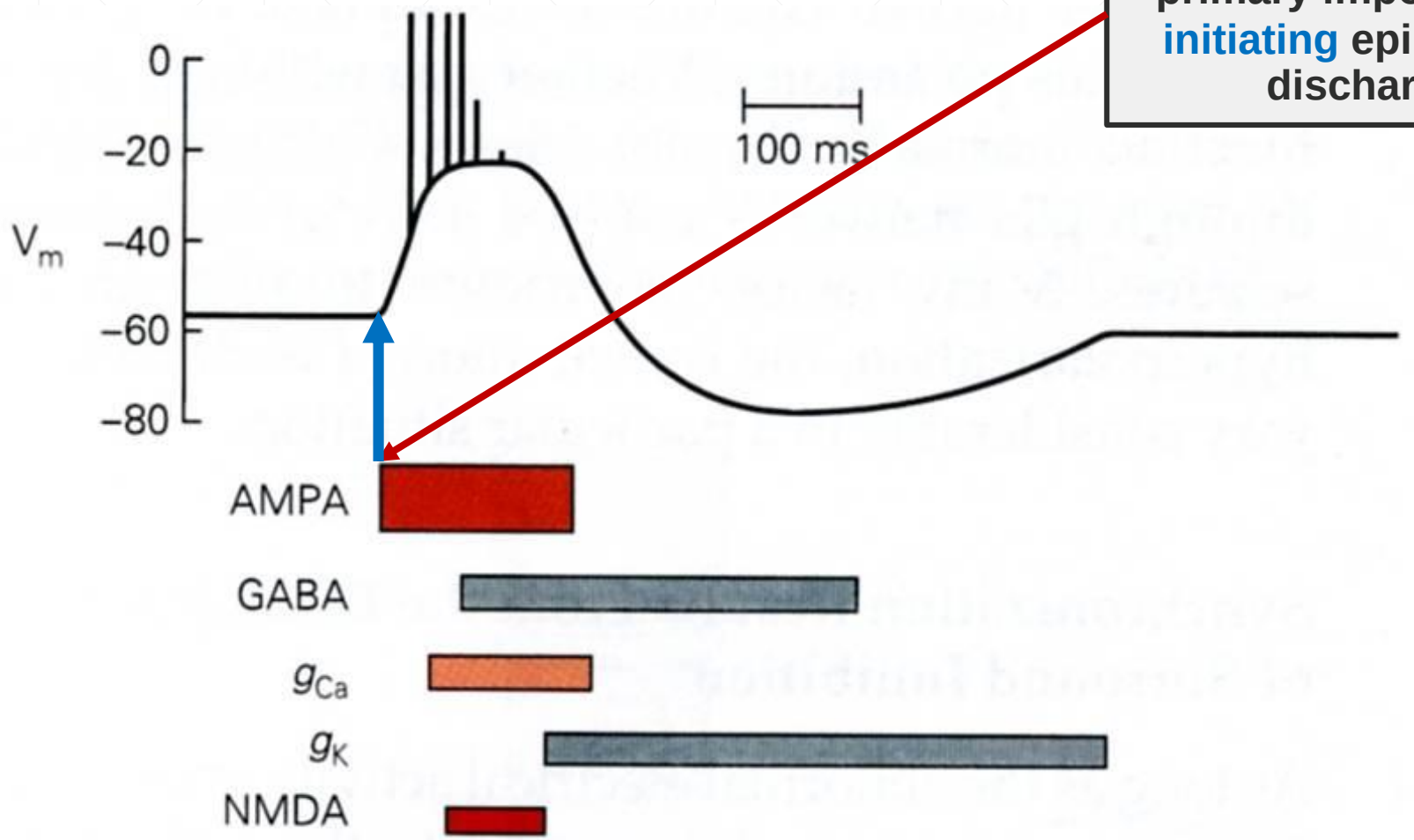
Figure 2–5. Agonist dose-response curves in the presence of competitive and irreversible antagonists. Note the use of a logarithmic scale for drug concentration. ***A.*** A competitive antagonist has an effect illustrated by the shift of the agonist curve to the right. ***B.*** A noncompetitive antagonist shifts the agonist curve downward.

https://my.bpcc.edu/content/blgy225/EnergyInTheHumanBody/EnergyInTheHumanBody2014_print.html; http://www.fycompa.eu/mode-of-action.php

Spike and Paroxysmal Depolarization Shift (PDS)



