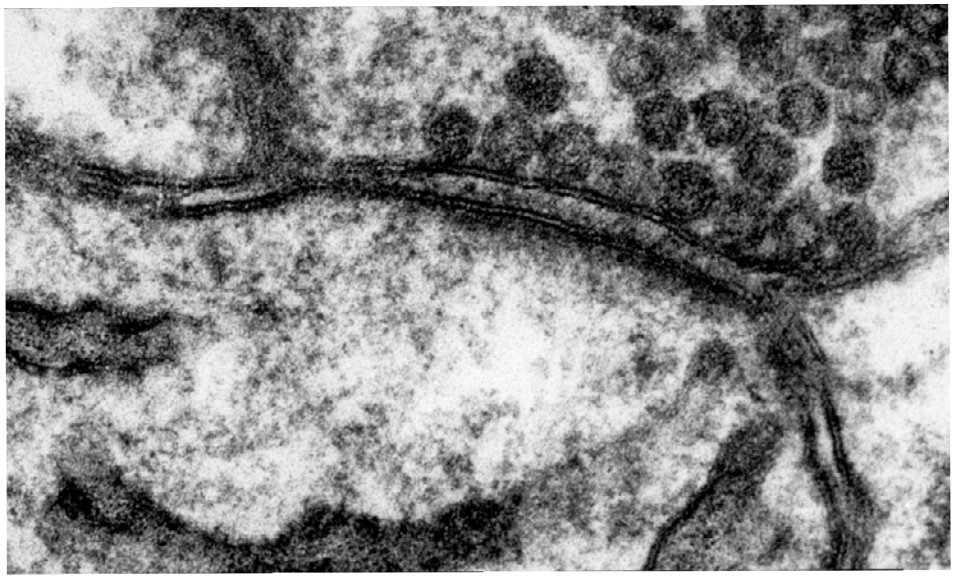
The postsynaptic density (PSD): A very complex structure



~1,000 different proteins

Receptors

Scaffolds

Adhesion proteins

Cytoskeleton

Signaling proteins

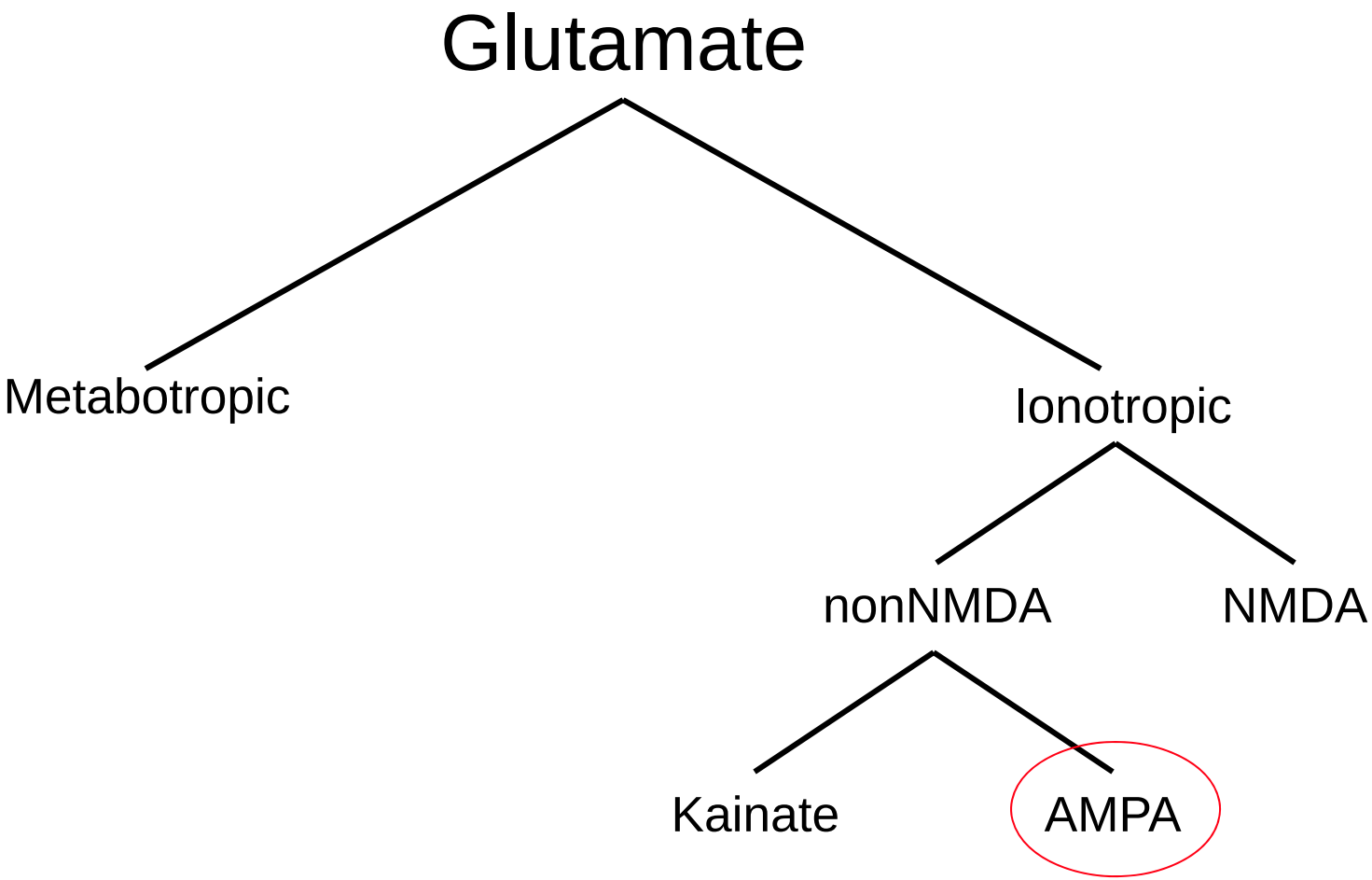
Receptor agonist

Receptor antagonist

Overexpression

Delete protein

Glutamate Receptor Subtypes



Cloning of a glutamate receptor

Cloning by functional expression of a member of the glutamate receptor family

Michael Hollmann, Anne O'Shea-Greenfield, Scott W. Rogers & Stephen Heinemann

Molecular Neurobiology Laboratory, The Salk Institute for Biological Studies, La Jolla, California 92037, USA

We have isolated a complementary DNA clone by screening a rat brain cDNA library for expression of kainate-gated ion channels in Xenopus oocytes. The cDNA encodes a single protein of relative molecular mass (M_r) 99,800 which on expression in oocytes forms a functional ion channel possessing the electrophysiological and pharmacological properties of the kainate subtype of the glutamate receptor family in the mammalian central nervous system.

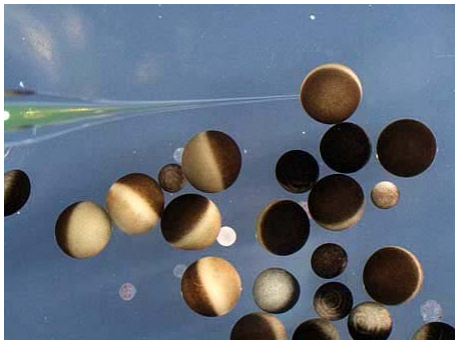
Recently, a G protein-coupled glutamate receptor representing a fifth class with a very different mechanism of action has been identified[6]. The fact that the subtypes show differences in their distribution in the brain[7] indicates that they represent distinct gene products with unique structures and functions.

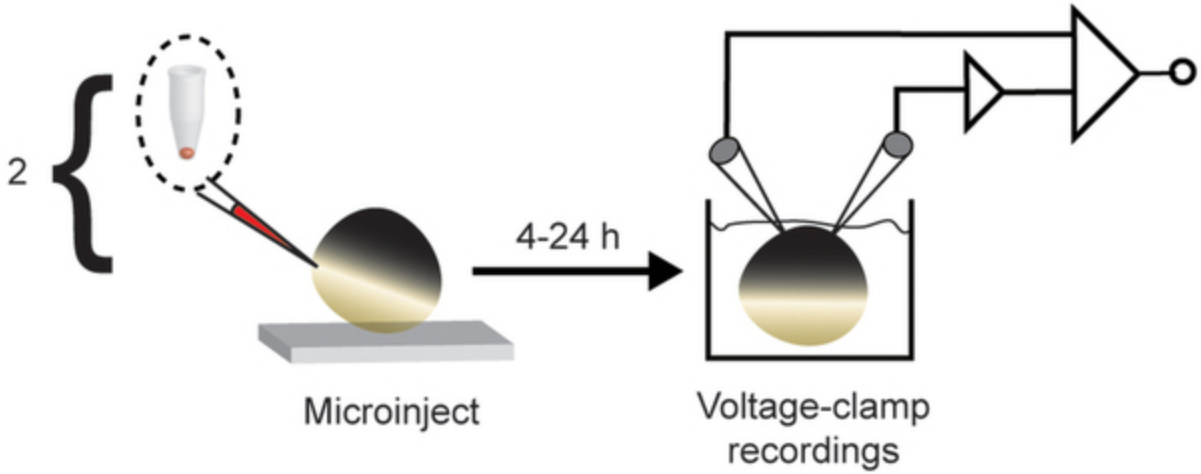
Despite the prominent part these receptors play in normal synaptic transmission as well as in neuronal plasticity, their molecular characteristics have remained elusive, mainly because of a lack of ligands that bind irreversibly and with high specificity. Conventional biochemical approaches to the isolation of these receptors have so far either not succeeded in progressing beyond crude receptor solubilization, as is the case for the NMDA[8] and quisqualate[9] subtypes, or have resulted in

NATURE · VOL 342 · 7 DECEMBER 1989

Expression Cloning of the glutamate receptors

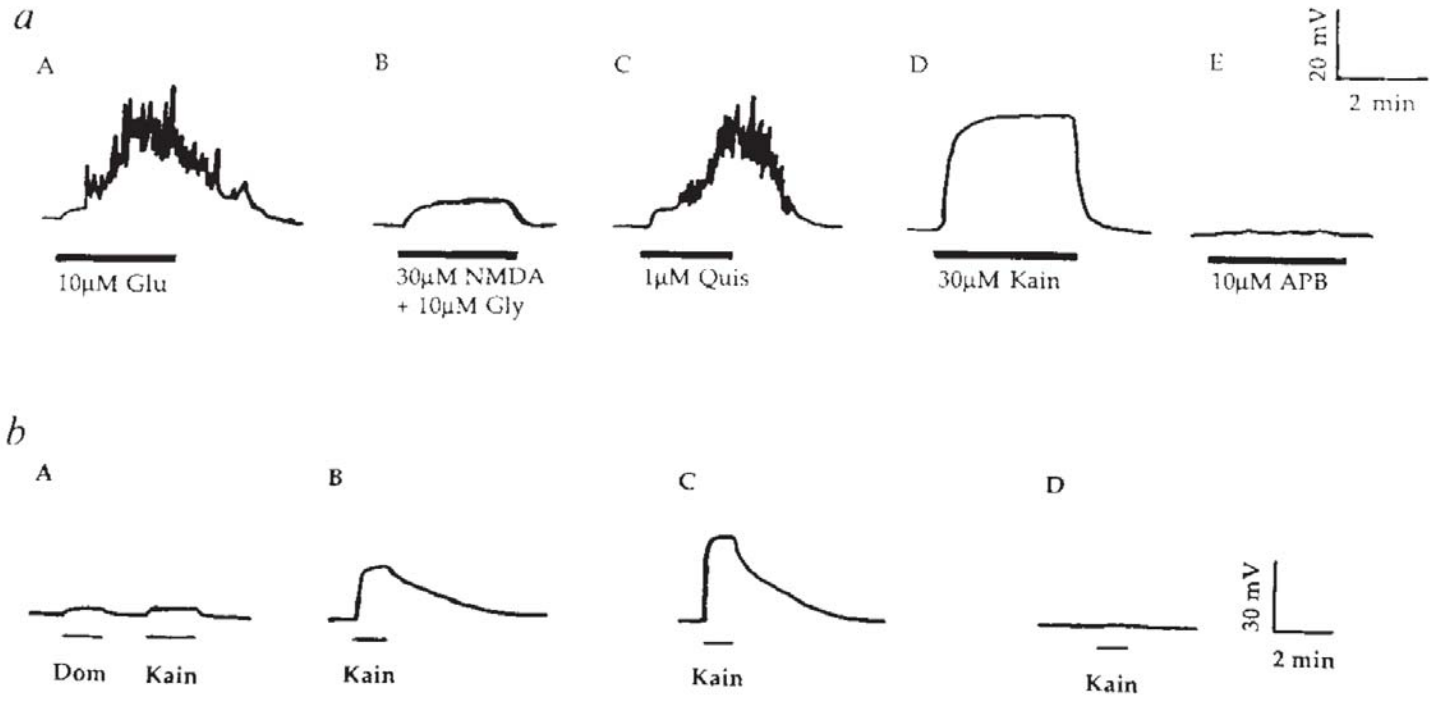
1. Prepare Rat brain mRNA extract
2. Prepare cDNA libraries
3. Inject large pools of cDNAs and measure glutamate gated currents
4. Refine pool until you get a single clone responsible for the glutamate response





Expression Cloning of the glutamate receptors

Total rat brain mRNA

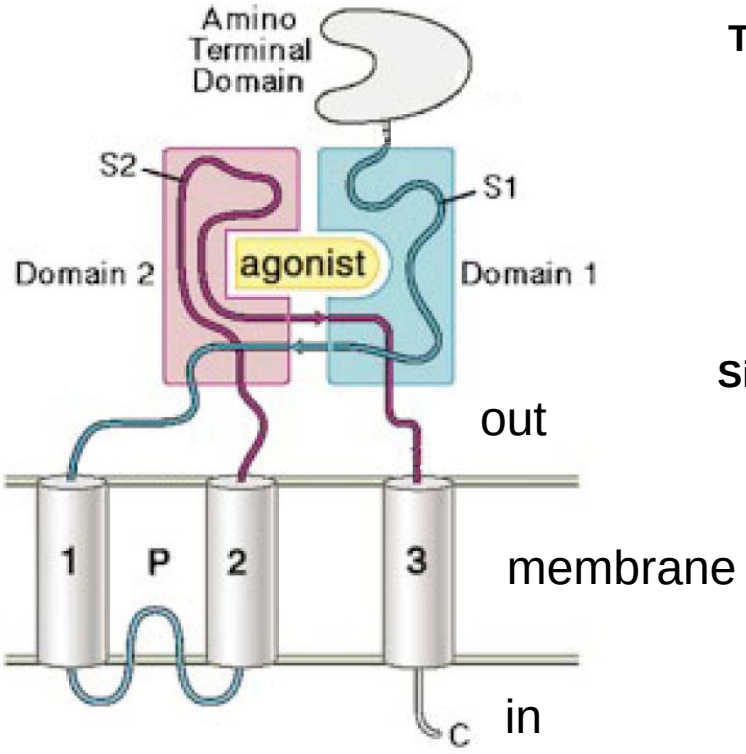


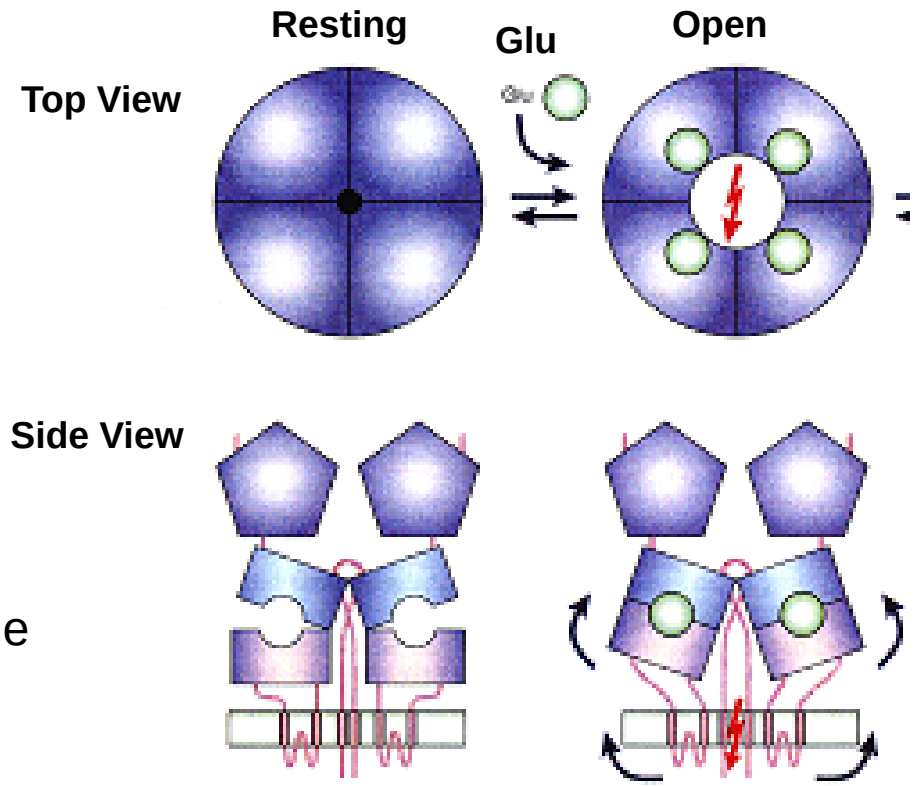
Enriched for a specific cDNA clone

Structure of glutamate receptors

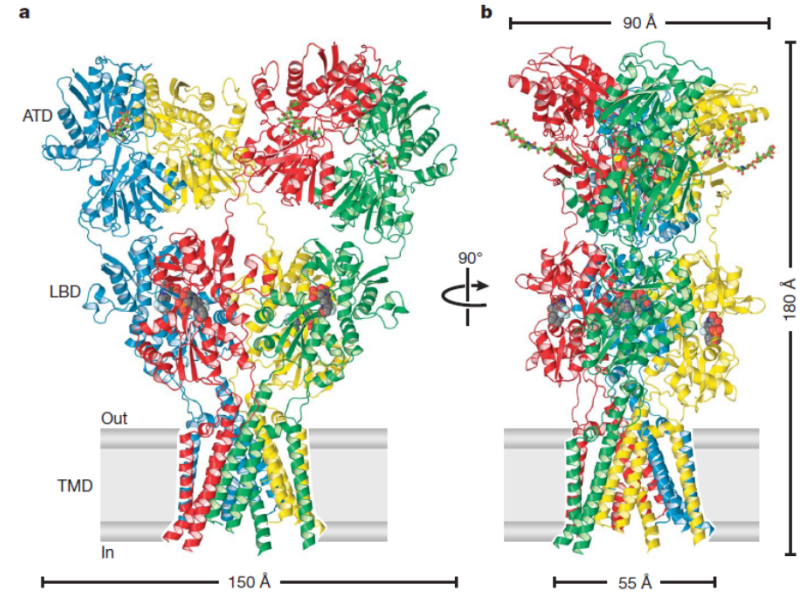
Four Genes Code For AMPARs (GluA1-4).
A functional AMPAR is made of four subunits (tetramer).

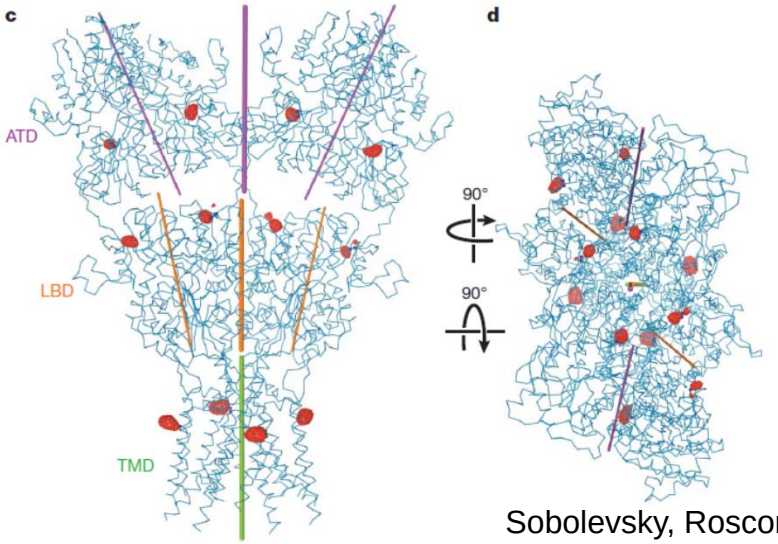
Domains of the AMPAR subunit





Structure of the AMPA receptor





Structure of the GluA2 homo-tetramer

-Y-Shaped with three major domains (ATD, LBD, TMD) arranged in layers

- Overall two-fold axis of symmetry perpendicular to membrane plane

- ATD dimer has two fold axis of symmetry ~24^{o} off main axis

- LBD dimer has two fold axis of symmetry ~19^{o} off main axis

-Ion channel domain with four fold axis of symmetry

-ATD: Amino Terminal Domain
-LBD: Ligand Binding Domain
-TMD: Trans Membrane Domain

Sobolevsky, Rosconi & Gouaux, Nature 2009

AMPA receptor gating

Resting

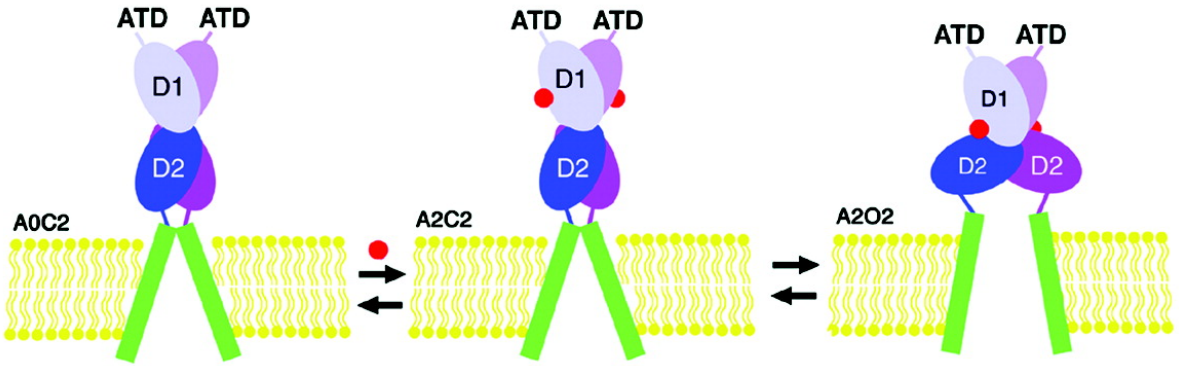
Open cleft
Constrained Dimer
Closed Channel

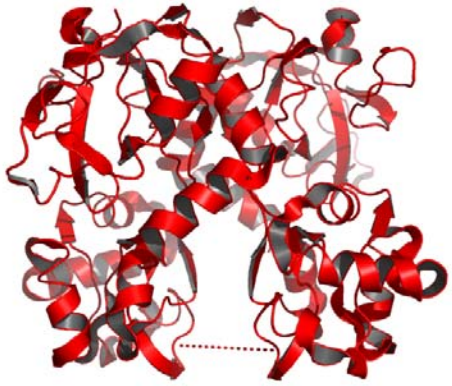
Activated

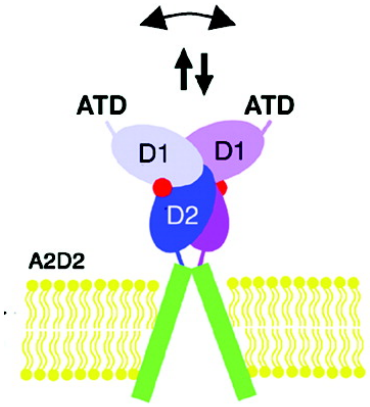
Open Cleft
Constrained Dimer
Closed Channel

Open

Closed Cleft
Constrained Dimer
Open Channel



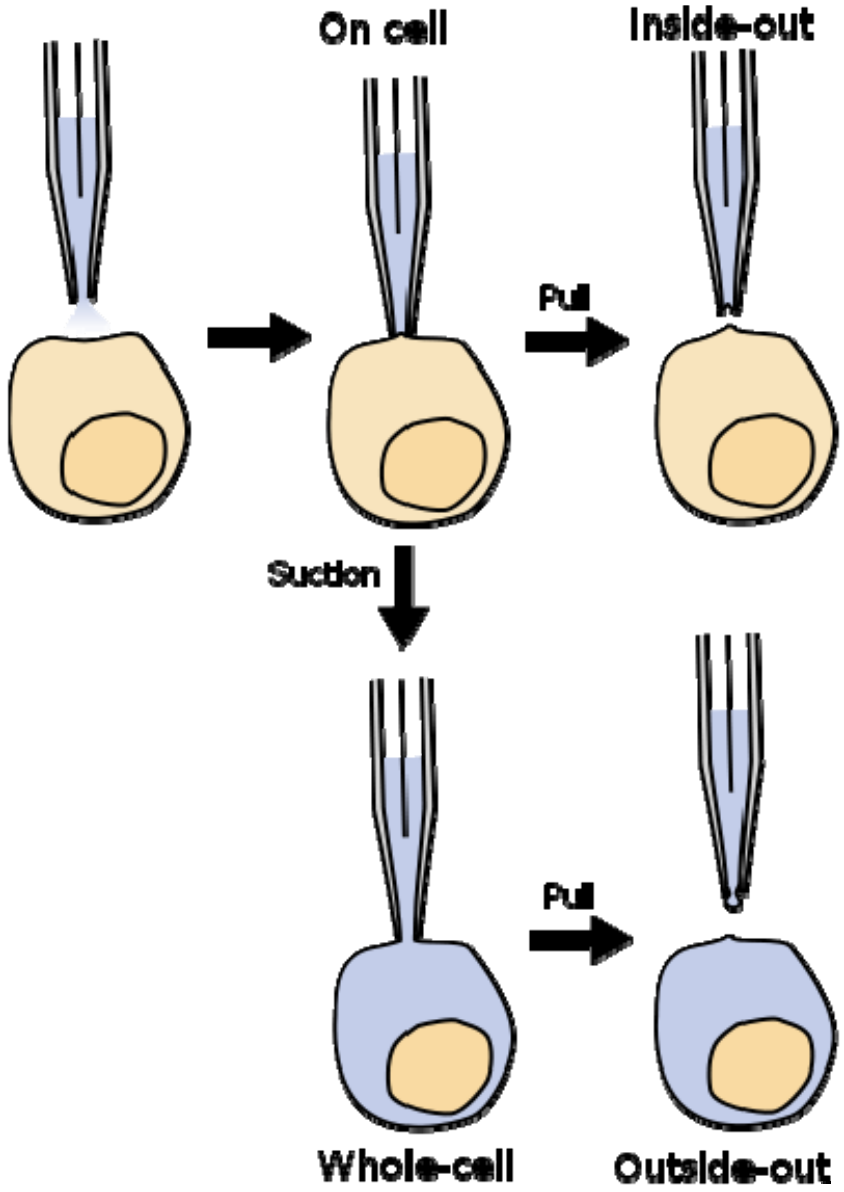




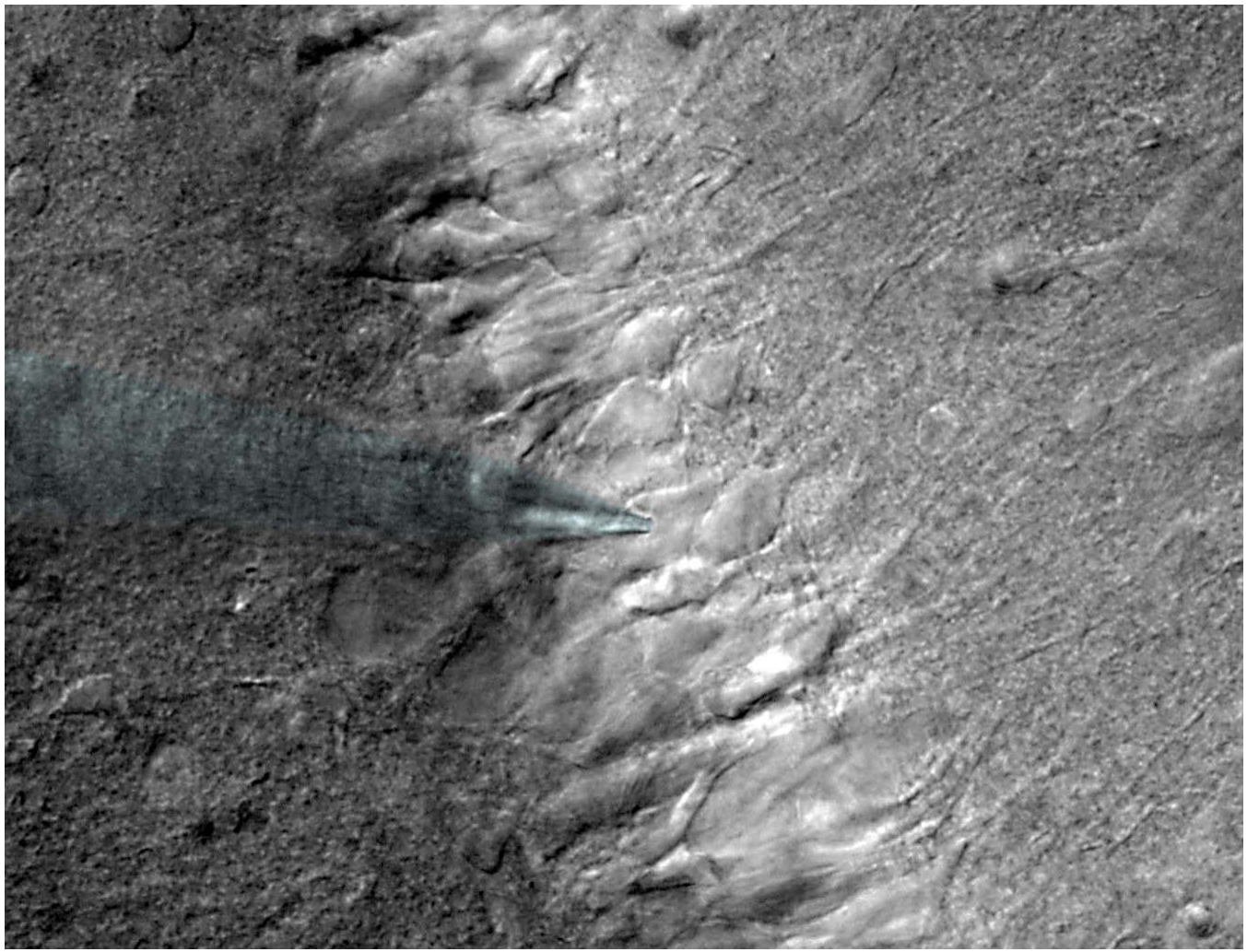
Closed Cleft
Relaxed Dimer
Closed Channel

Desensitized

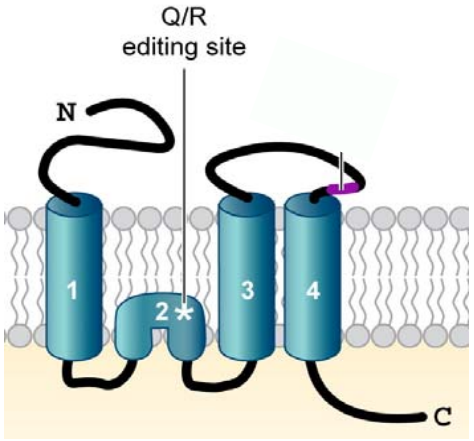
Patch clamp recording configurations



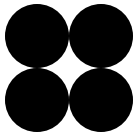
The hippocampal slice



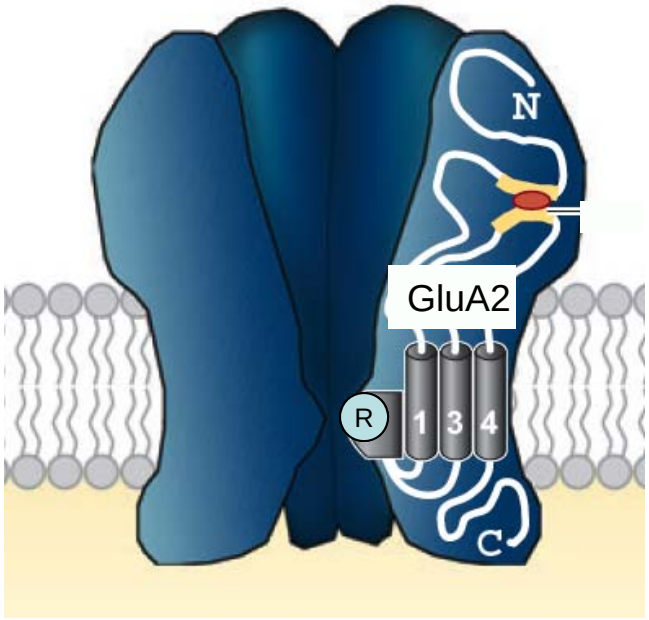
AMPA receptor subunit composition - biophysics



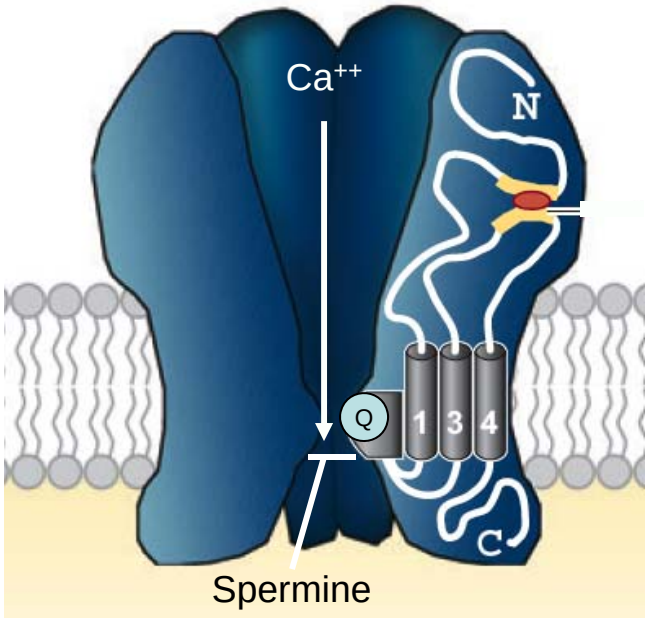
GluA1(Q)
GluA2(R)
GluA3(Q)
GluA4(Q)



Wild type

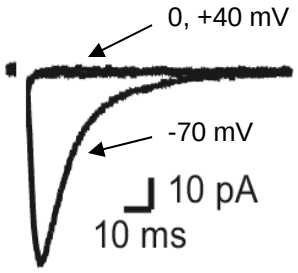


GluA2-lacking

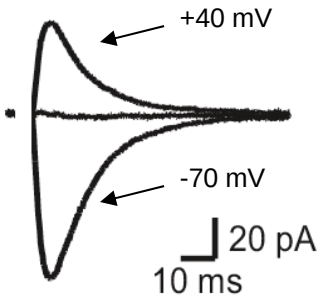


Synaptic

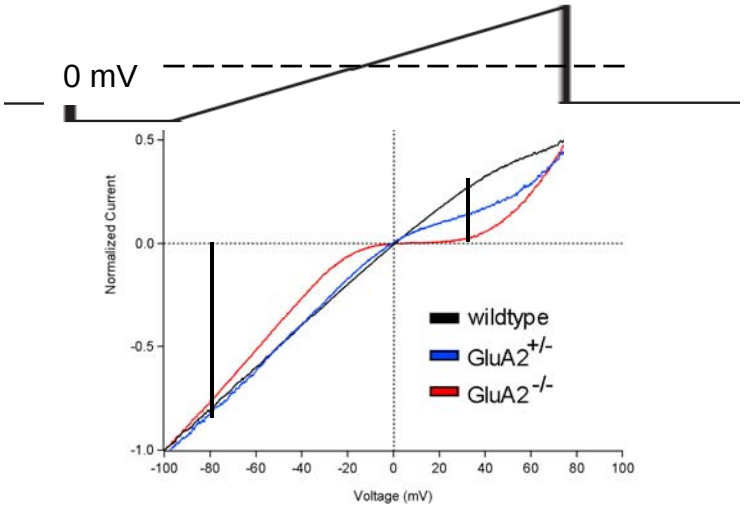
GluA2 KO



Wild type



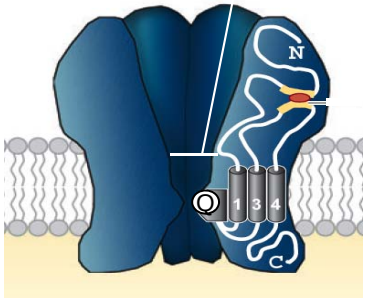
Outside-out patch



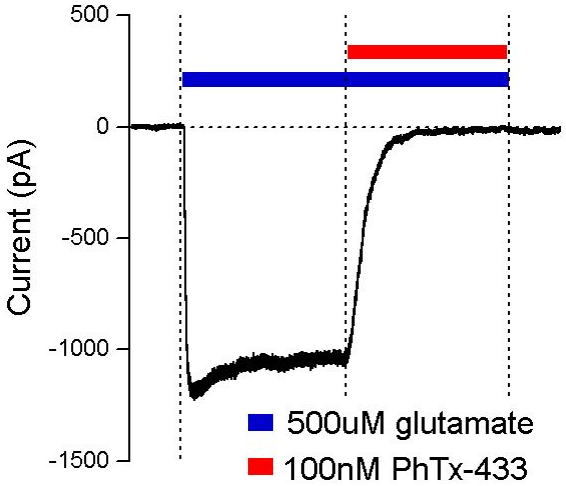
AMPA receptor subunit composition - pharmacology

Philanthotoxin-433

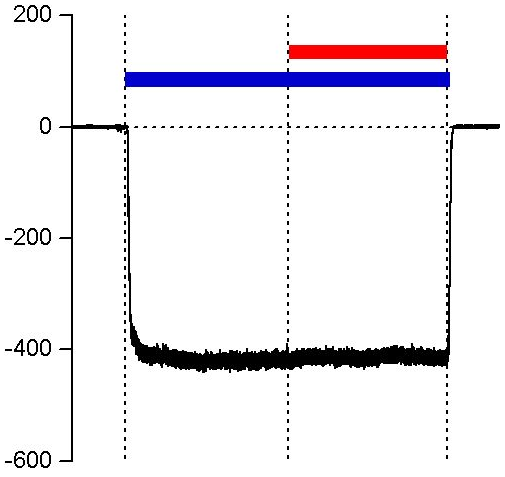
GluA2-lacking Phtx



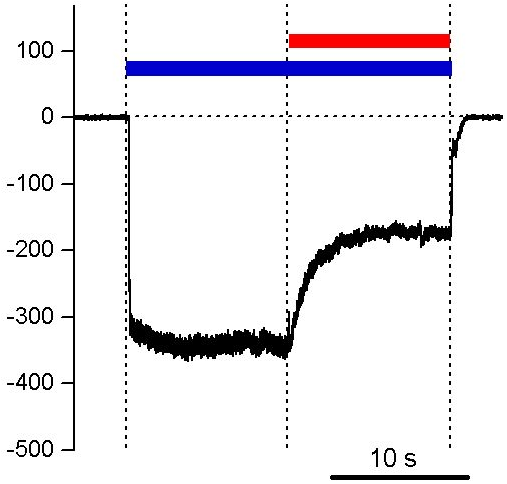
GluA2 KO



WT

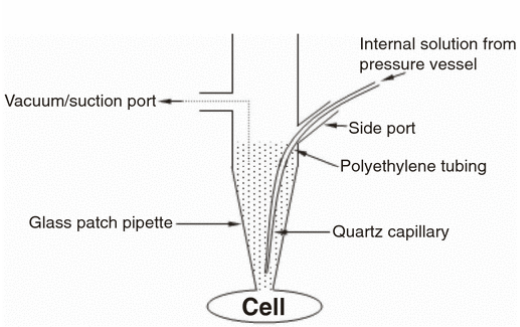


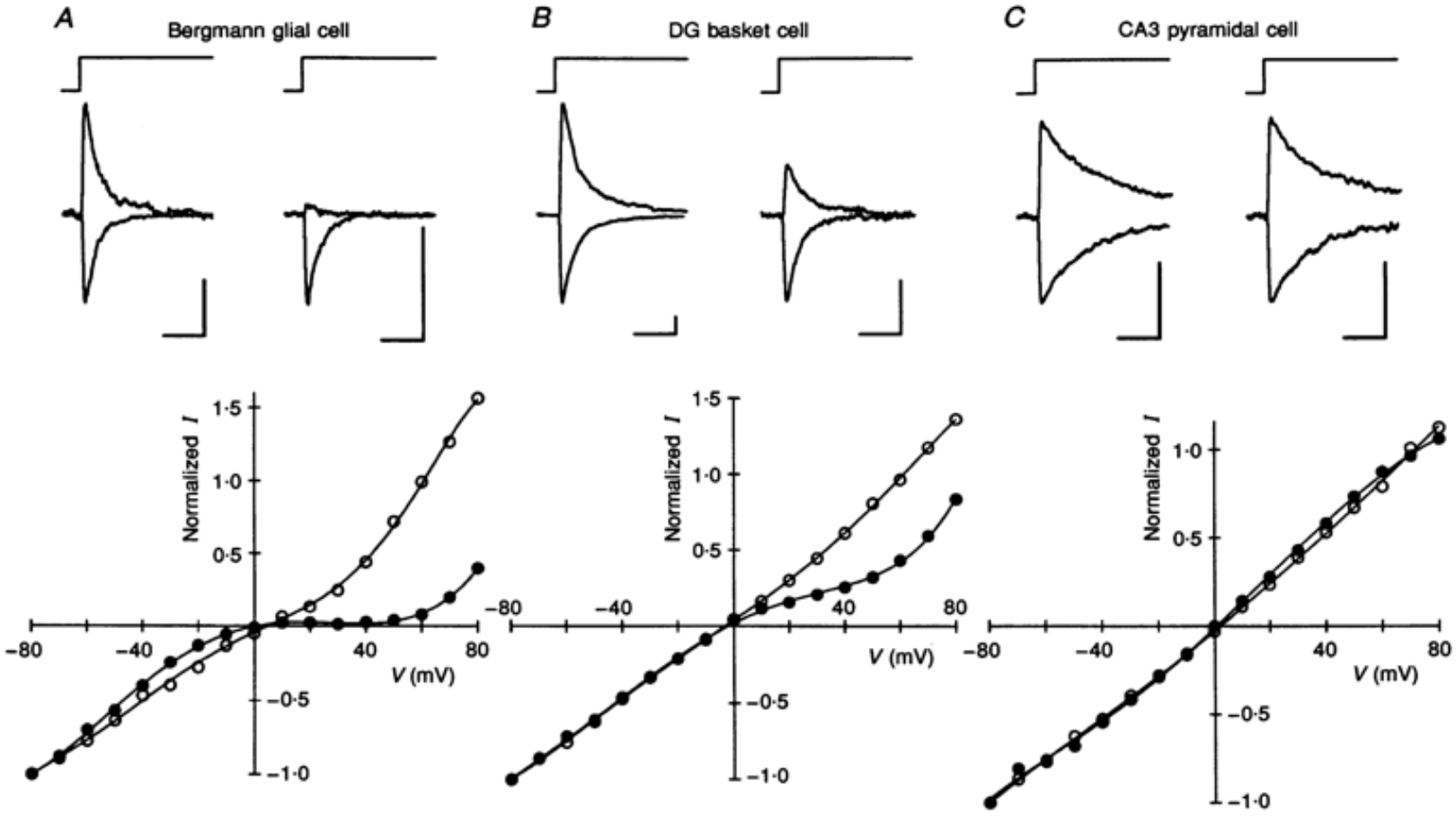
GluA2 het



All functional AMPA receptors on CA1 pyramidal cells contain the GluA2 subunit.