Pathophysiology of PDS



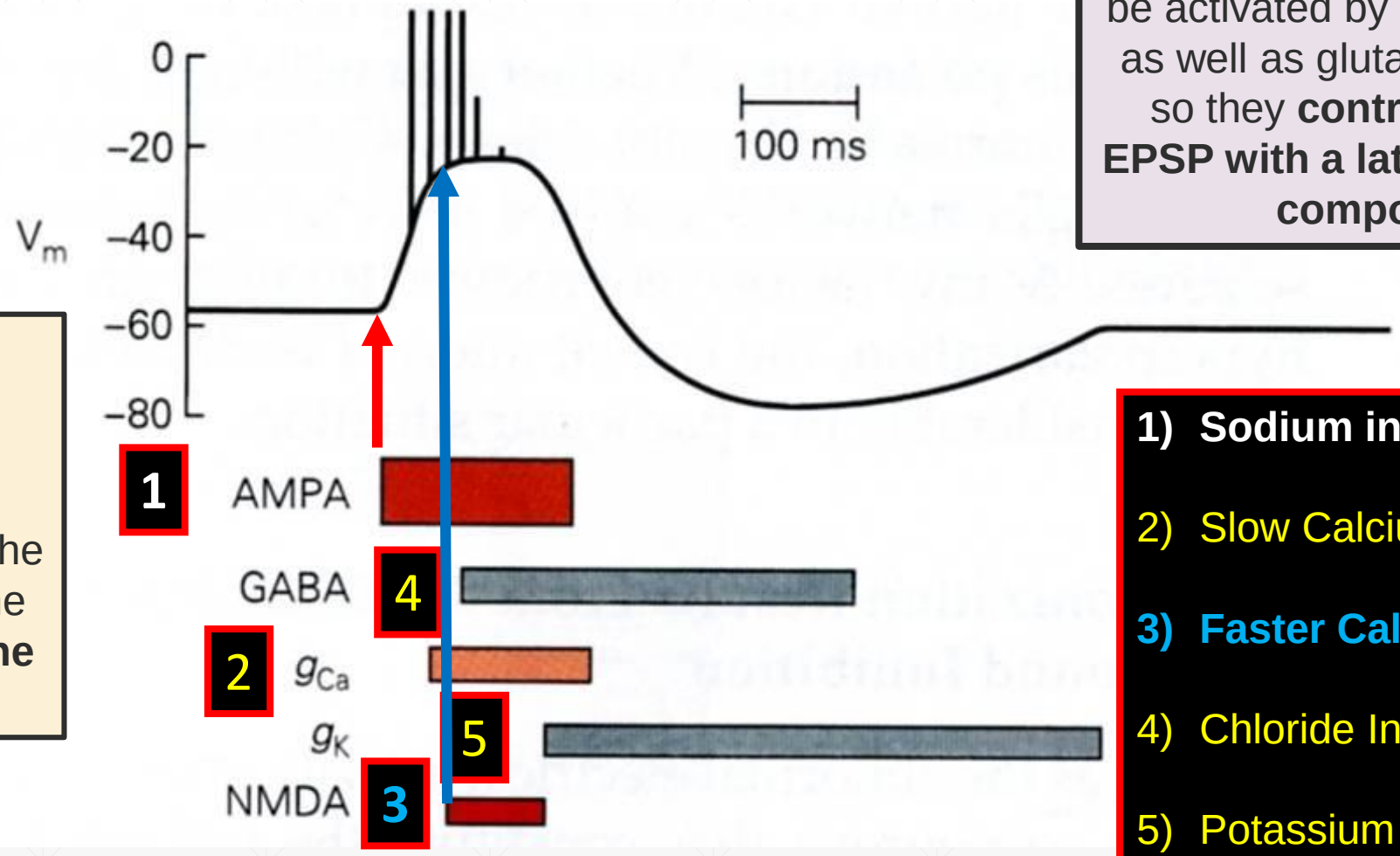
AMPA receptors are of primary importance in initiating epileptiform discharges

Pathophysiology of PDS

** Role of AMPA vs NMDA **

NMDA receptors <u>do not</u> contribute to synaptic transmission immediately; They need to be activated by depolarization, as well as glutamate binding, so they **contribute to the EPSP with a later and slower component**

Glutamate, via the **AMPA receptor,** mediates most fast excitatory neurotransmission in the CNS and underlies the **fast component of the EPSP**



1) **Sodium inflow**
2) Slow Calcium inflow
3) **Faster Calcium inflow**
4) Chloride Inflow
5) Potassium outflow

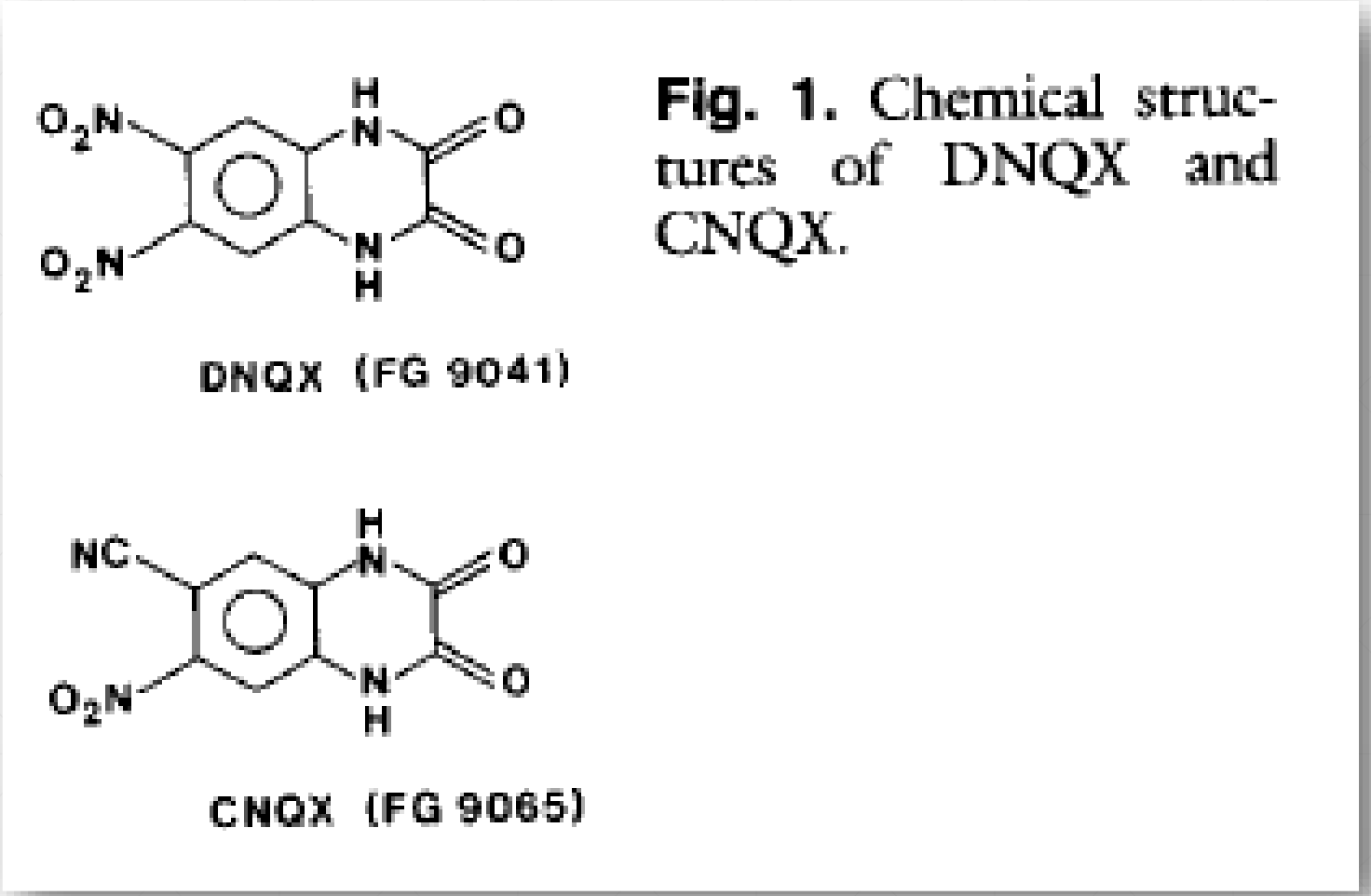
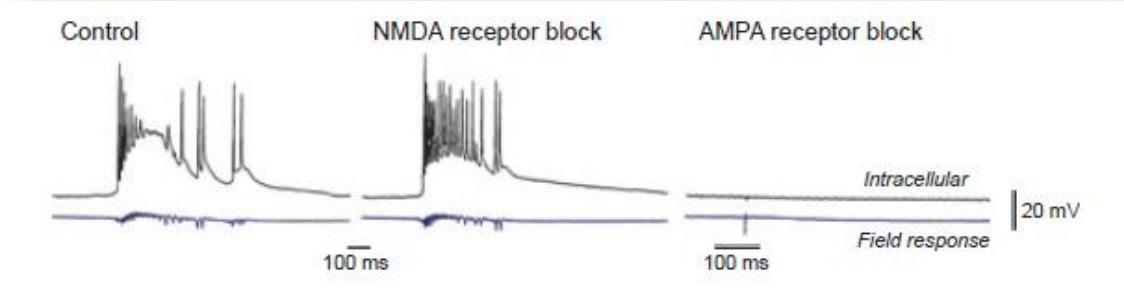