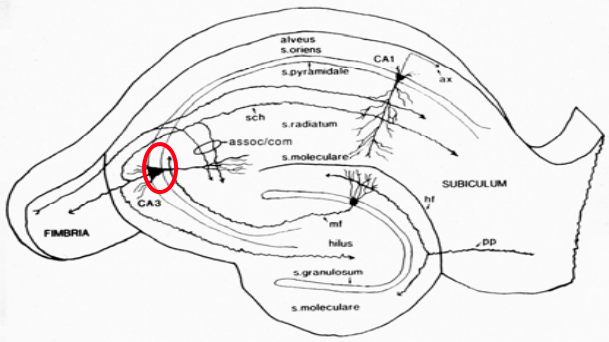
Different cells have AMPARs of different subunit composition

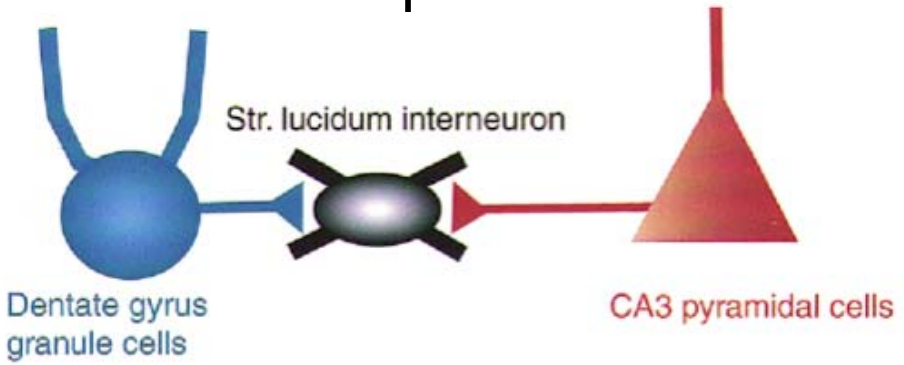


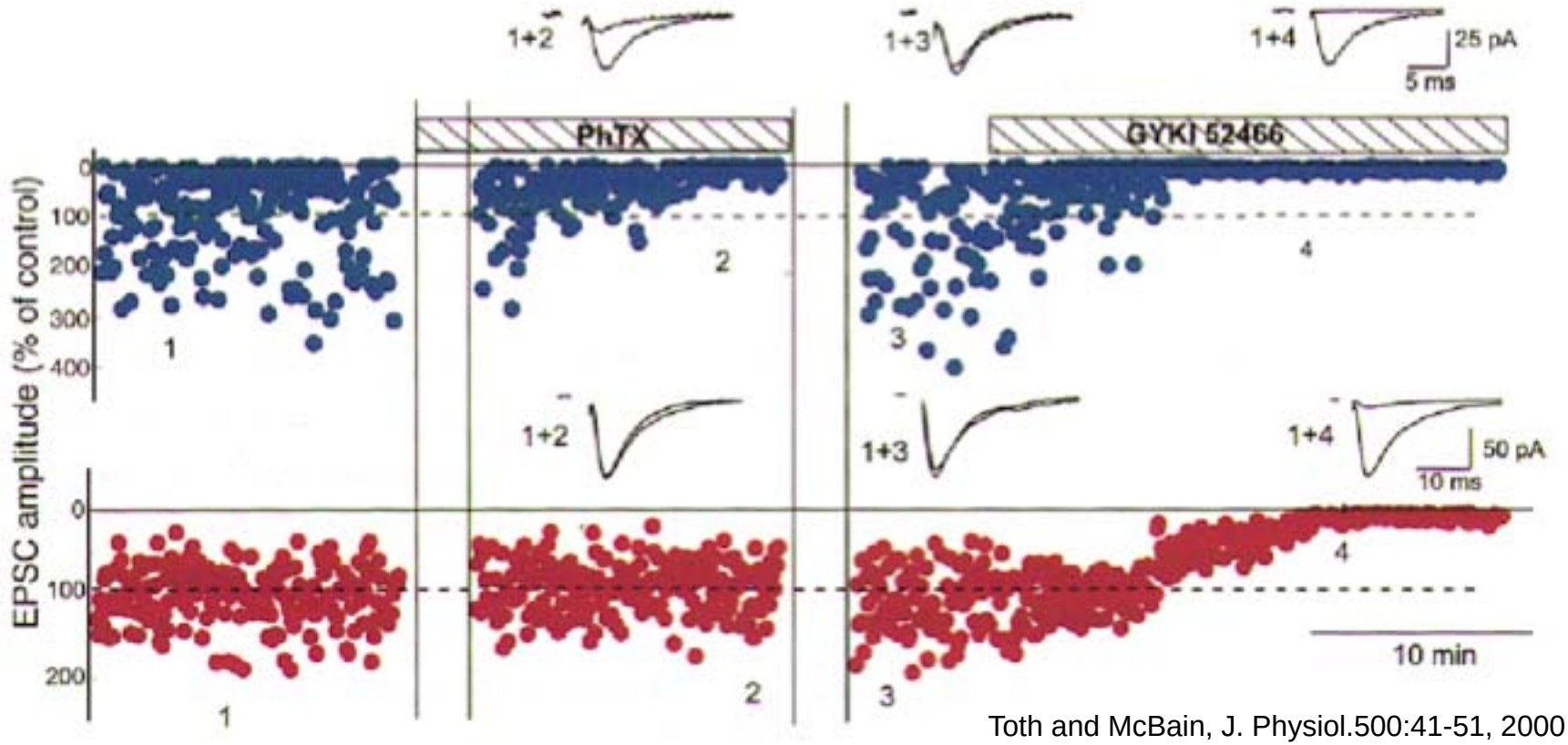


Koh, et al., J. Physiol. 486:305-312, 1995

Different synapses on the same cell have AMPARs with different subunit composition

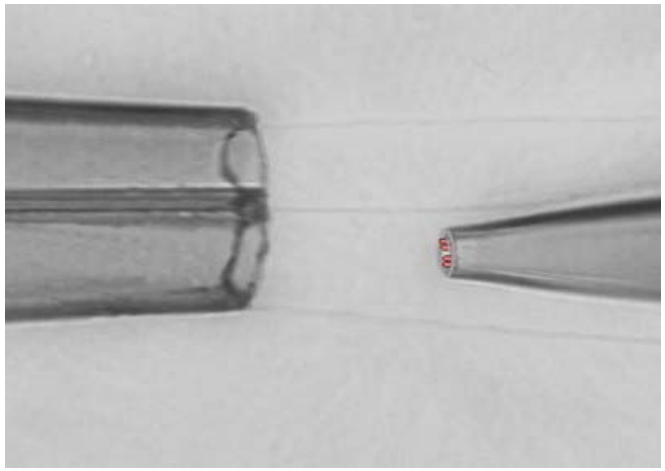


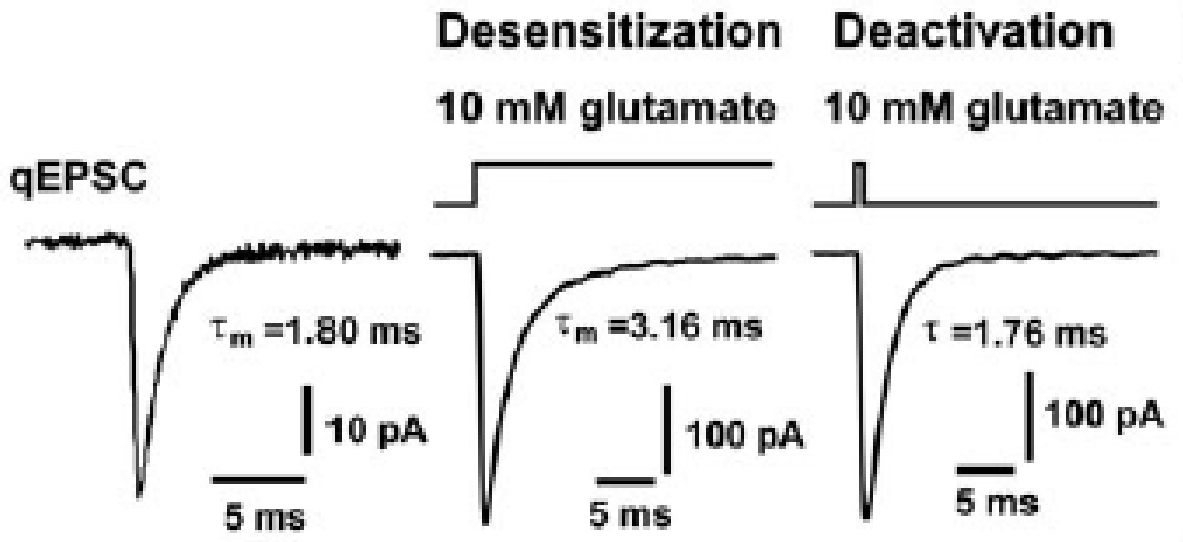




Toth and McBain, J. Physiol.500:41-51, 2000

What determines the time course of the EPSC?

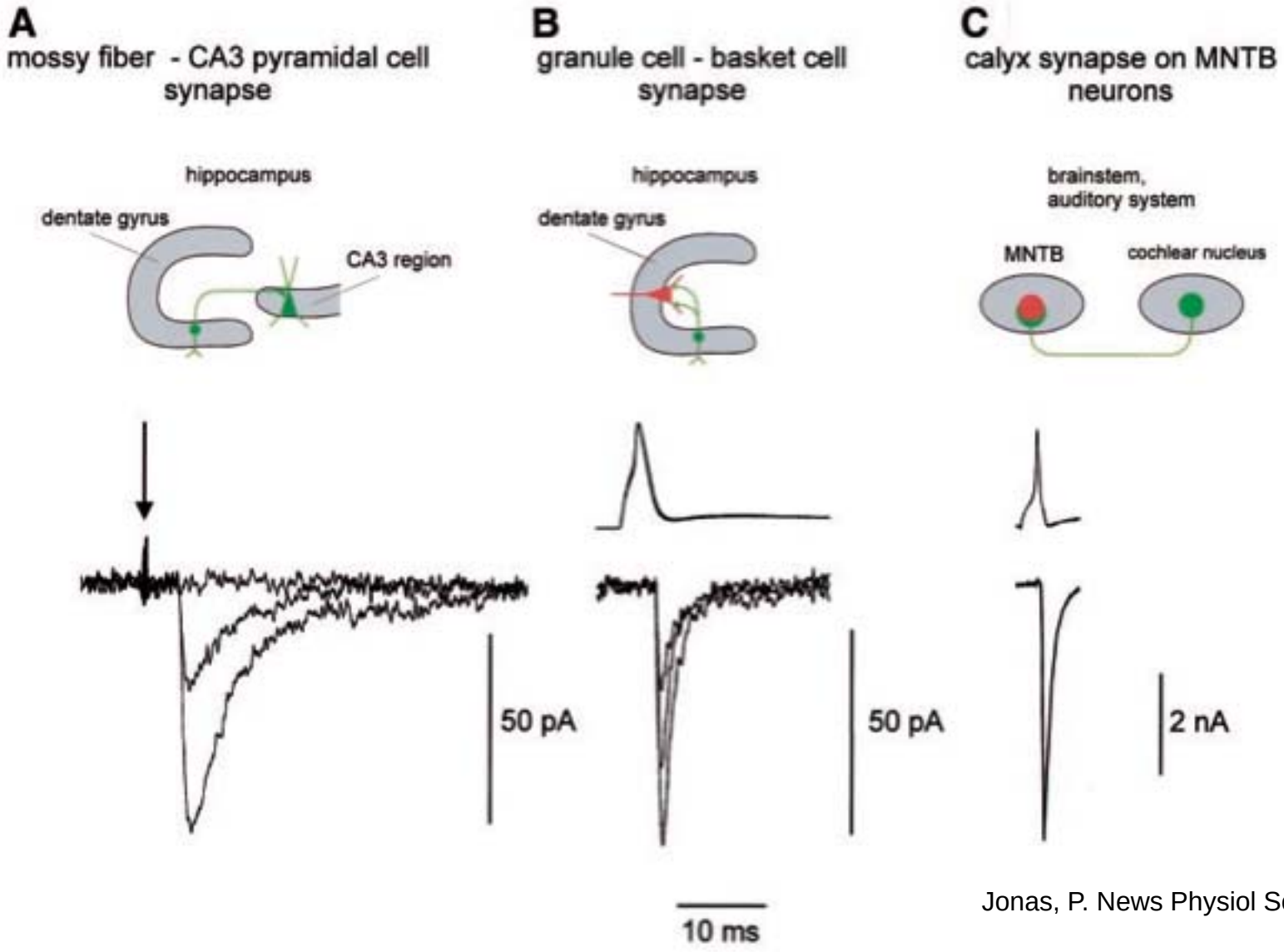




Deactivation is the primary determinant of EPSC time course

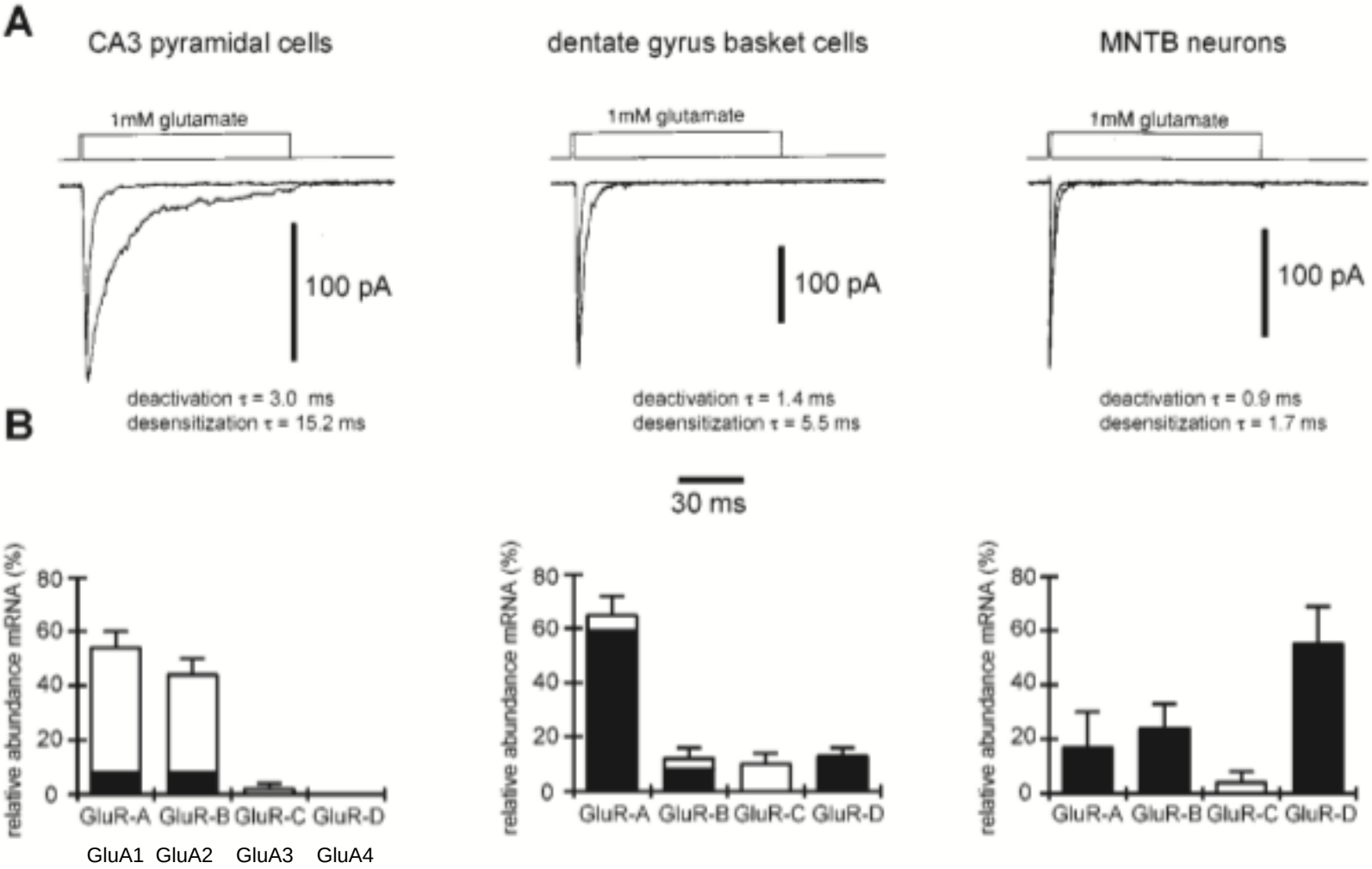
Koike-Tani, et al., J. Neurosci.2005

Different AMPAR-mediated synaptic currents have different kinetics



Jonas, P. News Physiol Sci., 2000

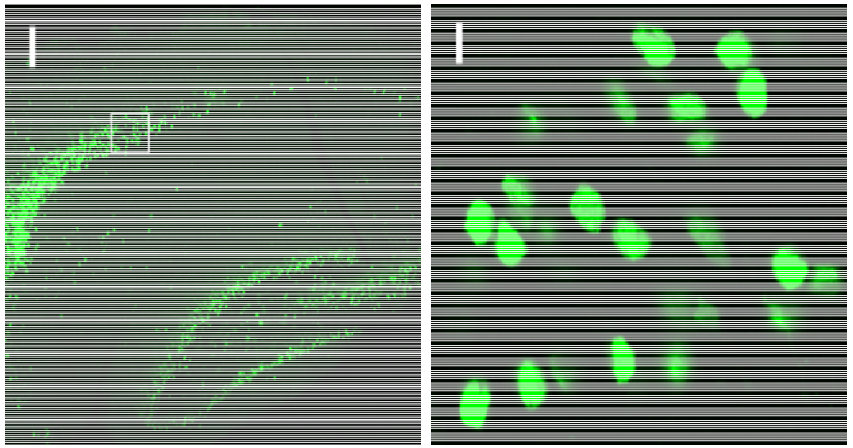
Different kinetics are mediated by different AMPAR subunit composition

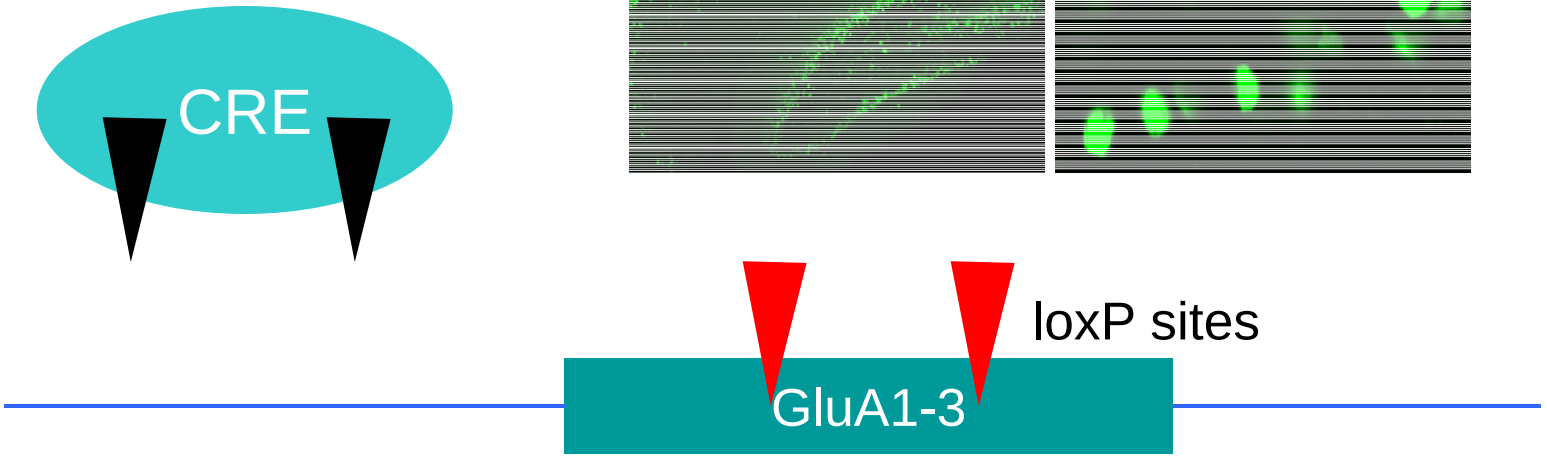


Jonas, P. News Physiol Sci., 2000

Genetic dissection of AMPA receptor subunit composition (hippocampal pyramidal cells)

P0 injection of AAV-CRE GFP

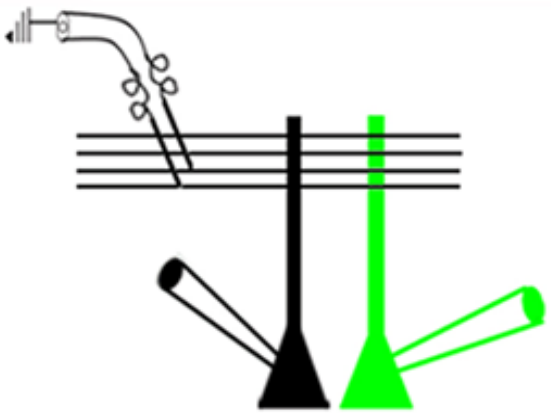


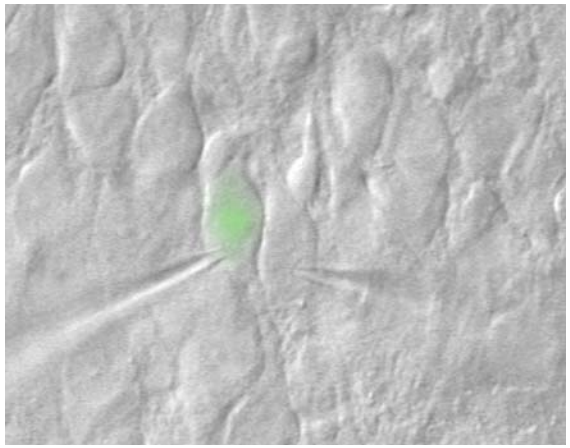


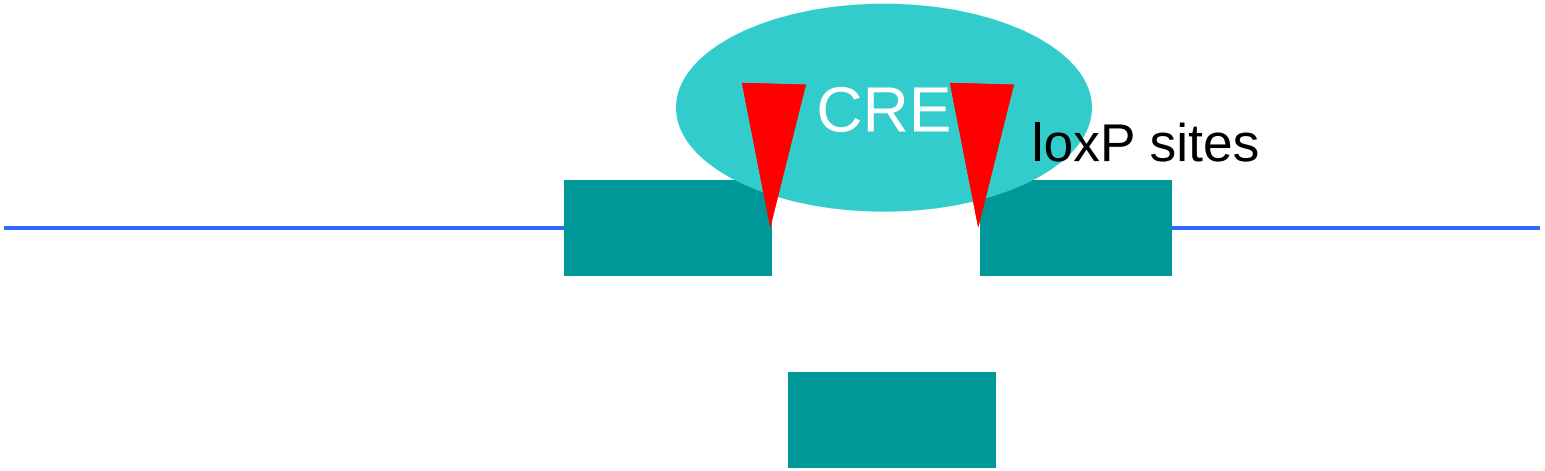
Genetic dissection of AMPA receptor subunit composition (hippocampal pyramidal cells)

P0 injection of AAV-CRE GFP

(2-3 weeks)

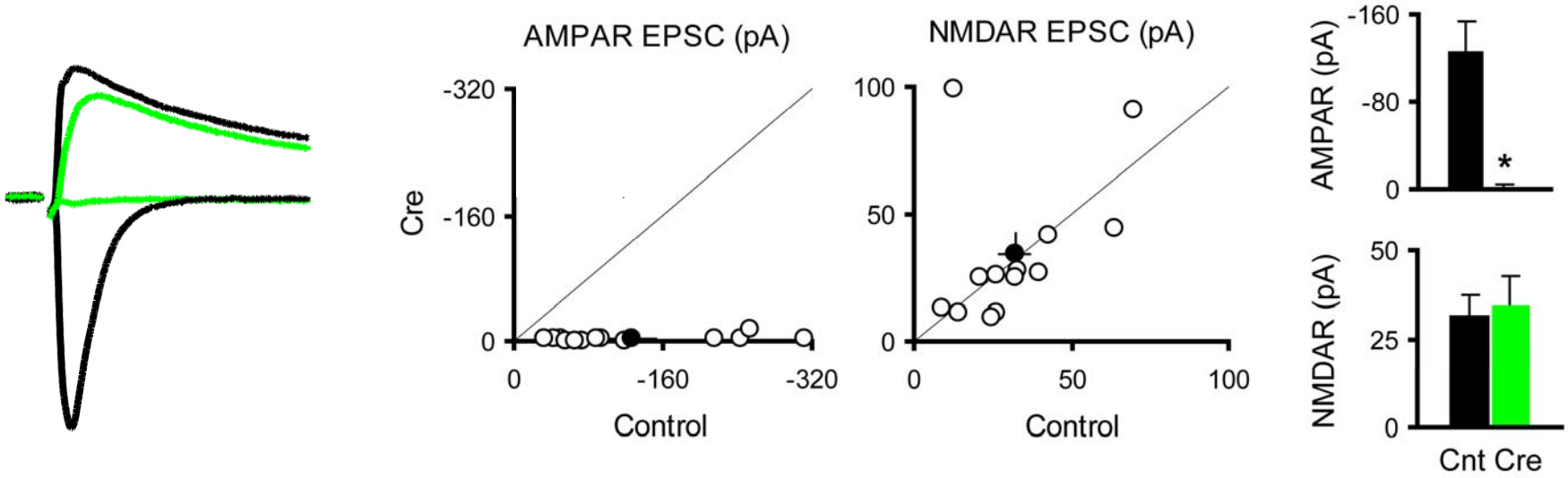


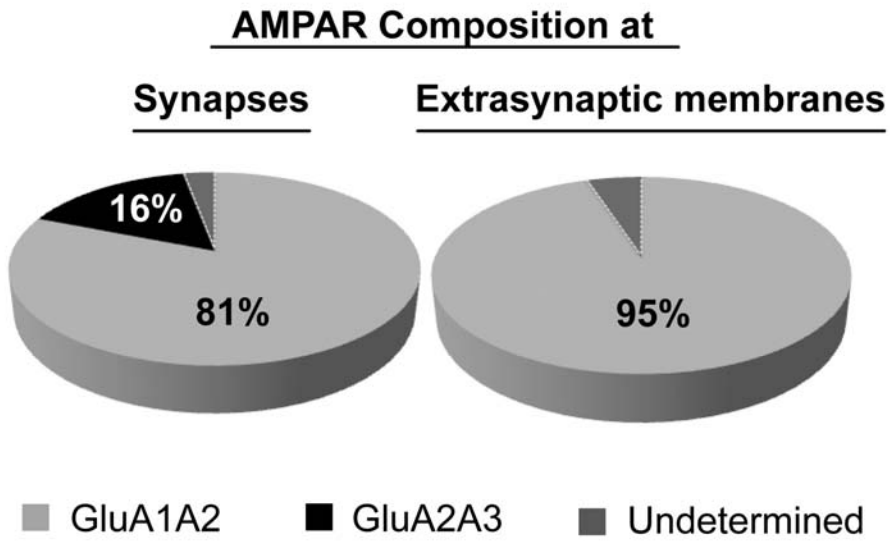




Protein is removed (<u>completely</u>) *where* and *when* CRE is expressed.

Floxed-GluA1A2A3





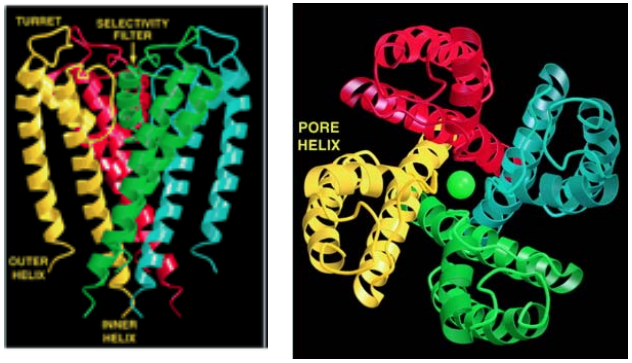
Why is GluA1A2 and GluA2A3 expressed in the same cell?

1. Have different kinetics.
2. Proposed to have different trafficking rules.

Lu, et al., Neuron (2009)

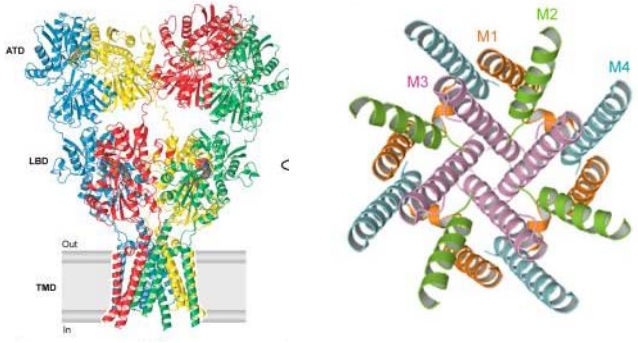
Ion channels and neuronal excitability

Voltage gated



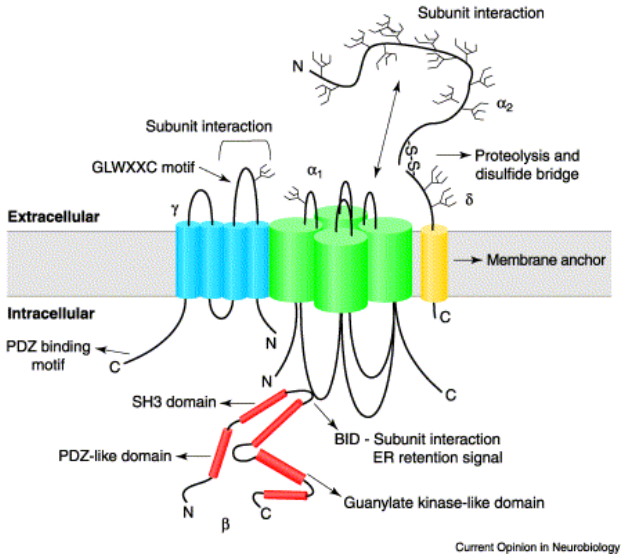
Doyle et al. Science 280:69-77, 1998

Ligand gated



Sobolevsky et al., Nature 462:745-756, 2009

<u>Sodium</u> <u>Potassium</u> <u>Calcium</u>

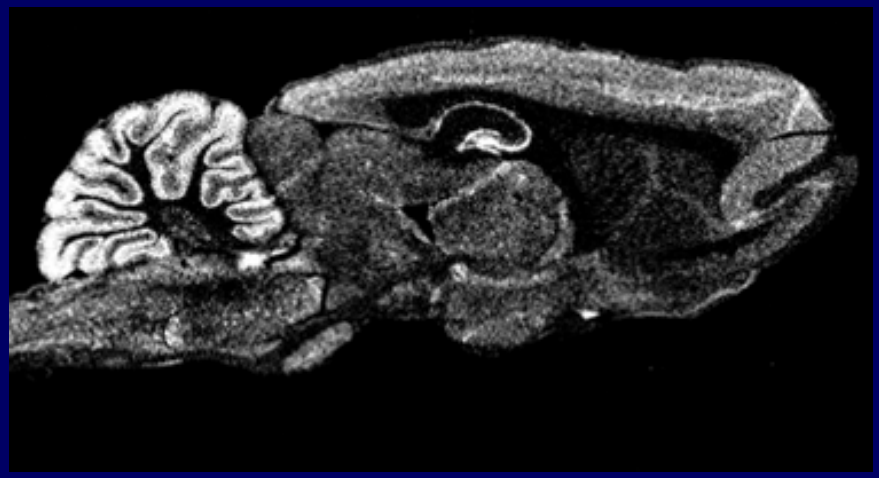


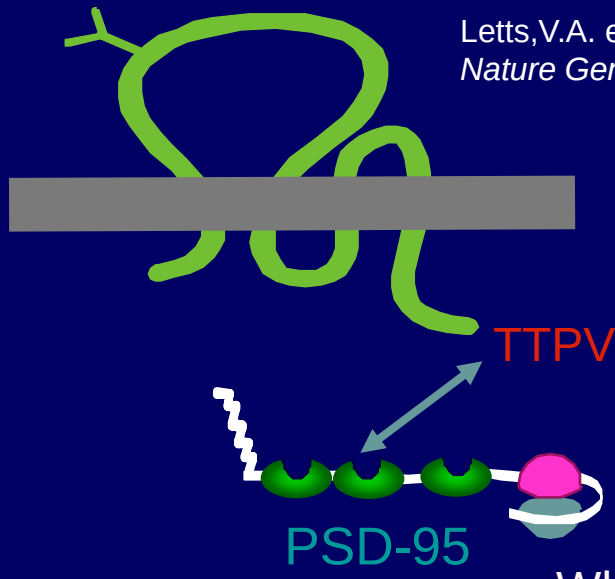
Nicotinic AchRs
GABARs
GlycineRs
glutamateRs
- AMPARs
- NMDARs
- kainateRs

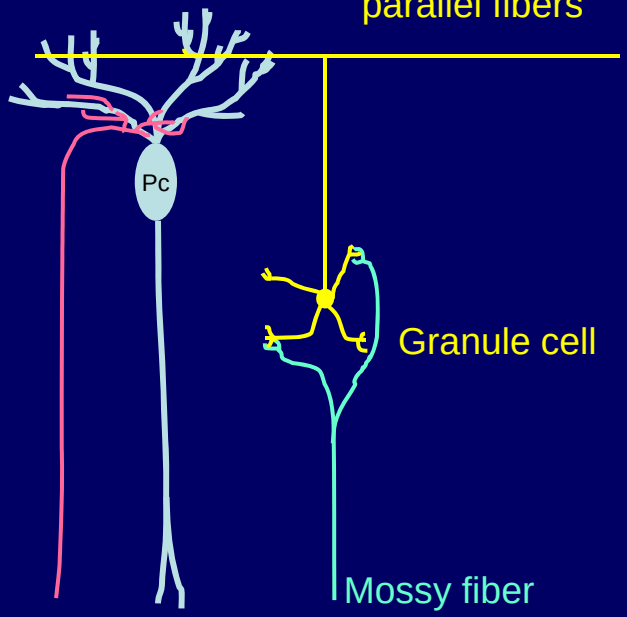
5-HT3Rs
P2XRs

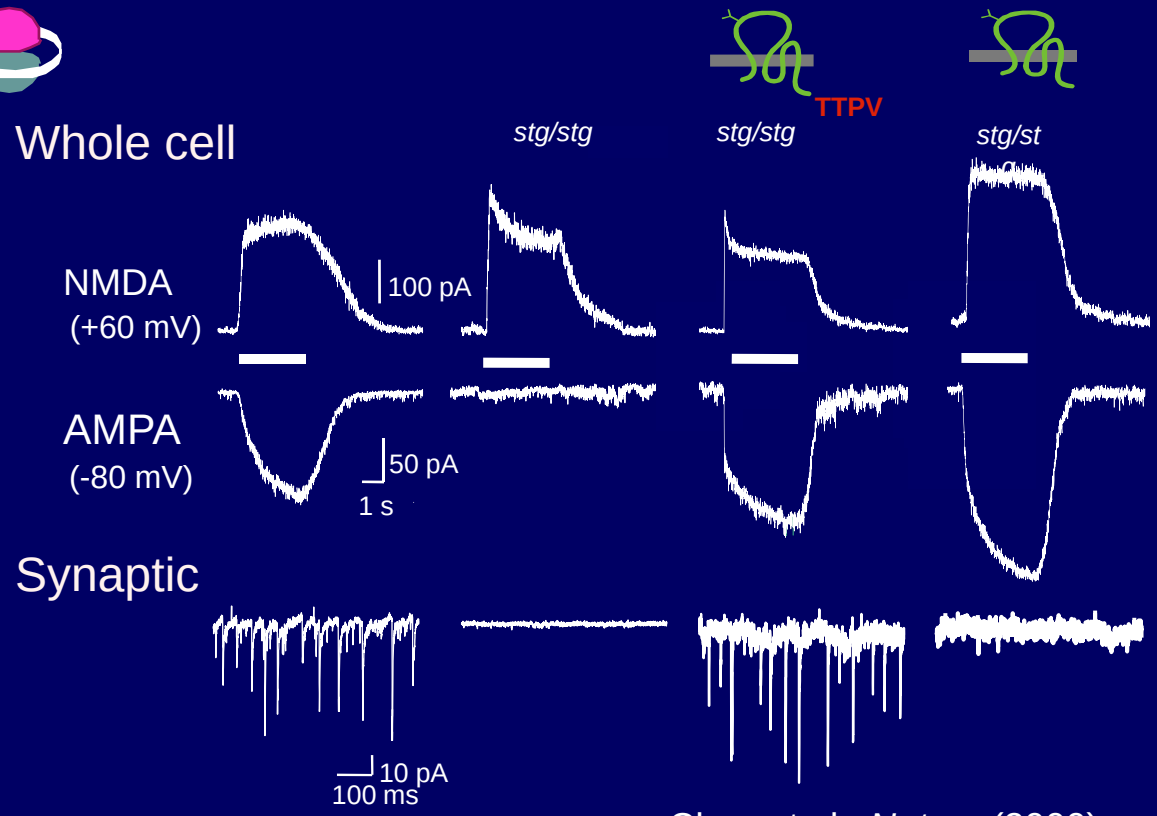
Stargazin (γ-2) and AMPA receptor trafficking