Fig. 1. Chemical structures of DNQX and CNQX.

First two **non-NMDA receptor antagonist**: **DNQX** and **CNQX**
- Inhibit glutamate binding to quisqualate (AMPA) receptors
- One-fifth as effective at kainite receptors

Honore T et.al; Science 1988

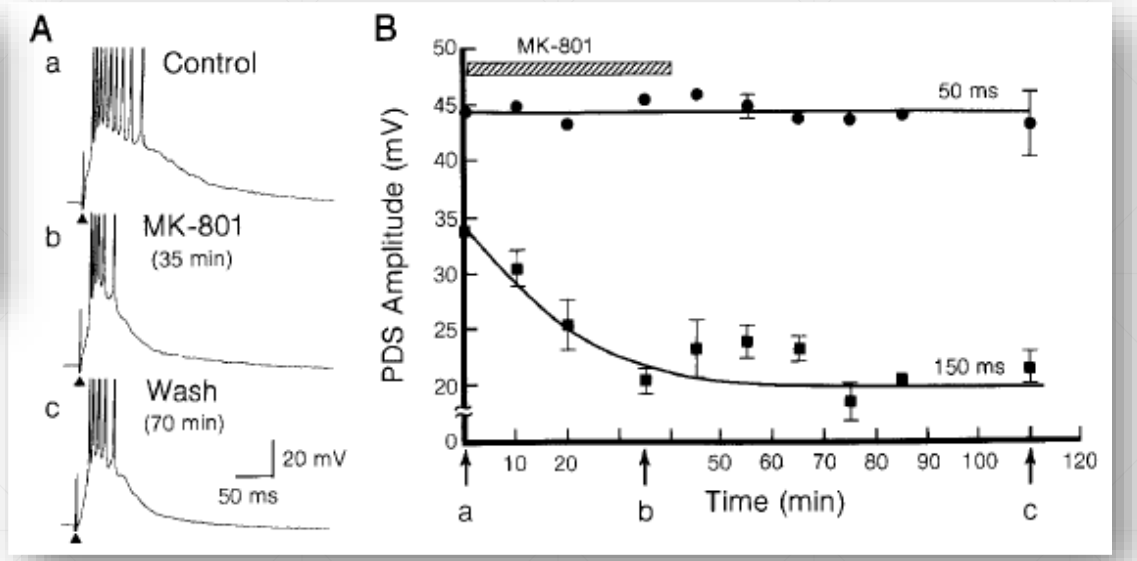
NMDA versus non-NMDA antagonists in suppression of seizures



- **CNQX** [non-NMDA (mainly AMPA) antagonist]