Letts,V.A. et al.
Nature Genetics (1998)

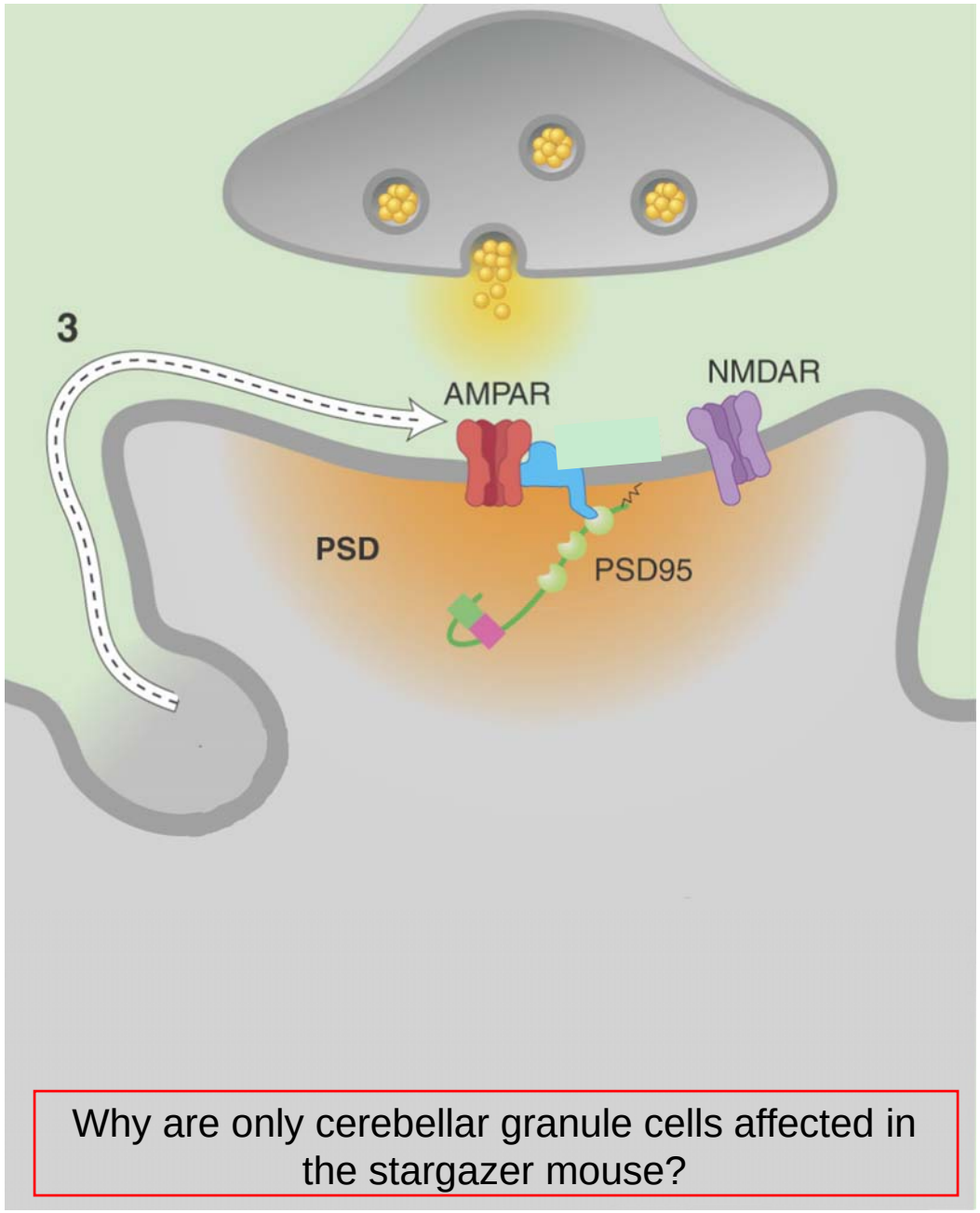






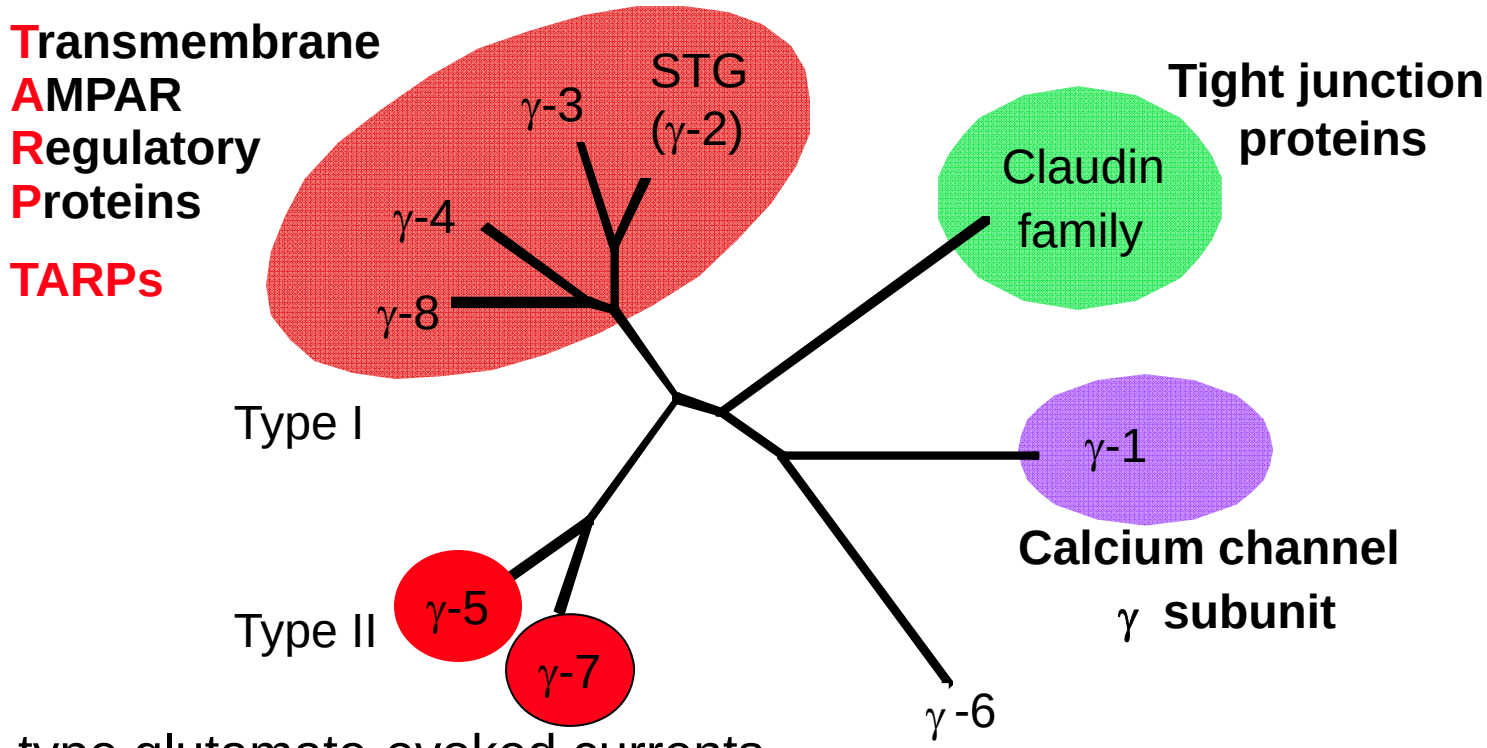


Chen et al., *Nature* (2000)

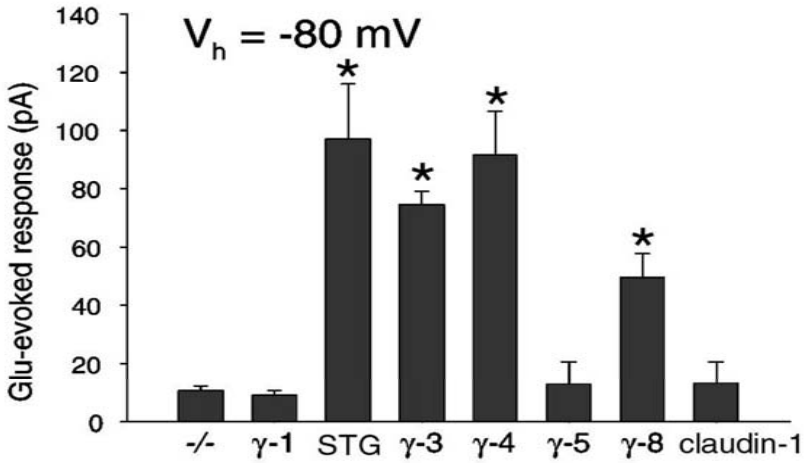


Why are only cerebellar granule cells affected in the stargazer mouse?

Superfamily of tetraspanning membrane proteins



AMPA-type glutamate-evoked currents



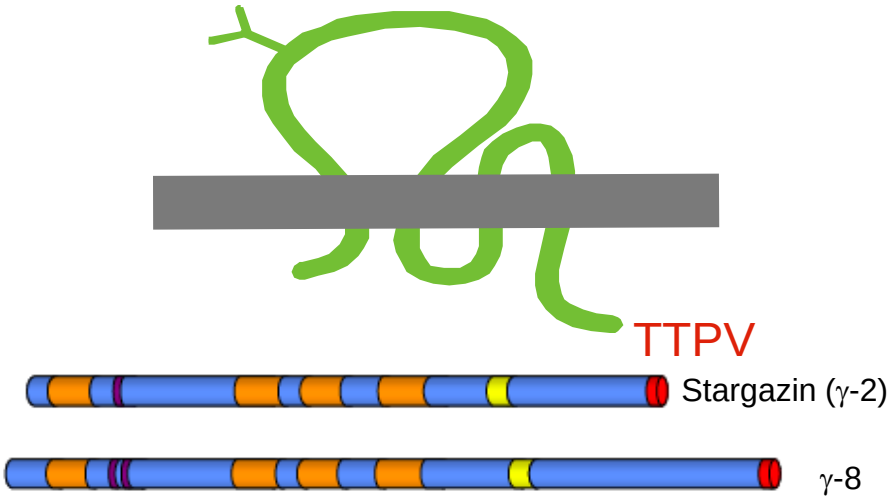
All TARPs can bind to all AMPAR subunits

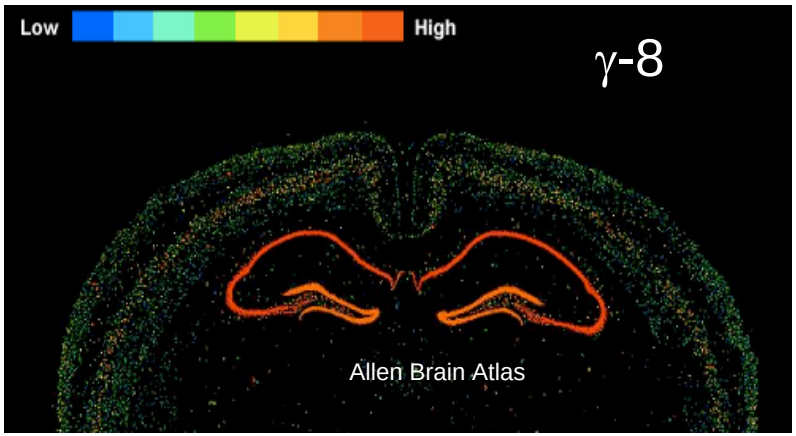
Differential anatomical distribution

Different developmental profiles

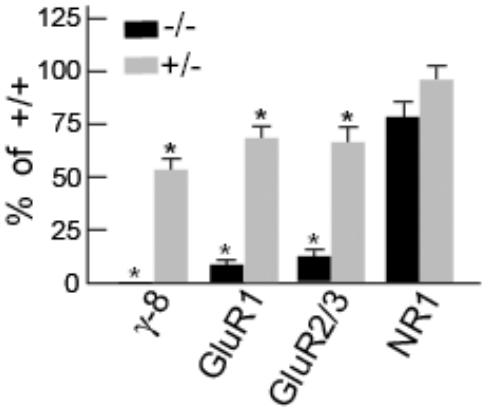
Knock out mice of the various TARPs establish their essential role in AMPAR targeting at other synapses

Role of TARP γ-8 in AMPAR trafficking in hippocampus

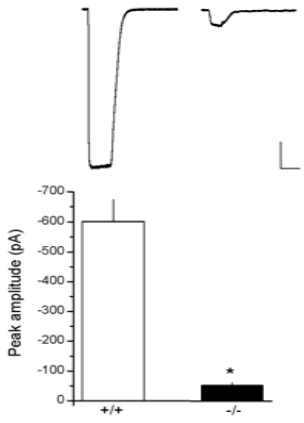


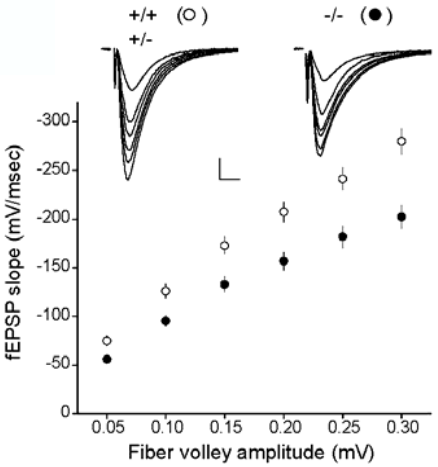


Hippocampal protein levels



outside out patch



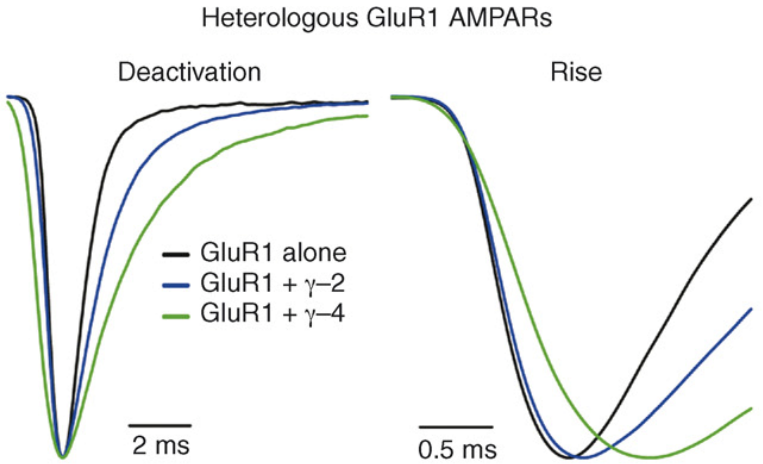


Is loss of surface receptors simply due to loss of AMPAR protein?

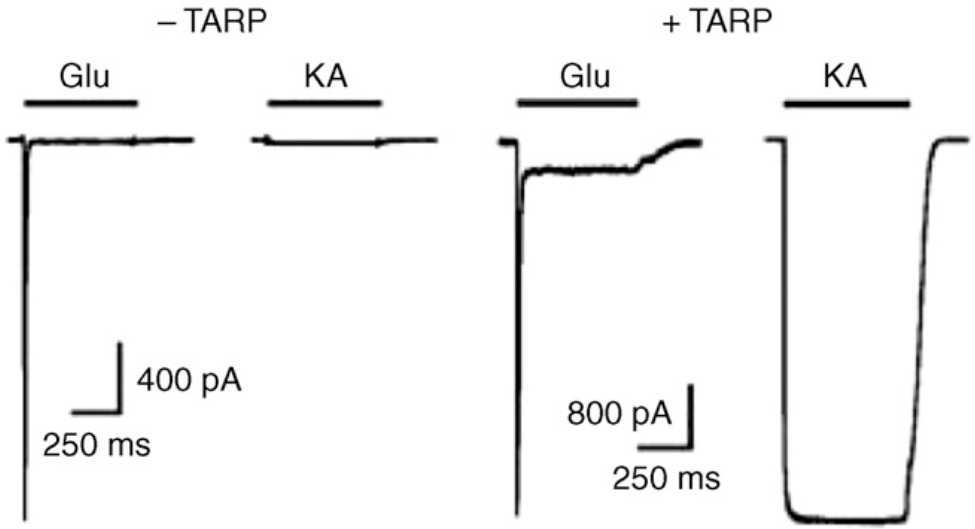
The remaining EPSC:
MAGUK independent?
TARP independent??