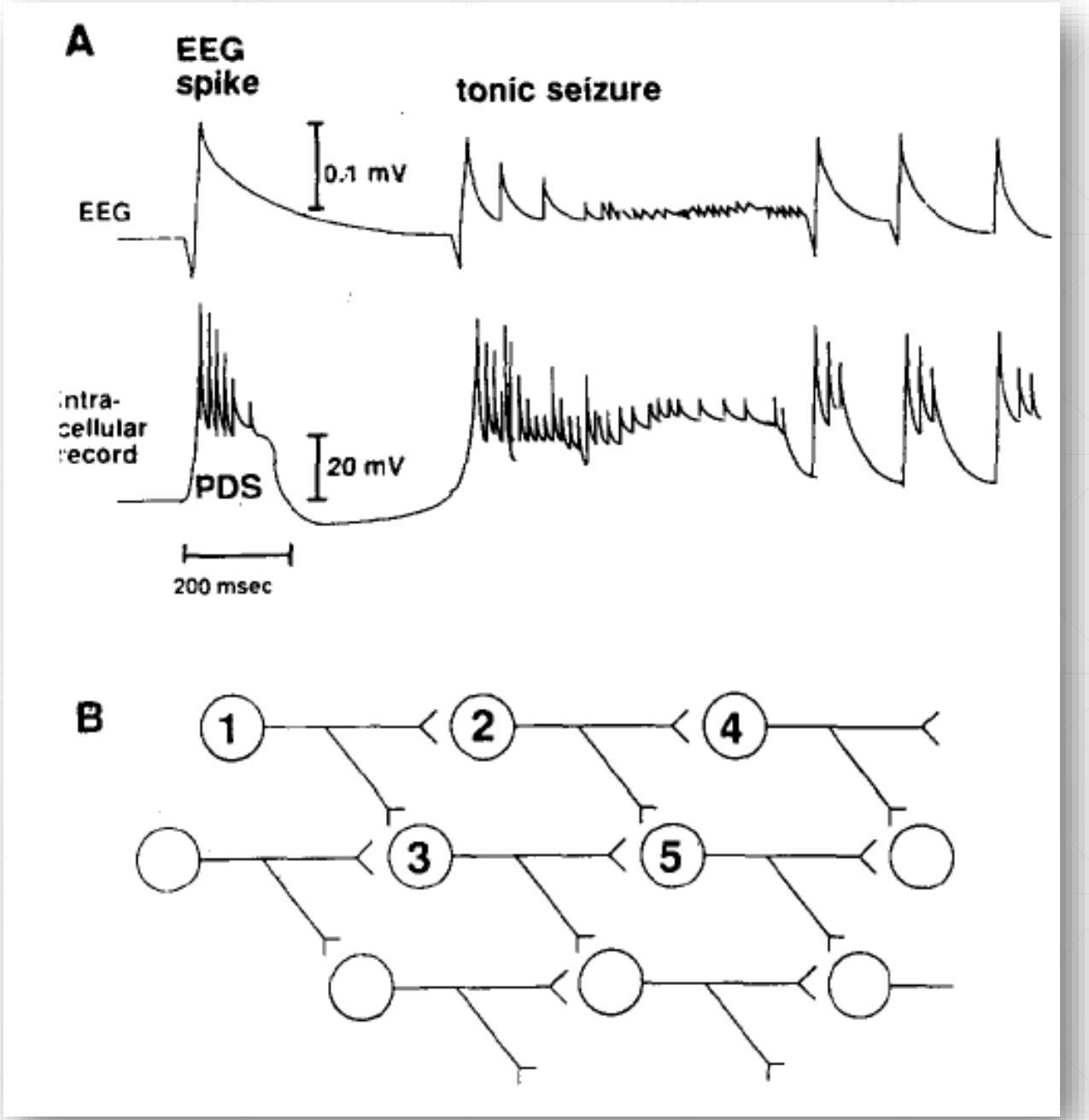
  - Potent anticonvulsant property by reducing the PDS

Traub RD et.al; J Physiol 1993
Rogawski MA; Acta Neurol Scand 2013



MK-801 (NMDA receptor antagonist)
– ineffective in abolishing the PDS
- attenuates the falling phase of the PDS but has no apparent effect on its early phase
- Not affect the triggering of the initial epileptiform discharge

Hwa GGC and Avoli M; Exp Brain Res 1991



Taylor CP; Trend in Neuroscience 1988

A transition from Spikes to Seizure

PDS (spike) and seizure are electrically similar, but brief EEG spikes do not interfere with normal behavior

Cascading excitation within a network of pyramidal neurons

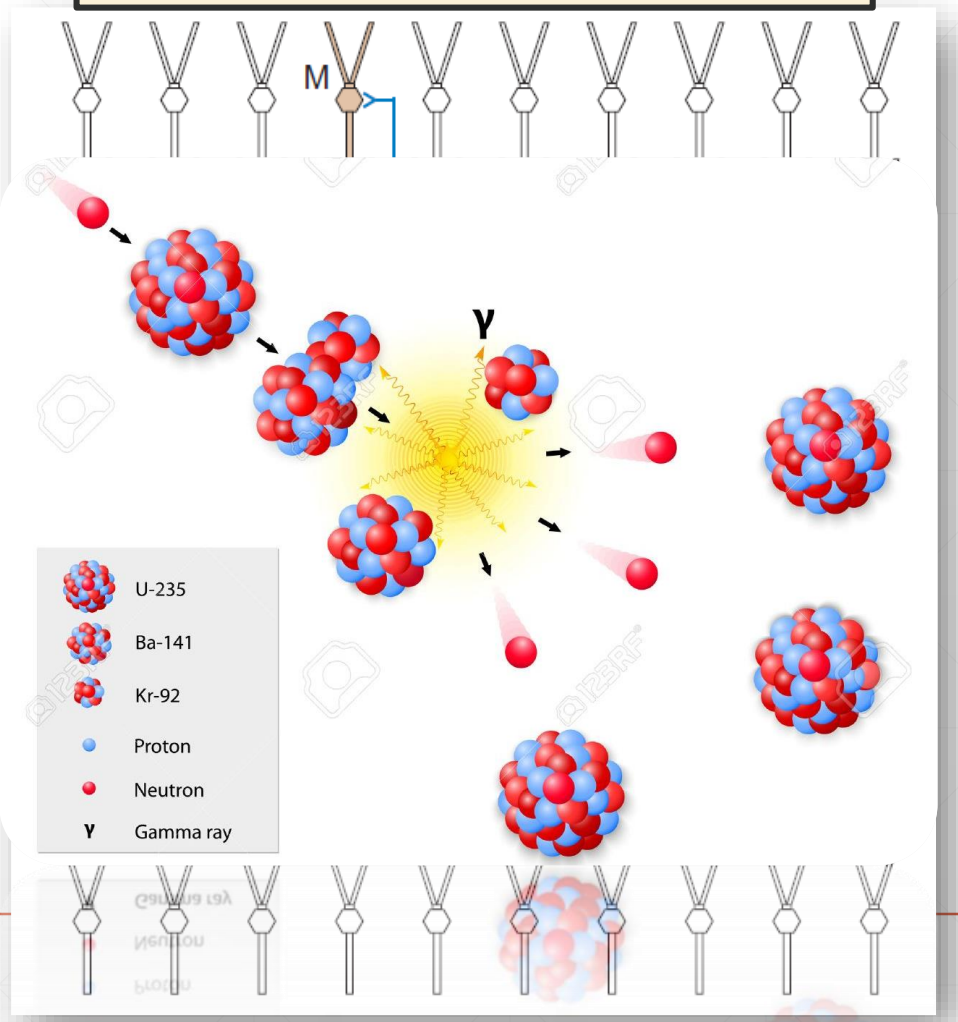
Excitation between a randomly chosen pair of neurons can be either **monosynaptic** (e.g. cell I to cell 2) or **polysynaptic** (e.g. cell I to cell 5)

Excitation normally stops after a single synapse but cascades across multiple synapses when **GABAergic inhibition is reduced**

AMPA receptors and epileptic synchronization

- Various pathological mechanisms lead to the **abnormal neuronal synchronization in epilepsy**
- Diverse interactions between neurons lead to epileptic synchronization. These interactions are both **non-synaptic and synaptic**
- AMPA receptors are critical to **epileptic synchronization** and the **generation and spread of epileptic discharges** in human epilepsy

Single neuron can initiate synchronized population discharge in hippocampus



The minimum ‘epileptic aggregate’ necessary to sustain synchronized epileptic discharges in the case of the CA3 region of the HC is ~1000–2000 neurons
“A ‘critical mass’ of neurons analogous to the **critical mass of a nuclear chain reaction**”

Rogawski MA; Neurol Scand 2013

AMPA - **Initiation of PDS** AND **Epileptic synchronization**

NMDA – **Prolong the epileptic burst activity**

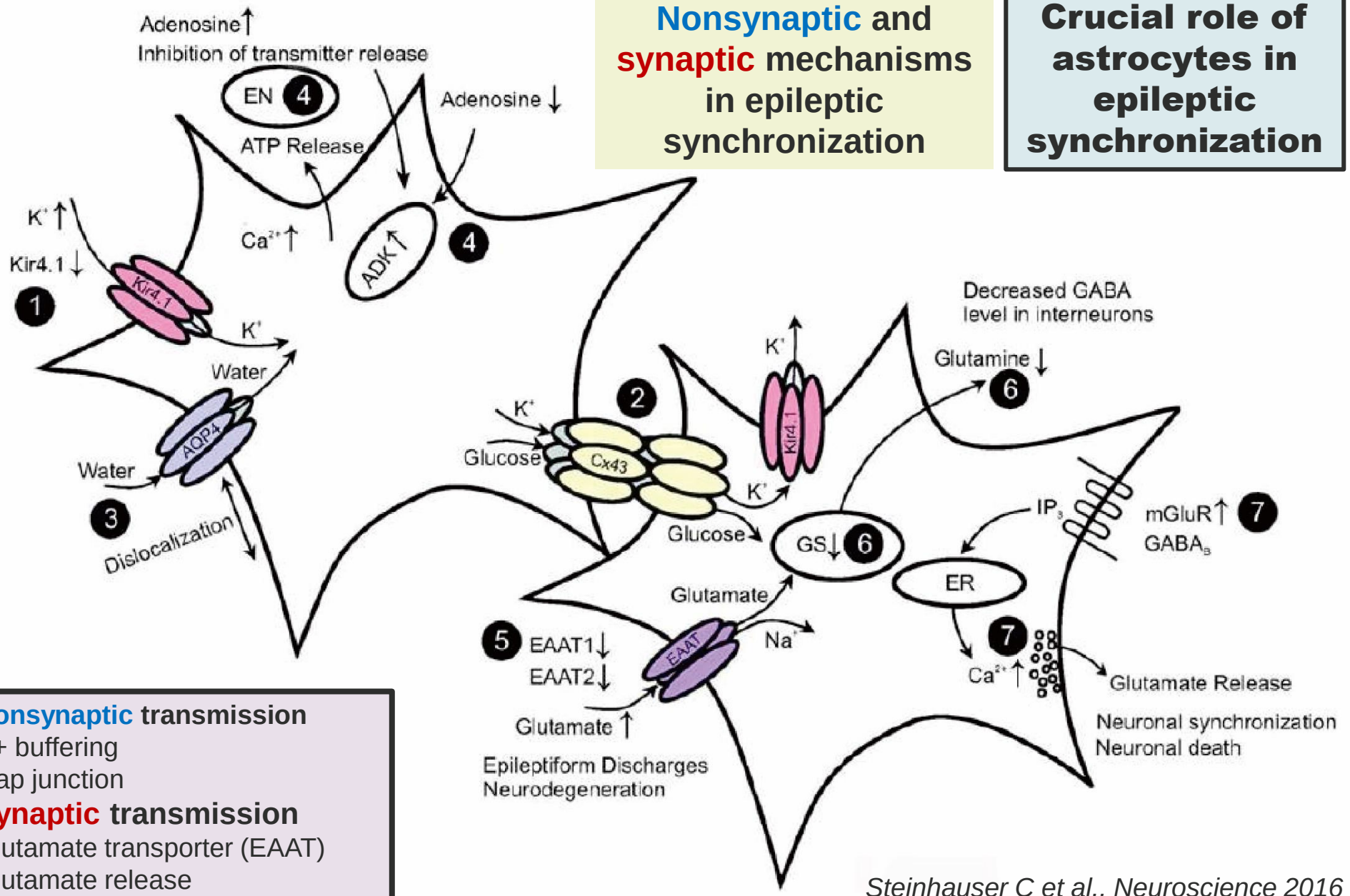
The discharges are **initiated and synchronized** by recurrent excitatory collateral connections primarily involving **AMPA receptors**.
Within **tens of milliseconds or less** of activation of even one of the neurons, all of the neurons fire and mutually excite each other via AMPA receptors to produce a synchronized burst;