Rouach et al. Nature Neurosci., 2005

TARPs control gating and pharmacology of AMPA receptors

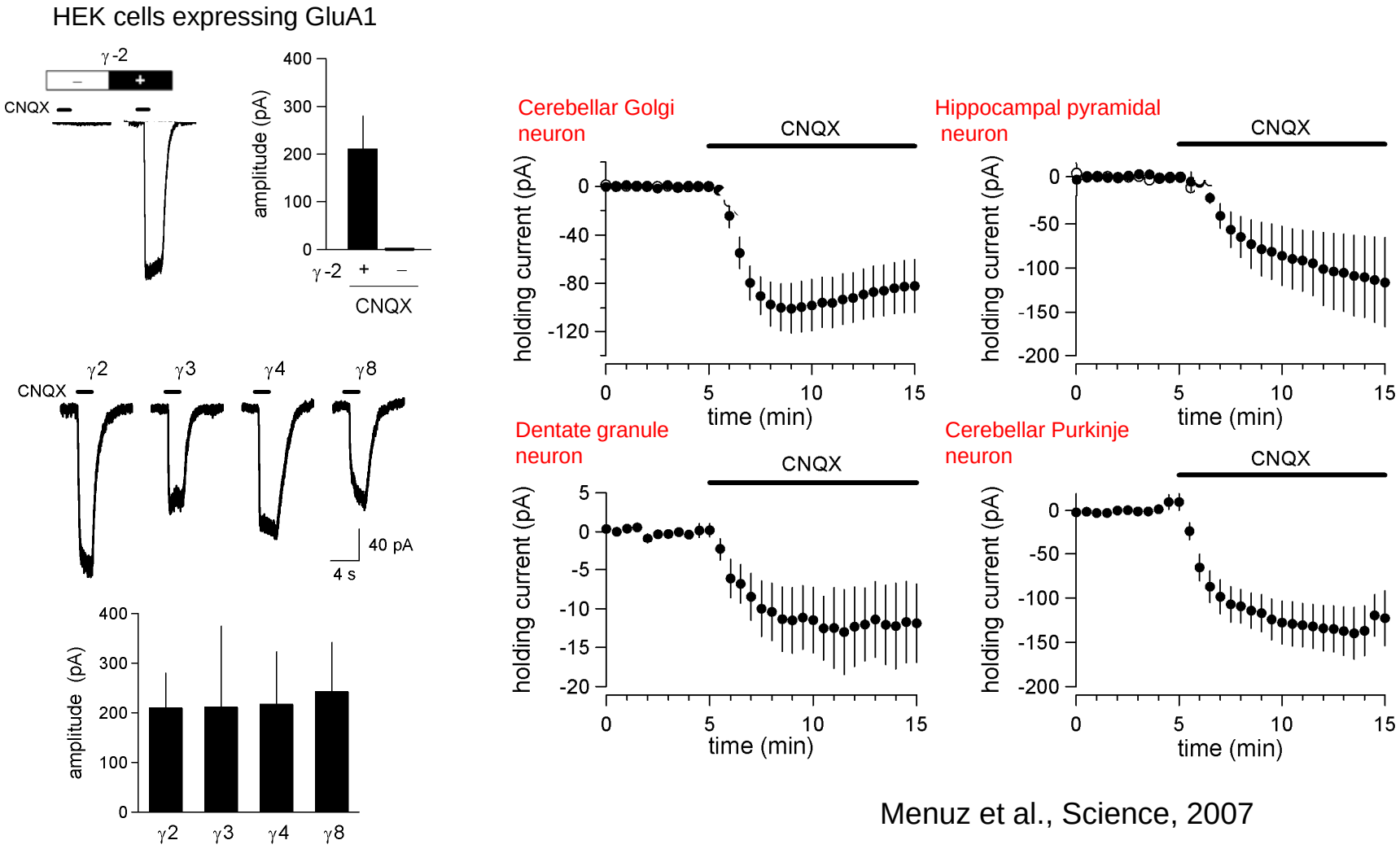


Milstein et al.,
Neuron, 2007



Turetsky et al.,
J. Neurosci., 2005

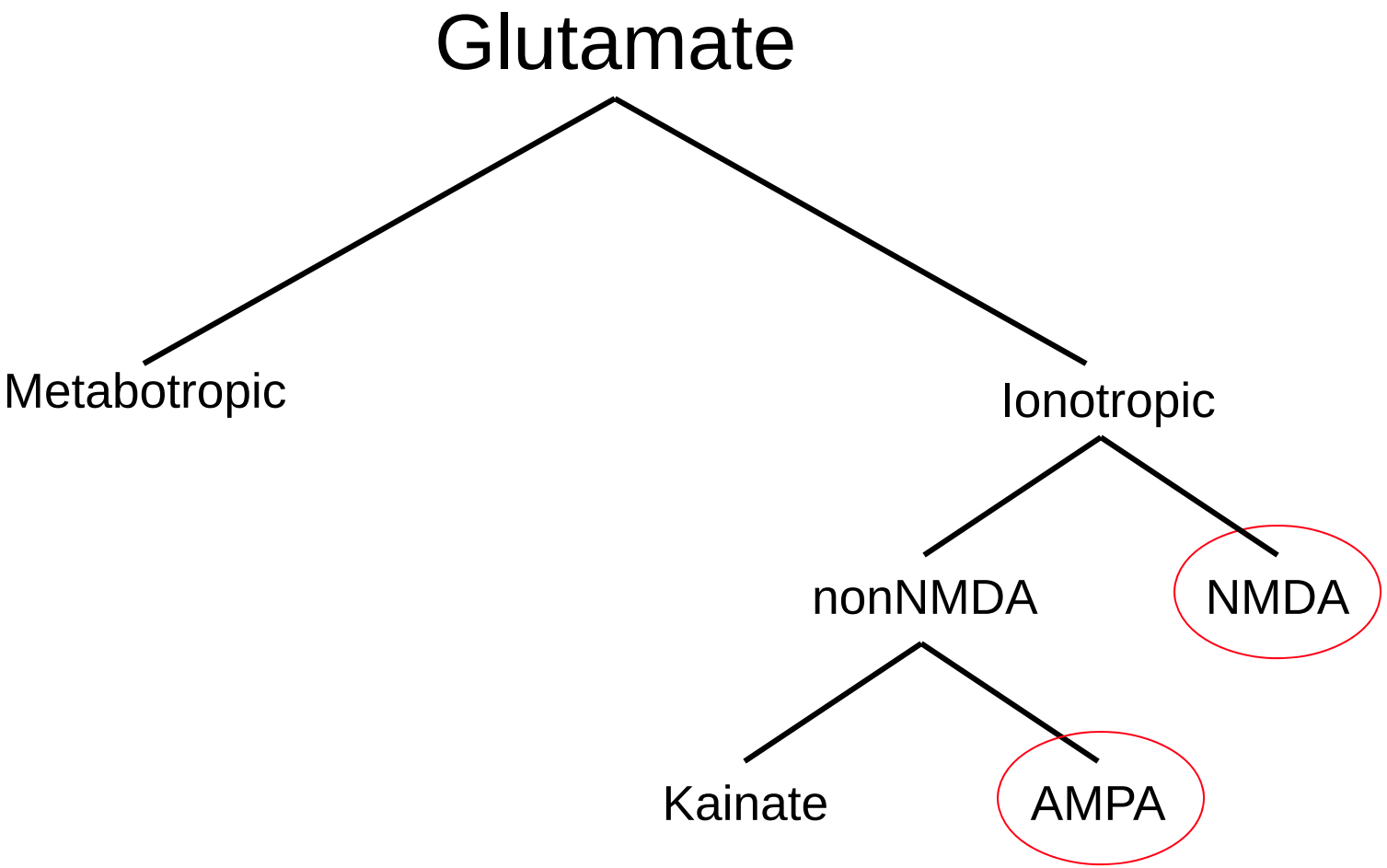
Assaying the presence of TARP/AMPAR association in neurons



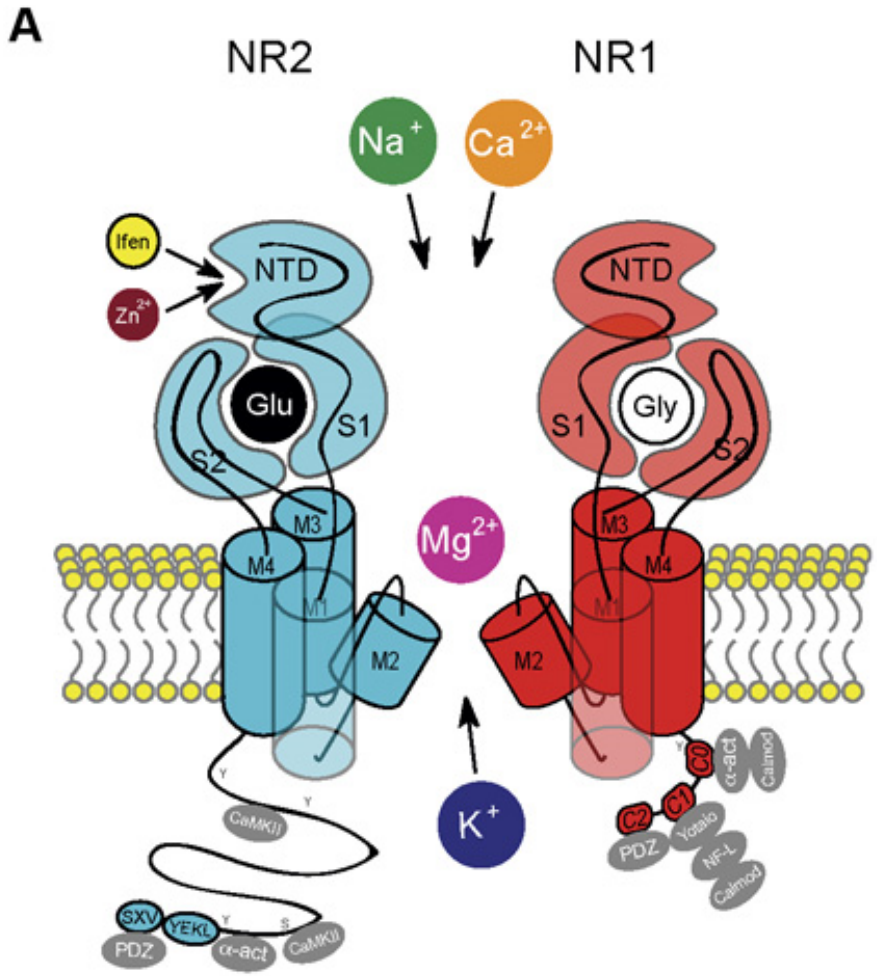
Menuz et al., Science, 2007

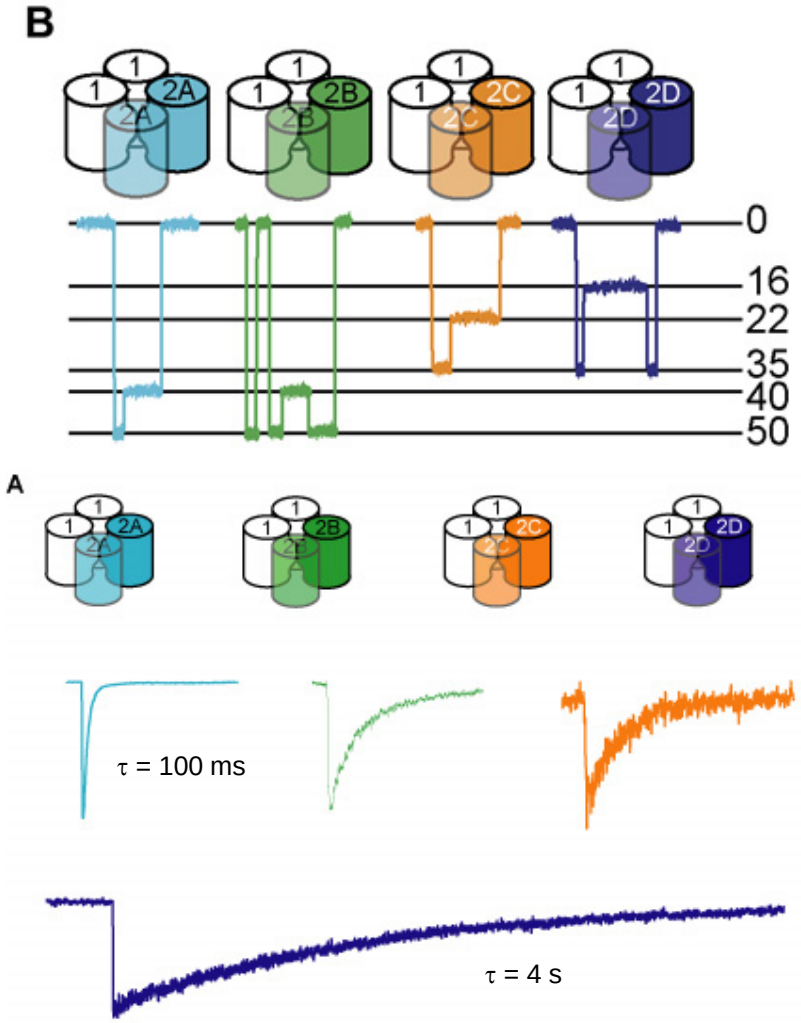
AMPARs in all neurons tested are associated with TARPs

Glutamate Receptor Subtypes



NMDA receptor subunits and properties





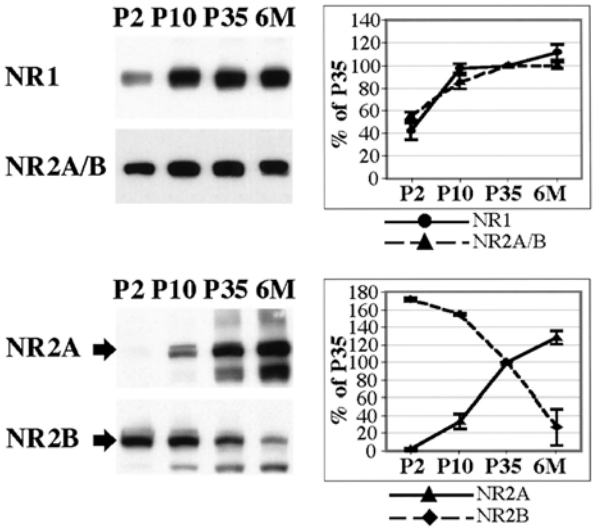
<u>NMDARs are heterotetramers</u>
GluN1- essential
GluN2- GluN2A
GluN2B
GluN2C
GluN2D

Cull-Candy and Leszkiewicz, Sci. STKE 2004 (255)