the burst is prolonged by the activity of NMDA receptors

Rogawski MA; Neurol Scand 2013

Nonsynaptic and synaptic mechanisms in epileptic synchronization

Crucial role of astrocytes in epileptic synchronization



Nonsynaptic transmission
K+ buffering
Gap junction

Synaptic transmission
Glutamate transporter (EAAT)
Glutamate release

Steinhauser C et al., Neuroscience 2016

NMDA versus non-NMDA antagonists in memory function

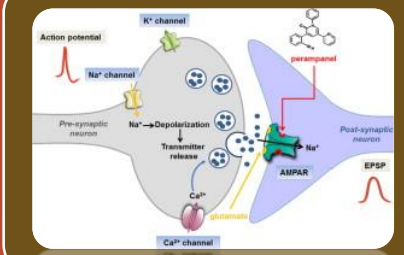
- Selective AMPA receptor antagonists is of clinical significance as NMDA antagonists are known to produce psychoactive effects, including schizophrenic-like symptoms and cognitive impairment

- **Long-term potentiation (LTP)** is believed to be a cellular mechanism underlying memory formation and NMDA receptor gating is required for the induction of LTP

  NMDA receptor antagonists eliminate LTP induction

  AMPA receptor antagonists were not found to impair memory function or retrieval, even at doses that affected motor performance

Kapus G et.al; Brain Res Bull 2000
Pitsikas N et.al; Pharmacol Res 2002
Parada-Turska J and Turski WA; Neuropharmacology 1990



Perampanel in selected epilepsies

PER is promising AED, but requires further clinical studies

ADJUNCTIVE THERAPY FOR THE TREATMENT OF PARTIAL-ONSET SEIZURES WITH OR WITHOUT SECONDARY GENERALIZATION

Primary generalized tonic-clonic seizures in IGE

Aged ≥ 12 years

AES & AAN Refractory epilepsy 2018

Practice guideline update summary: Efficacy and tolerability of the new antiepileptic drugs II: Treatment-resistant epilepsy

Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology and the American Epilepsy Society

Andres M. Kanner, MD, Eric Ashman, MD, David Gloss, MD, MPH&TM, Cynthia Harden, MD, Blaise Bourgeois, MD, Jocelyn F. Bautista, MD, Bassel Abou-Khalil, MD, Evren Burakgazi-Dalkilic, MD, Esmeralda Llanas Park, MD, John Stern, MD, Deborah Hirtz, MD, Mark Nespeca, MD, Barry Gidal, PharmD, Edward Faught, MD, and Jacqueline French, MD

Correspondence
American Academy of Neurology
guidelines@aan.com

Head-to-head trials are lacking

A lack of placebo-controlled and head-to-head trials of newer AEDs in pediatric patients

Jan 2003 – Nov 2015

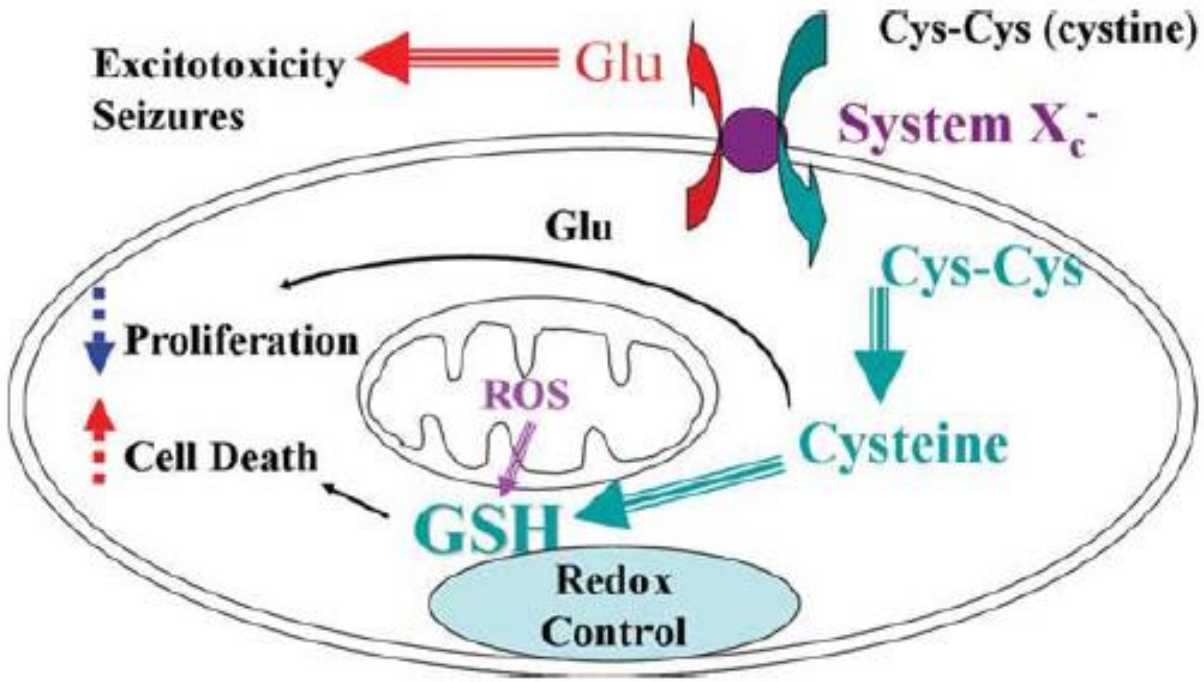
CLB, VGB, and the **8 second-generation** and **6 third-generation AEDs** **(ESL, EZG, LCM, PER, PGB, RFN)**

AES & AAN Refractory epilepsy 2018

Seizure type or epileptic syndrome	Level of evidence	AEDs
Treatment-resistant (TR) adult focal epilepsy	A B	PGB, **PER** ESL, LCM, TPM
Lennox-Gastaut syndrome	A B	RFN CLB (add-on)
Generalized epilepsy with treatment-resistant GTC	A B	None LTG
TR childhood focal epilepsy	A B	None LEV (add-on), ZNM, OXC
TR GTC seizures and JME	A B	None LEV

BRAIN TUMOR RELATED EPILEPSY

de Groot J, Sontheimer H; Glia 2011



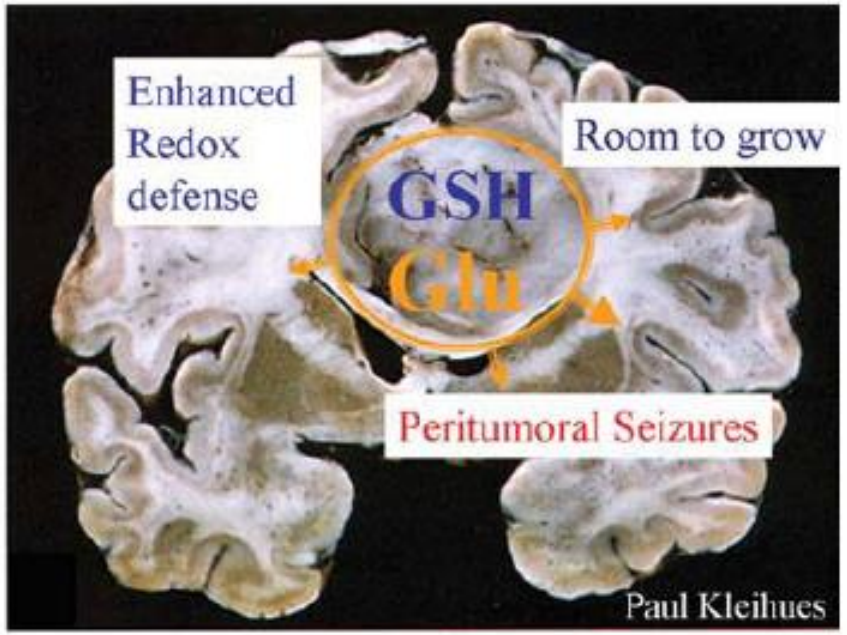
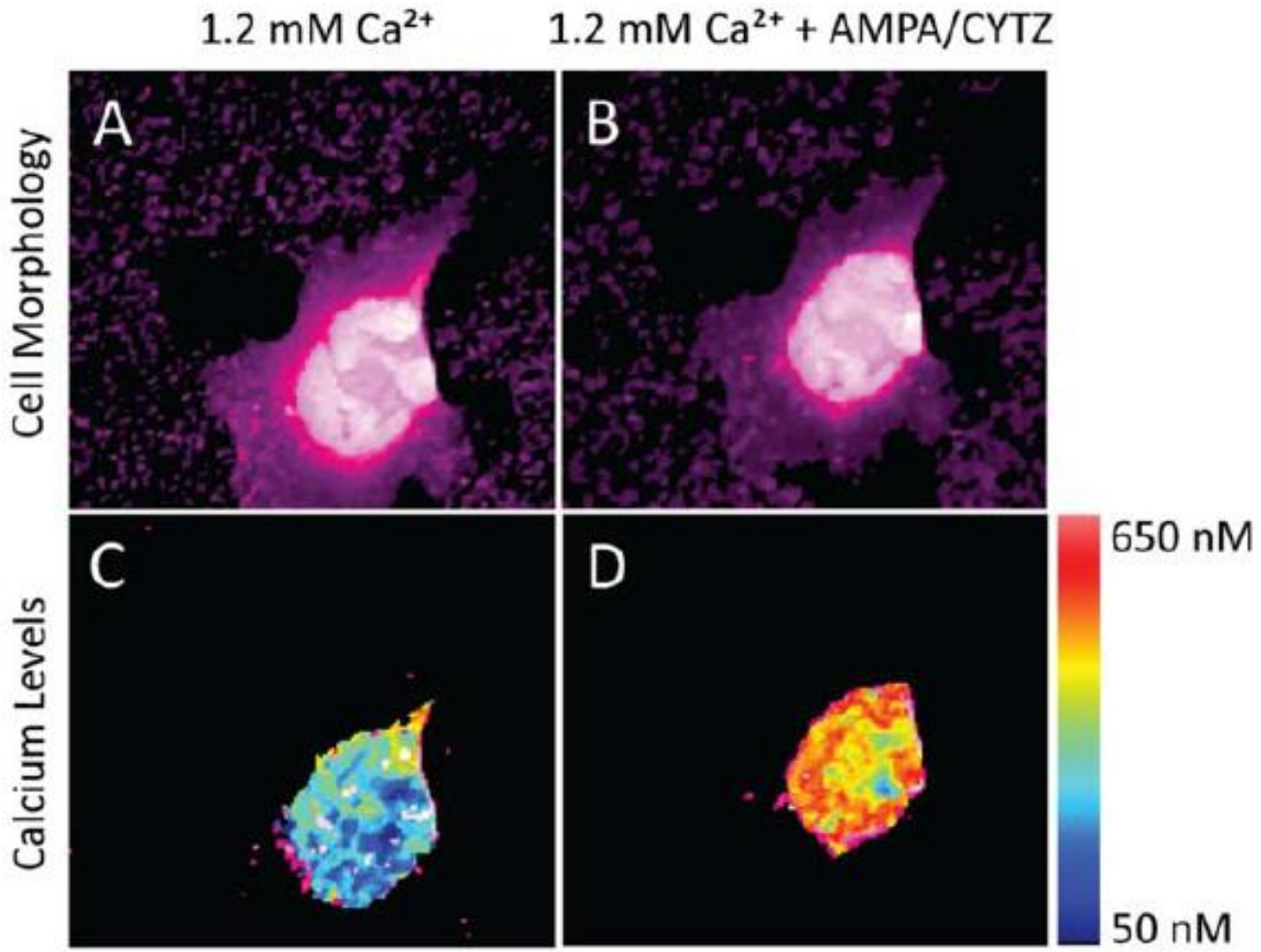