Developmental switch of NMDAR subunit composition

A

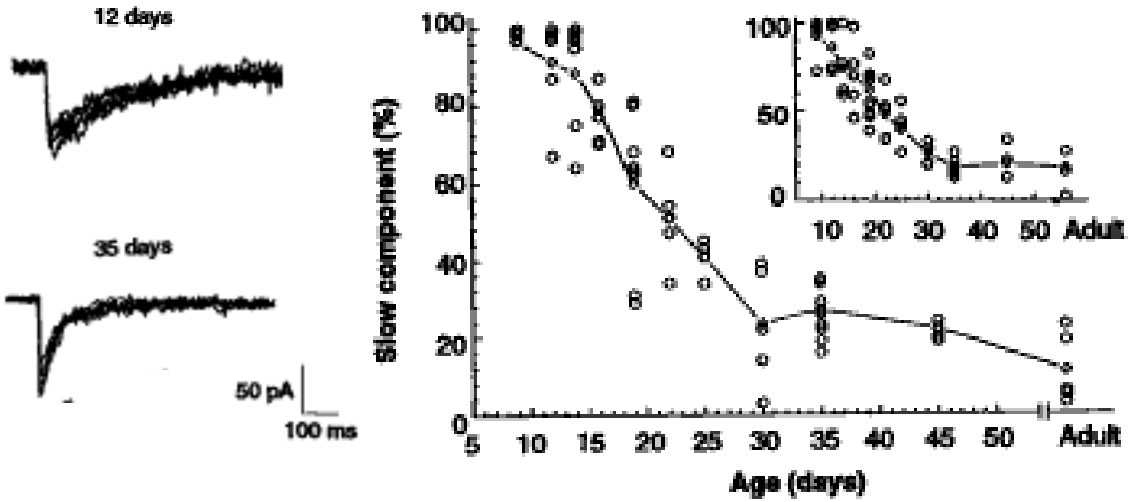
Expression pattern



Sans N et al. J. Neurosci. 2000;20:1260-1271

B

Developmental speeding of kinetics

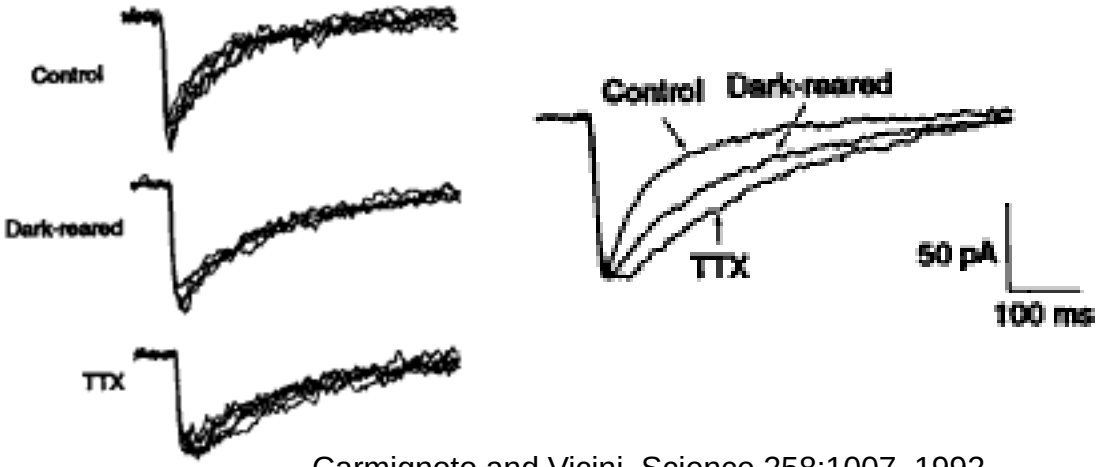


Carmignoto and Vicini, Science 258:1007, 1992

C

Activity dependence of speeding

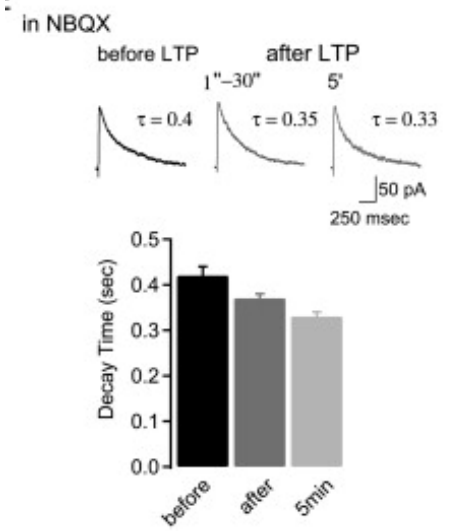
25 days



Carmignoto and Vicini, Science 258:1007, 1992

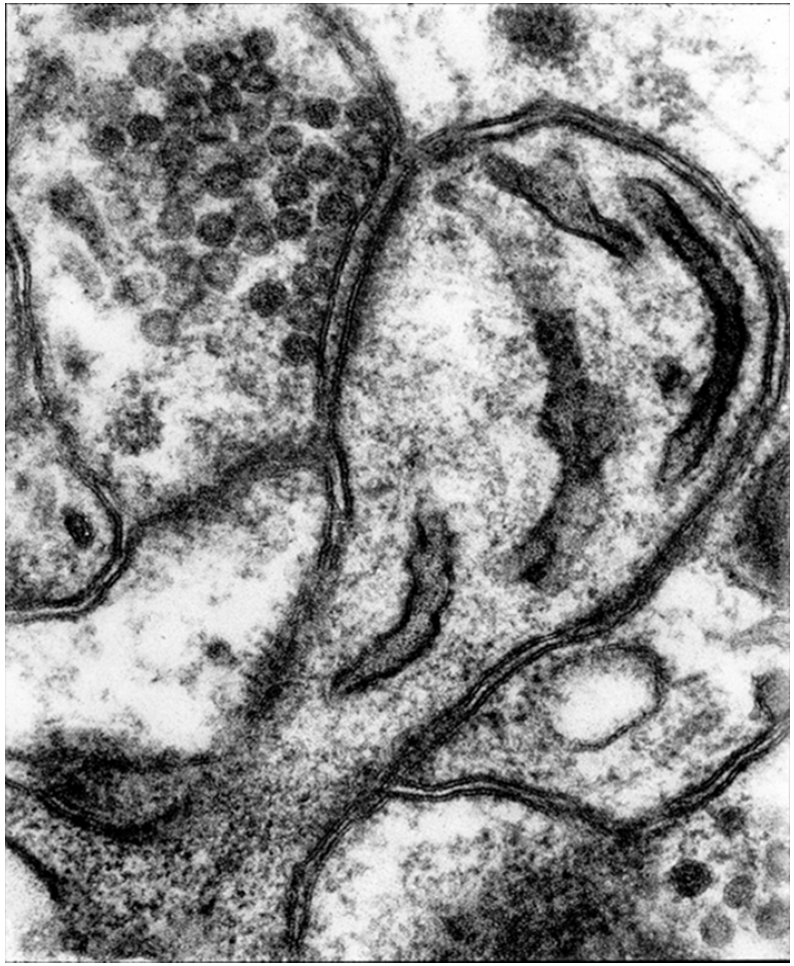
D

Time course of switch



Bellone and Nicoll Neuron 55:779, 2007

A molecular dissection of the postsynaptic density



~1,000 Receptors

Scaffolds

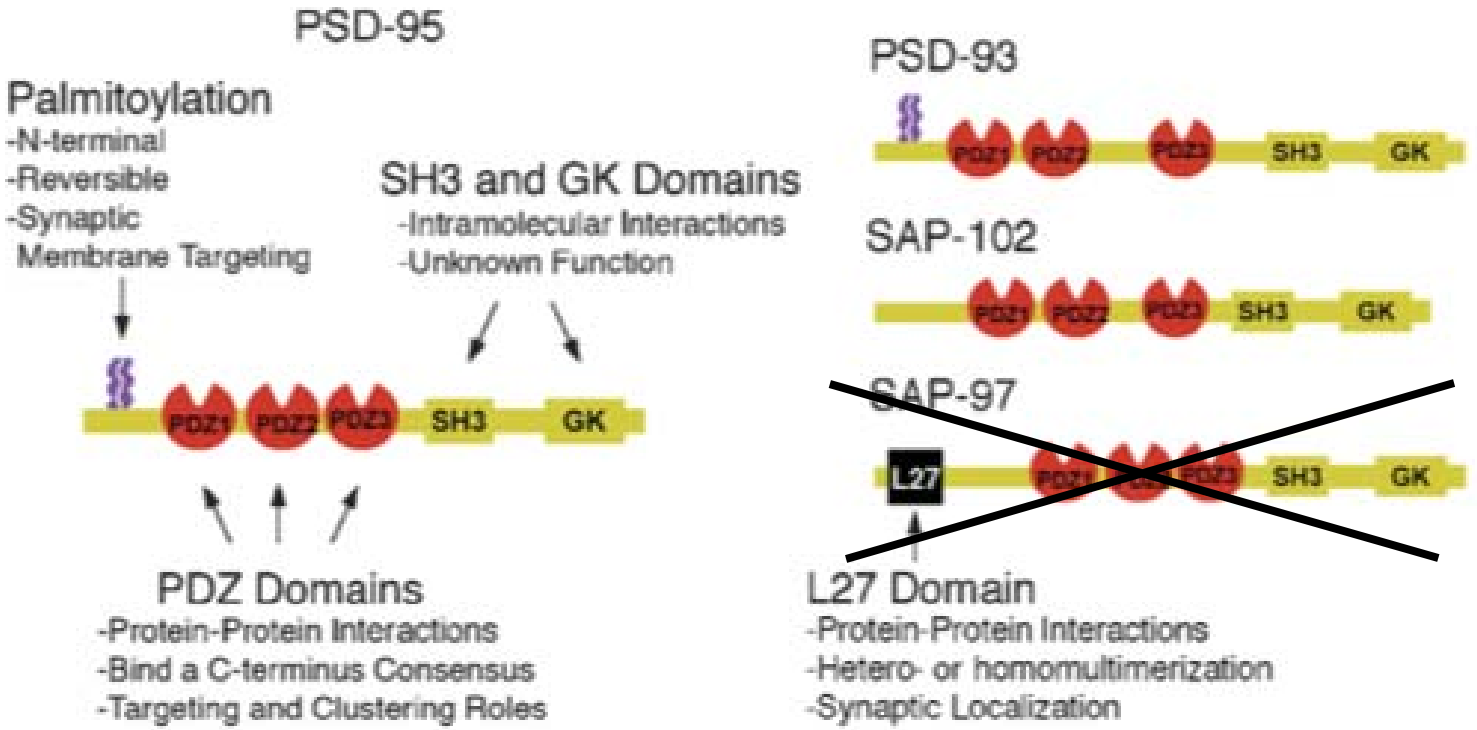
Adhesion proteins

Cytoskeleton

Signaling proteins

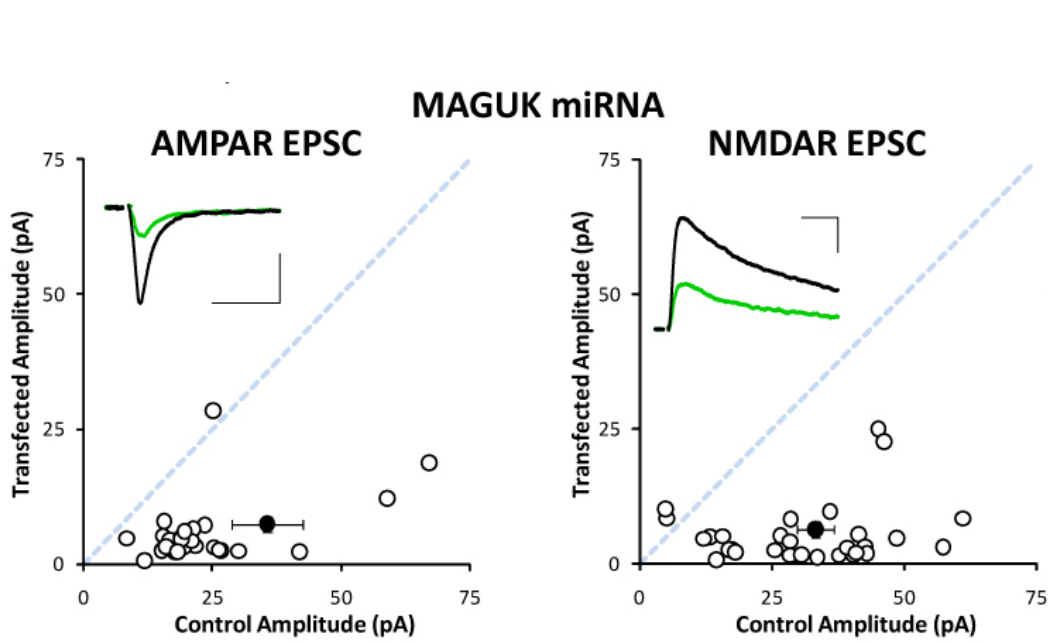
Agonist = overexpression
Antagonist = gene deletion/RNAi

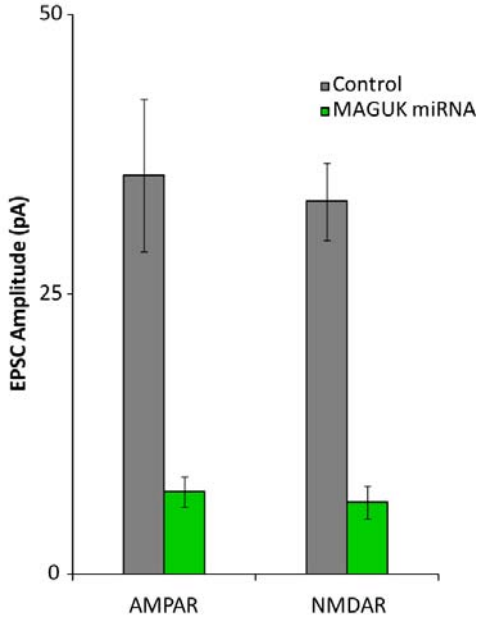
Membrane Associated Guanylate Kinases (MAGUKs)
Synaptic scaffolding proteins

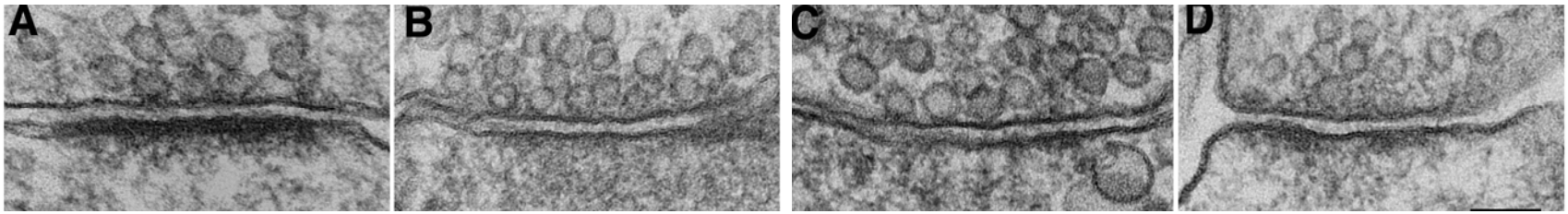


MAGUKs are important

Triple knock down (PSD-95, PSD-93 and SAP102)



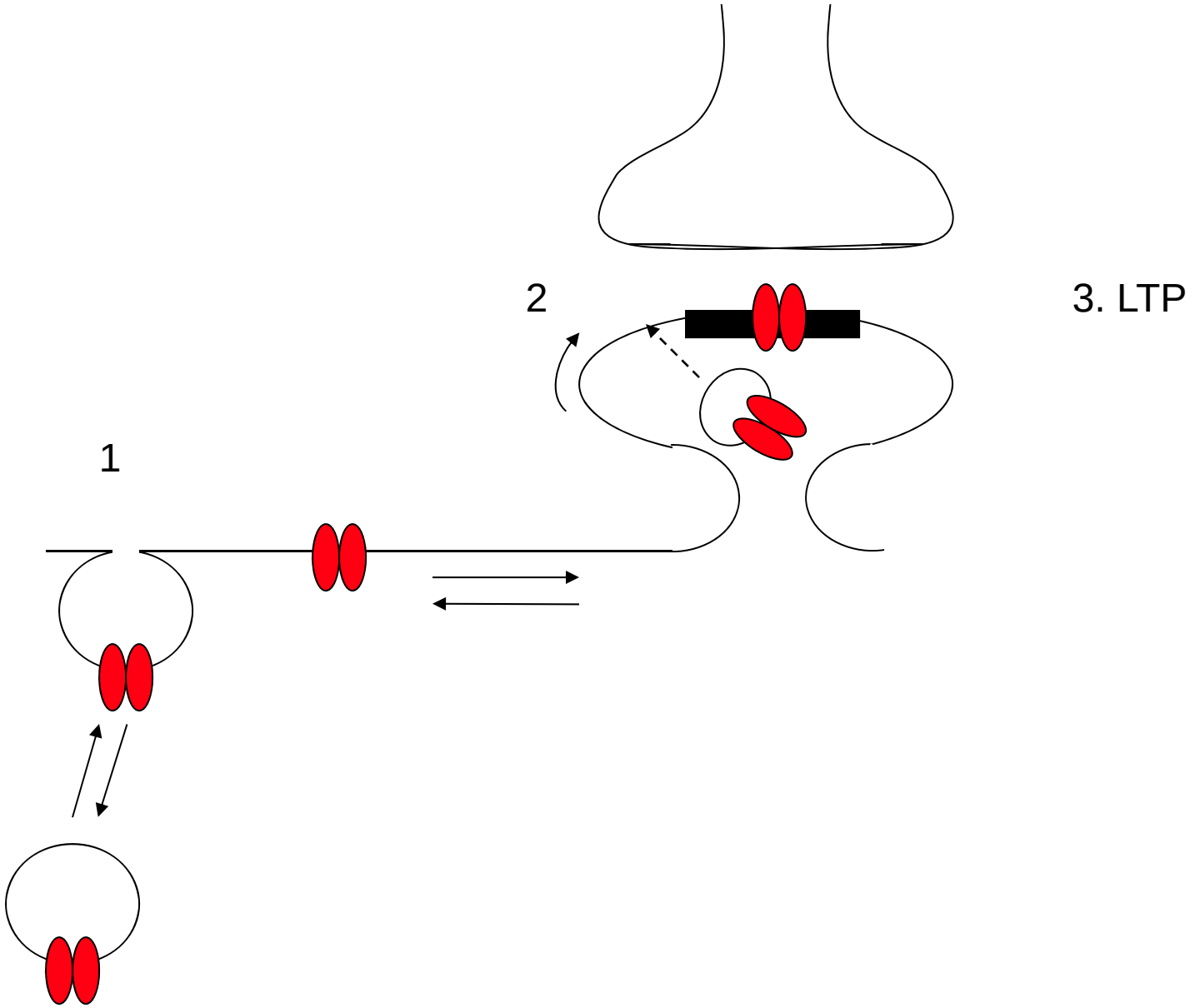




Chen, et al., PNAS, 2015

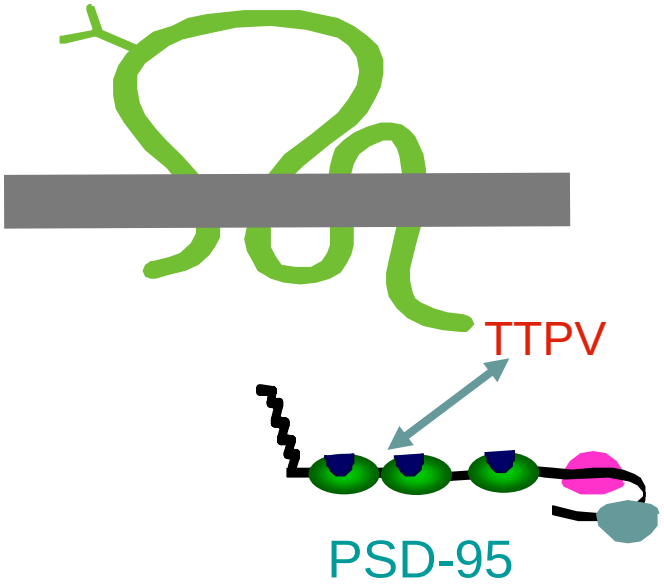
How do MAGUKs traffic glutamate receptors?

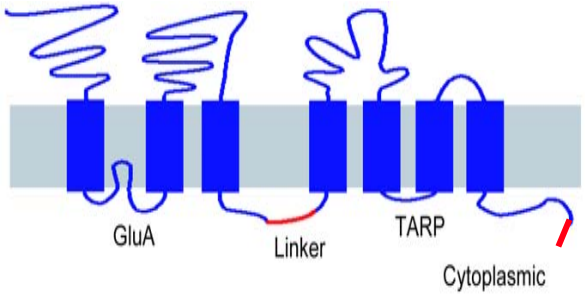
Synaptic AMPA receptor trafficking - Three steps.



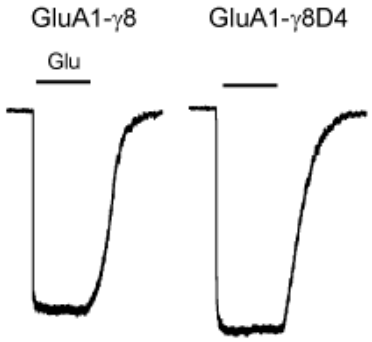
Synaptic targeting of AMPARs via TARPs

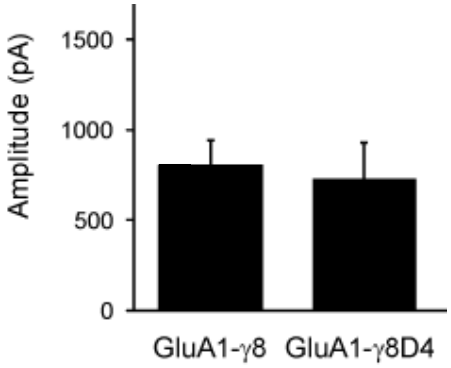
(on a GluA1A2A3 triple floxed background)

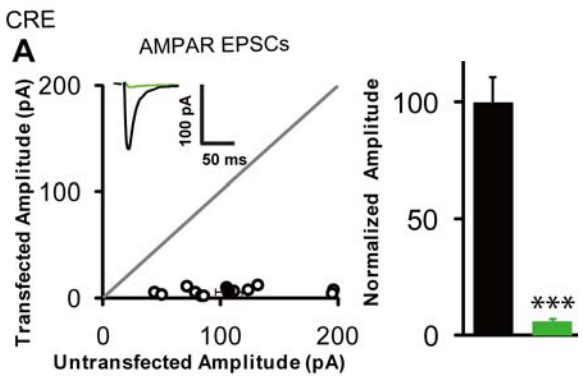


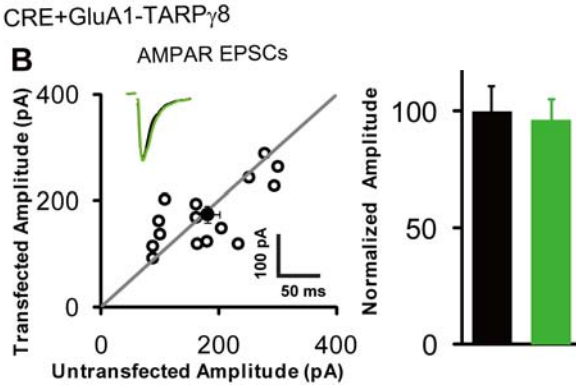


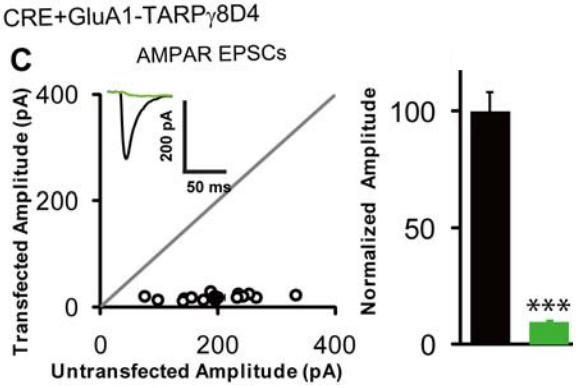
Surface receptors





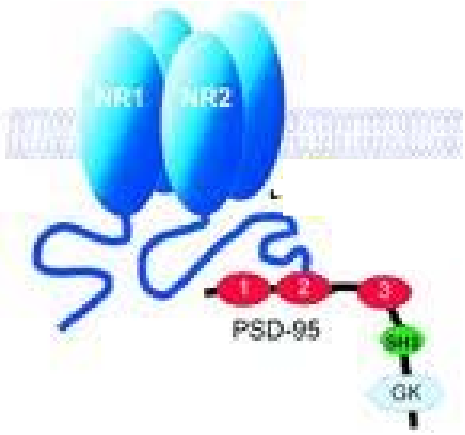


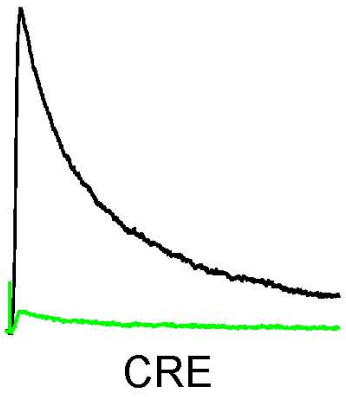


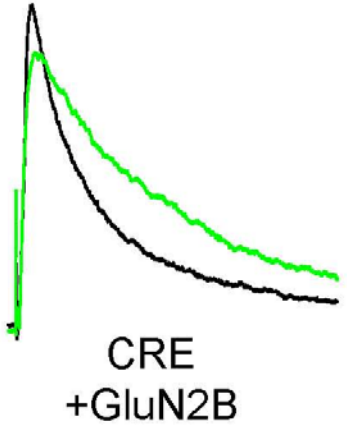


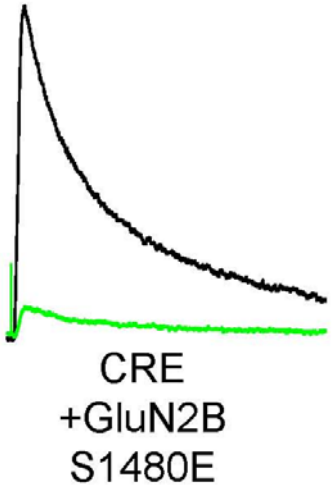
Synaptic targeting of NMDARs

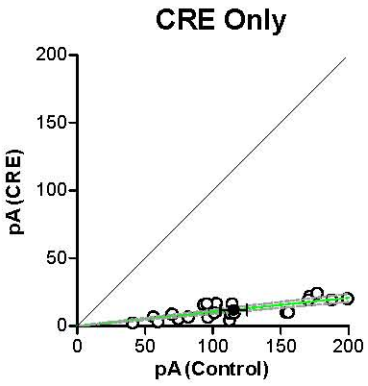
(on a GluN2A/2B double floxed background)