Fig. 2. Glutamate is released from glioma cells in conjunction with the uptake of cystine for the cellular synthesis of the antioxidant glutathione. It is hypothesized to initiate excitotoxicity in the peritumoral brain.

By binding to peritumoral neuronal glutamate receptors, **glutamate is responsible for seizure induction and similarly causes excitotoxicity**, which aids the expansion of tumor cells into the space vacated by destroyed tissue

Reduced uptake from glial cells due to the **diminished expression of the glutamate transporter EAA**T, which leads to neuronal hyperexcitability surrounding the tumor



Glioblastoma brain tumor-initiating cells express
high concentrations of functional **calcium-permeable AMPA receptors**, which further contribute to increase excitotoxicity

Oh MC et al., PLoS One 2012

MESIAL TLE

With HS

Perspectives on treatment options for mesial temporal lobe epilepsy with hippocampal sclerosis

Caterina Palleria, Antonietta Coppola, Rita Citraro, Luigi Del Gaudio, Salvatore Striano, Giovambattista De Sarro & Emilio Russo

- **Perampanel and retigabine** are very effective drugs in animal models of TLE
- **Concepts**: Agents that inhibit or decrease AMPAR activity have the potential to **decrease excessive excitatory responses** providing **neuroprotection and seizure suppression**
- There are no specific data on the use of PER in MTLE-HS patients

Exp Opin Pharmacotherapy 2015

STATUS EPILEPTICUS

The transition from seizures to SE

"Complex Interaction Mechanism"

- **Cellular level**
  - ✓ Four key mechanisms
    - **Na^+/ K^+ ATPase (↑ $[Na]_i$ and ↑ $[K+]_e$)**
    - **Sustained excitatory GABAergic signaling**
      (impaired function KCC2 and upregulation NKCC1)
    - **Astrocyte (K^+ buffering; lactate shuttle; glutamate transporter)**
    - **Change in receptor expression (receptor trafficking)**

Glutamate

- **Network level**
  - ✓ Hippocampus
  - ✓ Thalamus

Burman RJ et.al, Seizure 2020

Perampanel for treatment of status epilepticus in Austria, Finland, Germany, and Spain

- Clinical cases where perampanel was used in established SE, refractory SE (RSE), or super-refractory SE (SRSE)
- 5 European hospitals between 2011 and 2015
- Of 1319 patients identified as experiencing SE, 52 (3.9%) received PER
- Median latency from SE onset to PER initiation was 10 days
- Median initial PER dose was 6 mg/d, up-titrated to a median maximum dose of 10 mg/d
- PER was the last drug added in 32/52 (61.5%) patients, with **response attributed to PER in 19/52 (36.5%) patients**

Strzelczyk A et.al.; Acta Neurol Scand 2018

Perampanel in patients with RSE and SRSE in a neurological intensive care unit: A single center audit of 30 patients

- PER was administered via nasogastric tube, crushed and dissolved in water
- 16/30 patients (53%) received a "**standard dose**" of median 4 mg (range = 2-12, dose increase = 2-4 mg/d).
- 14/30 patients (47%) received **"higher initial doses"** with median 32 mg (range = 16-32 mg)
  - ✓ A loading dose of PER 32 mg on the first day
  - ✓ PER 24 mg on the second day and
  - ✓ thereafter reduction to a maintenance dose of 12 mg (in absence of enzyme-inducing AEDs) or 16-20 mg (in presence of enzyme inducers like PHT)

No significant difference in treatment response, outcome, or stay on the NICU

- **PER terminated SE in 17% of patients with RSE or SRSE in this single-center case series.**
- **Time from perampanel administration to treatment response ranged between 6 and 72 hours**

Rohracher A et.al.; Epilepsia 2018

Perampanel in the treatment of status epilepticus: A systematic review of the literature

- 10 articles were included,with a total of 69 episodes of SE occurring in 68 patients (aged 18 to 91 years)
- **Varied in confounding factors among studies**;
  - ✓ The type and etiology of SE
  - ✓ Number of drugs used prior to PER (range 1-9)
  - ✓ Time from SE onset to PER administration (range 9.25 h – 35 d)
  - ✓ Initial PER dose (2-32 mg)
- The proportion of patients achieving clinical SE cessation varied from

  17% to 100%

The currently available evidence supporting the use of PER in SE is weak and hampered by several confounding factors

Brigo F et.al.; Epilepsy&Behav 2018

Thank you for your attention