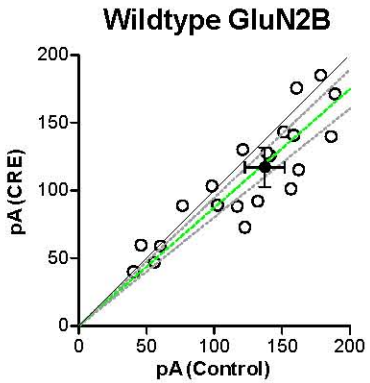


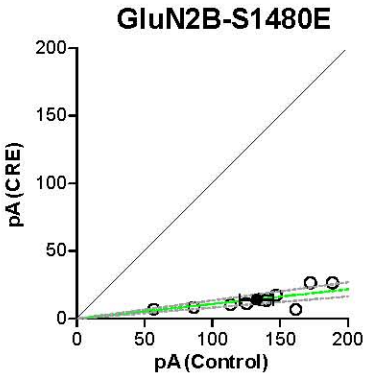


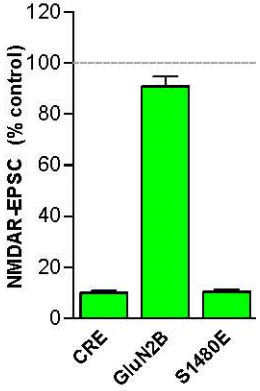




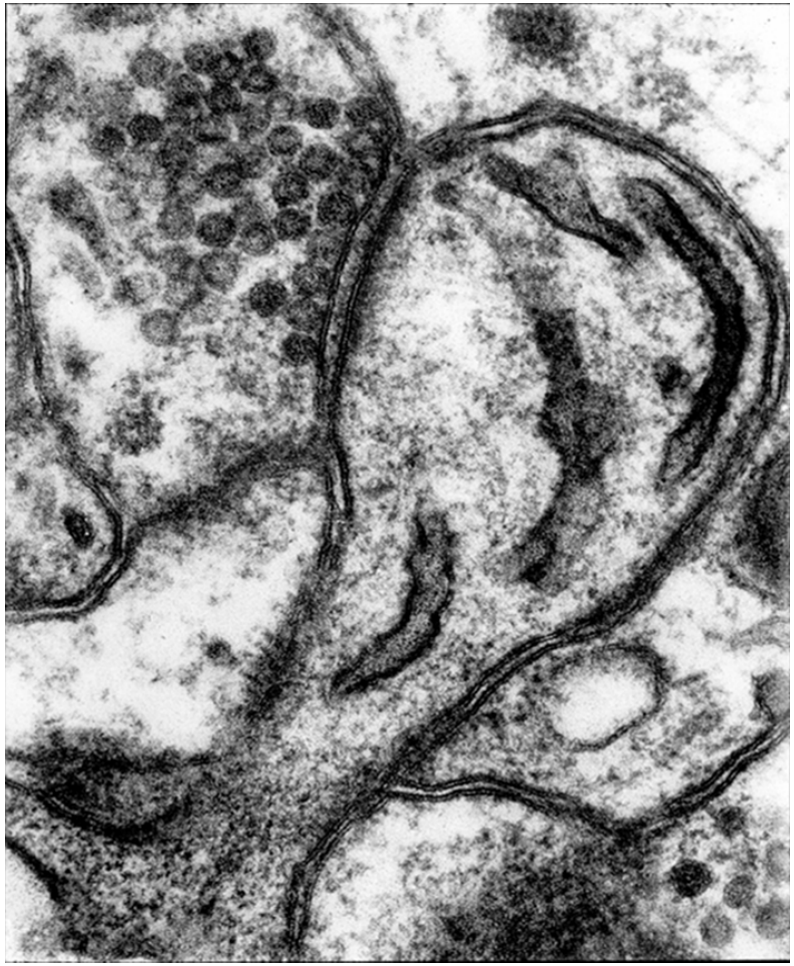








A molecular dissection of the postsynaptic density



~1,000 Receptors

Scaffolds

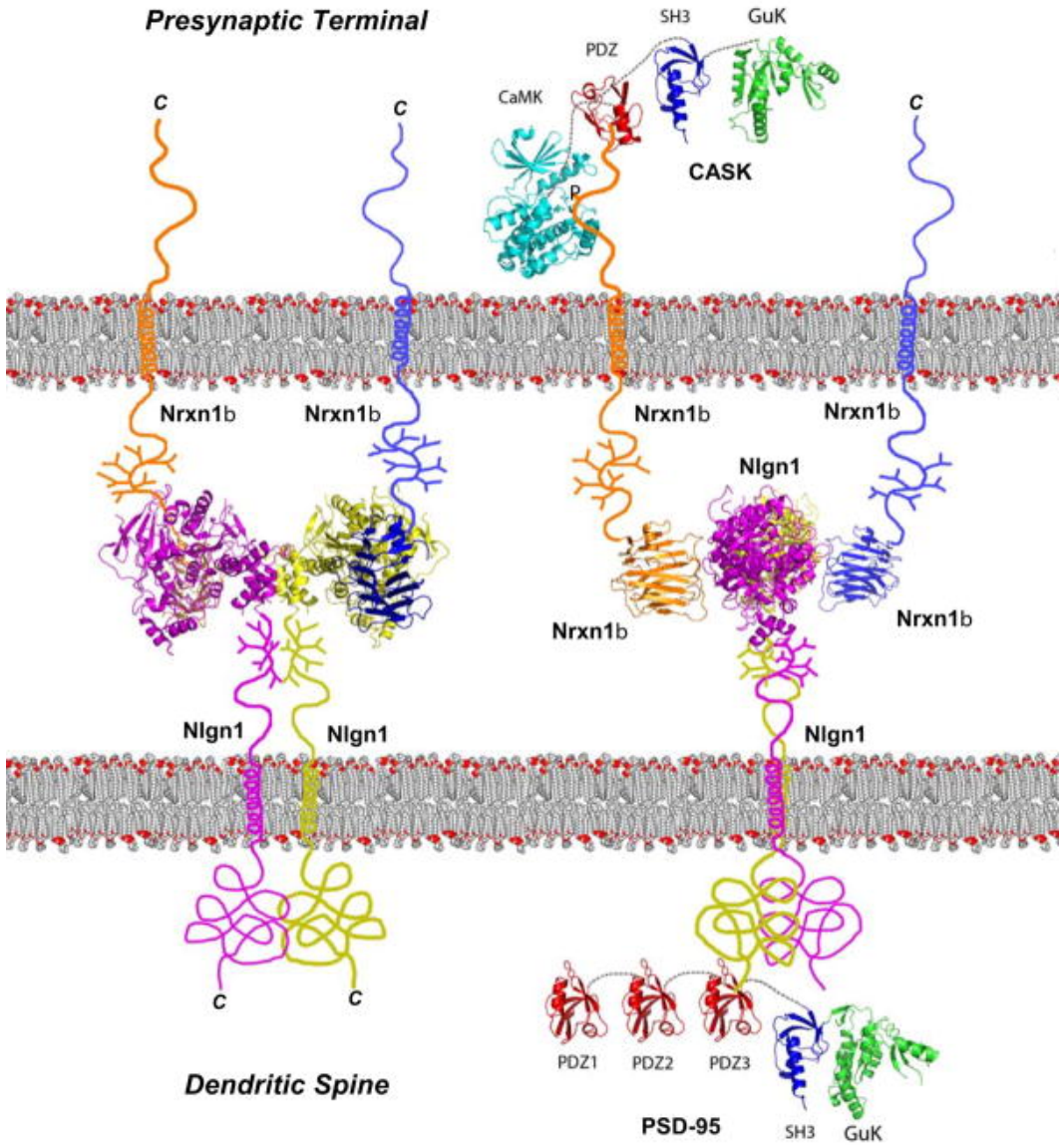
Adhesion proteins

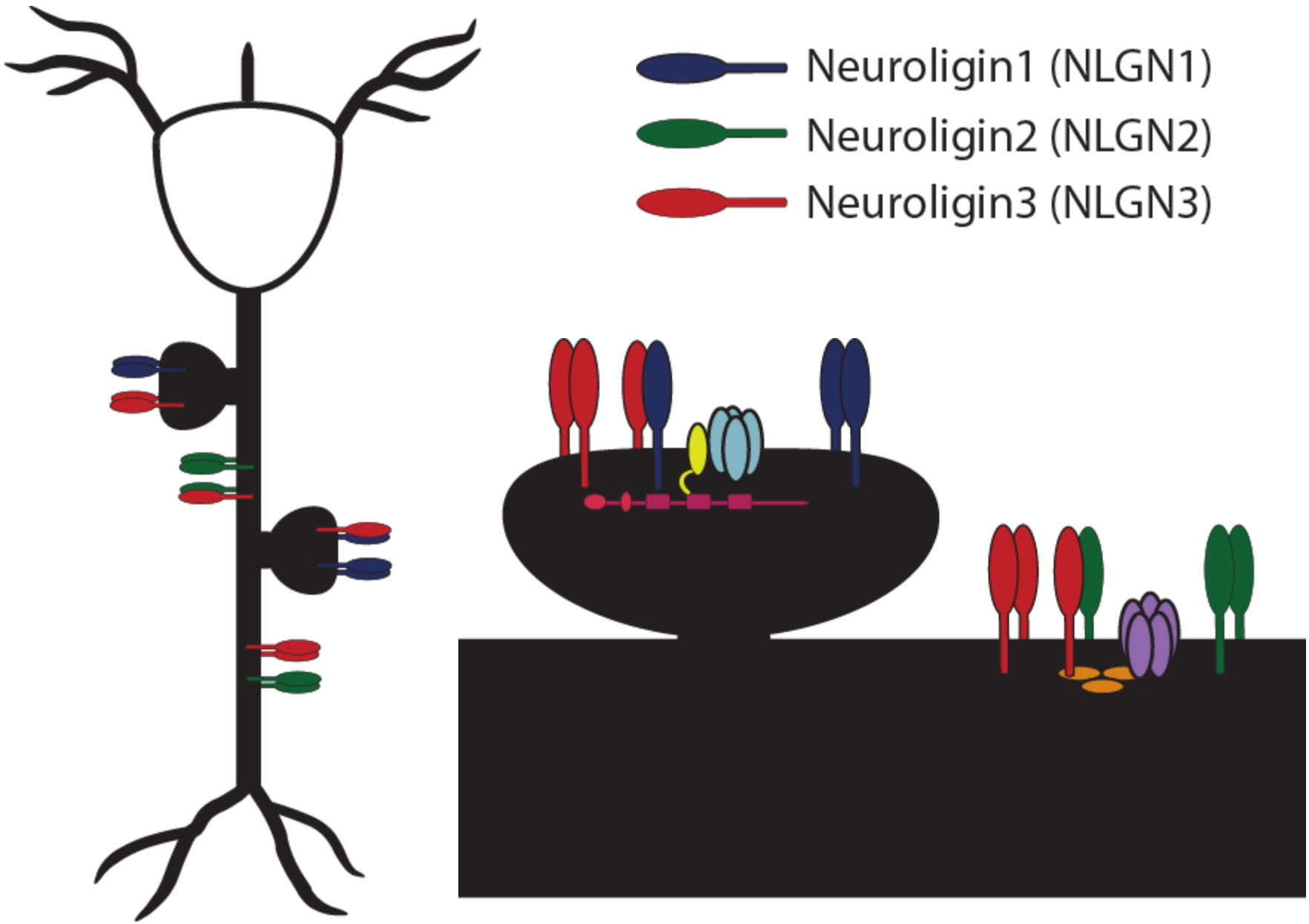
Cytoskeleton

Signaling proteins

Agonist = overexpression
Antagonist = gene deletion/RNAi

Neuroligins/neurexins bridge the synaptic cleft

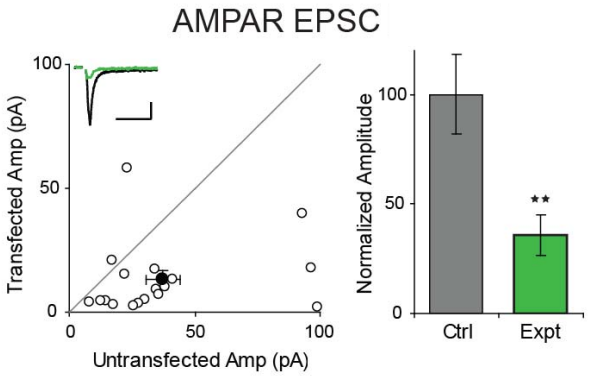


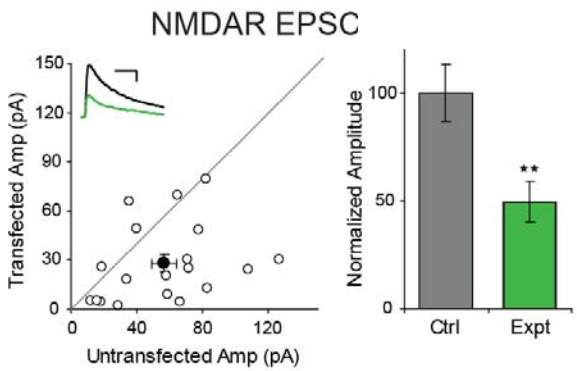

Neuroligin1 (NLGN1)
Neuroligin2 (NLGN2)
Neuroligin3 (NLGN3)

Neuroligins are important

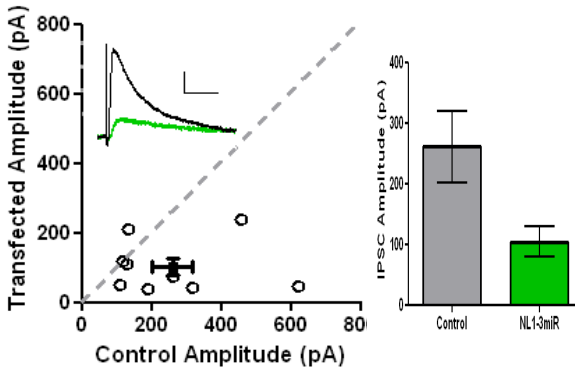
Triple knock down (NL1-3)

EPSCs





IPSCs

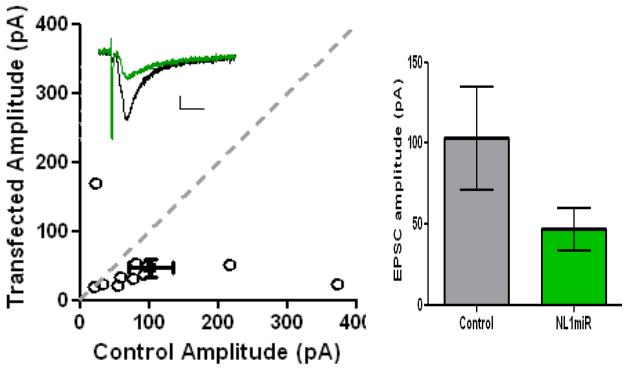


NL1 knock down

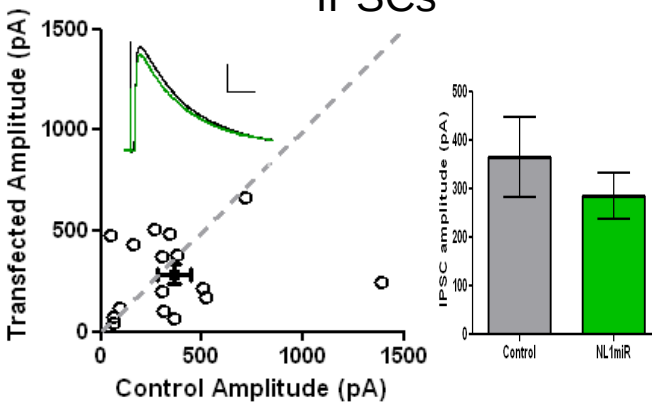
Record EPSCs and IPSCs in the same cell.

E_{EPSC} = 0 mV
E_{IPSC} = -70 mV

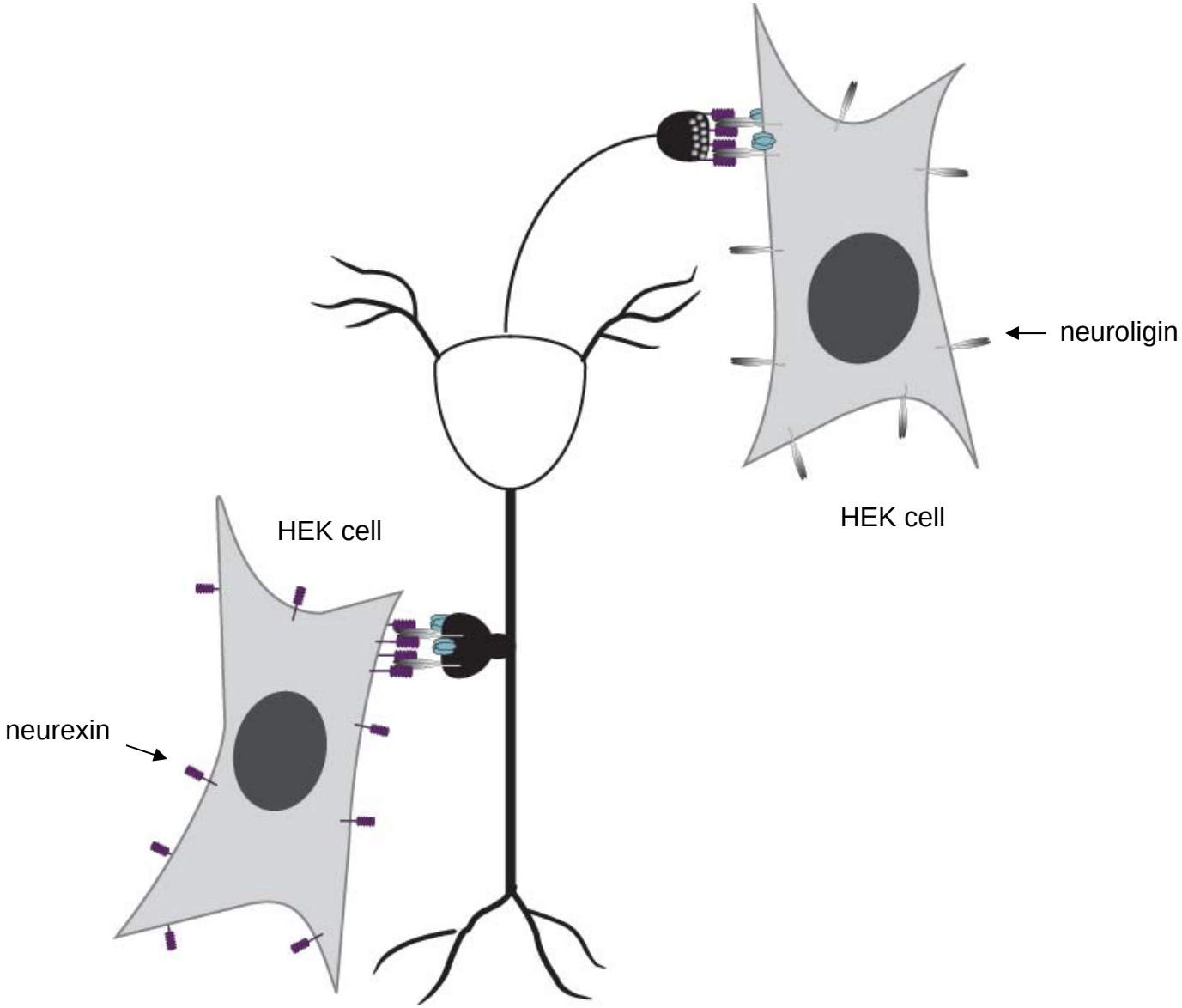
AMPA EPSCs

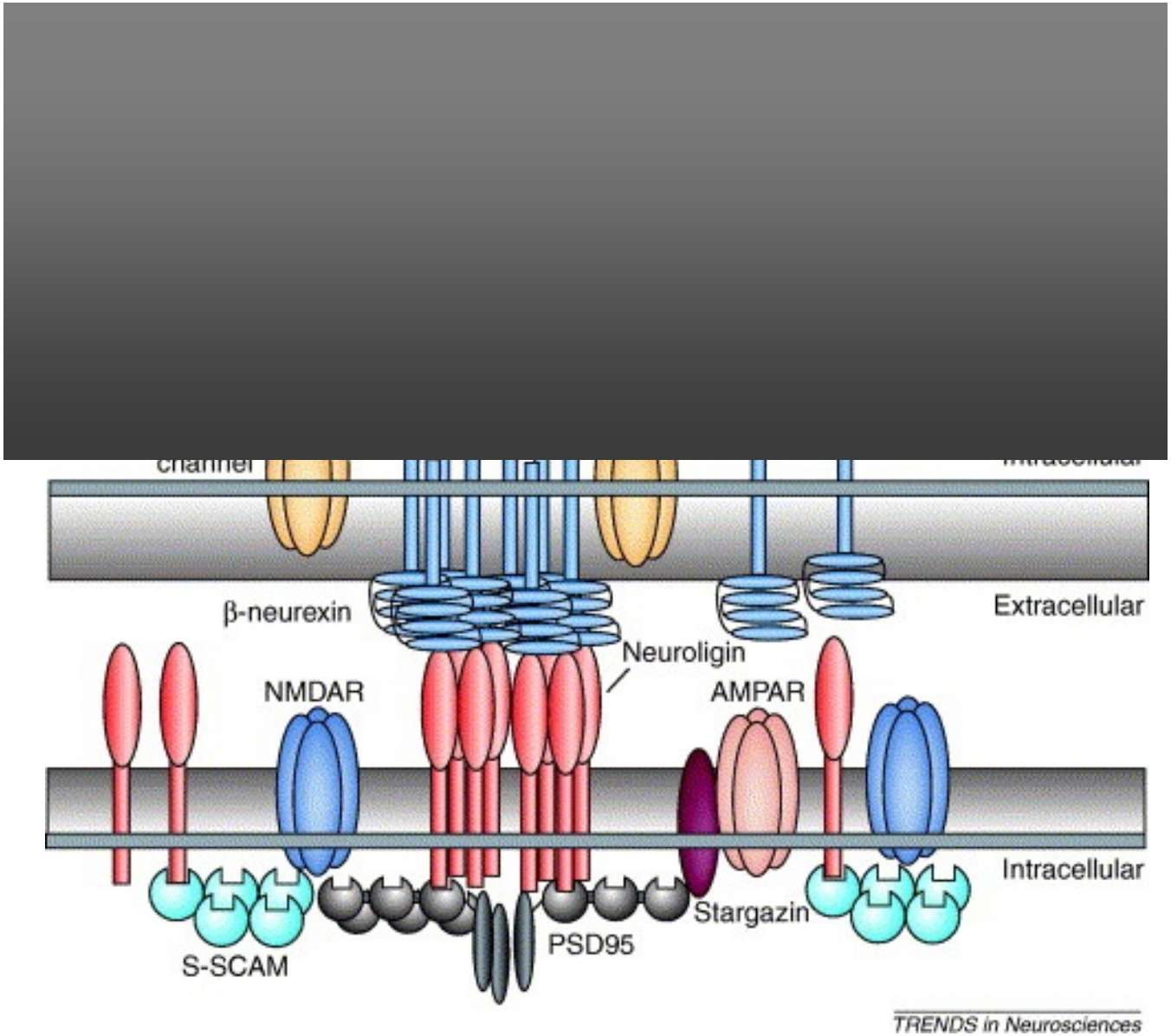


IPSCs



Neuroligins/neurexins are sufficient for synapse formation




β-neurexin
Extracellular
Neuroligin
NMDAR
AMPAR
Intracellular
Stargazin
PSD95
S-SCAM
TRENDS in Neurosciences