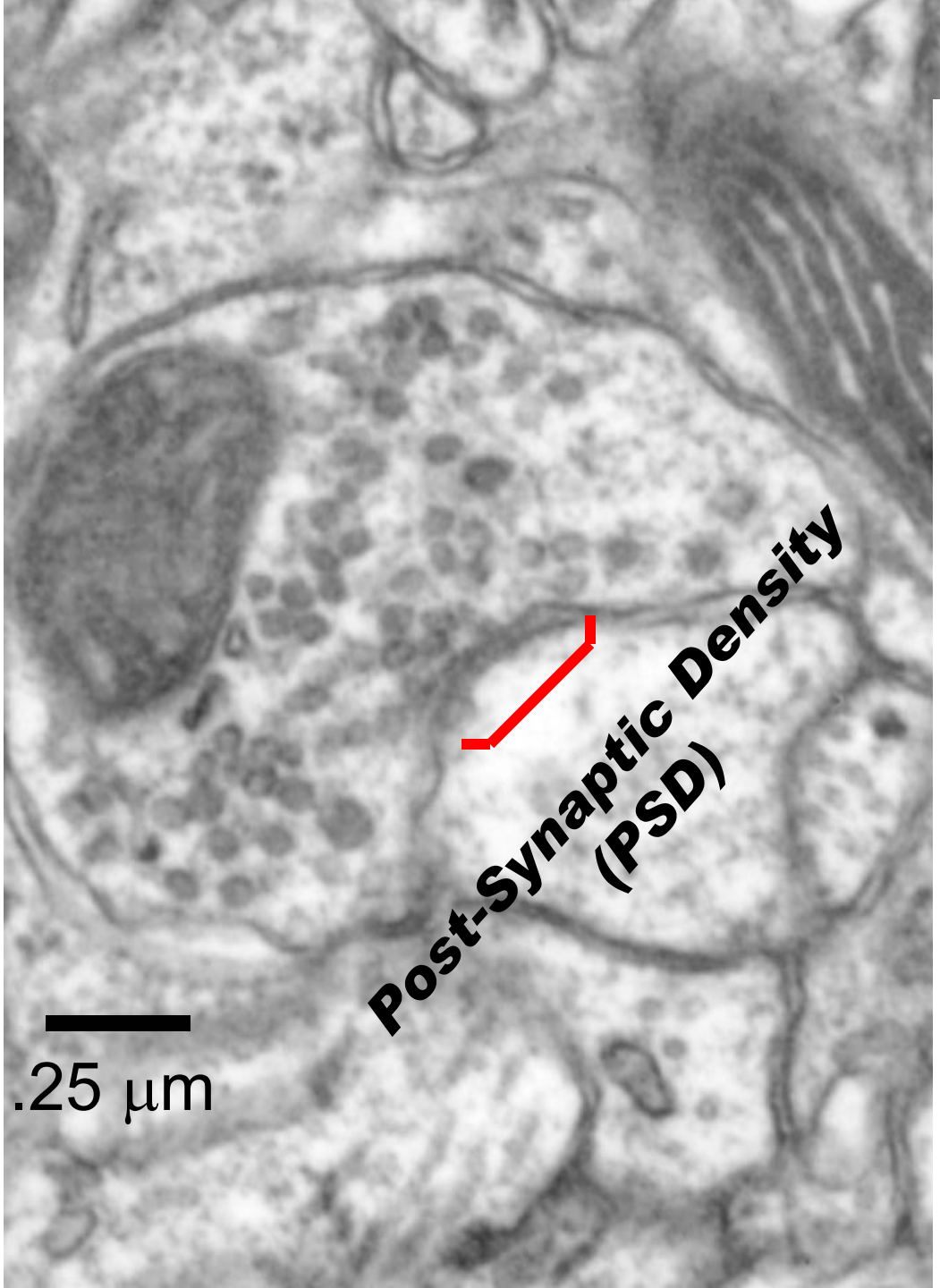
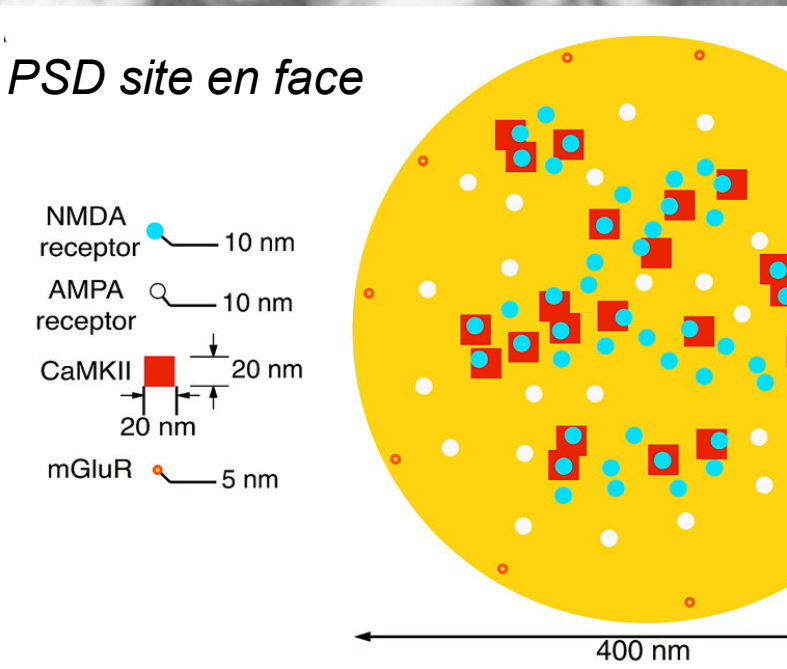
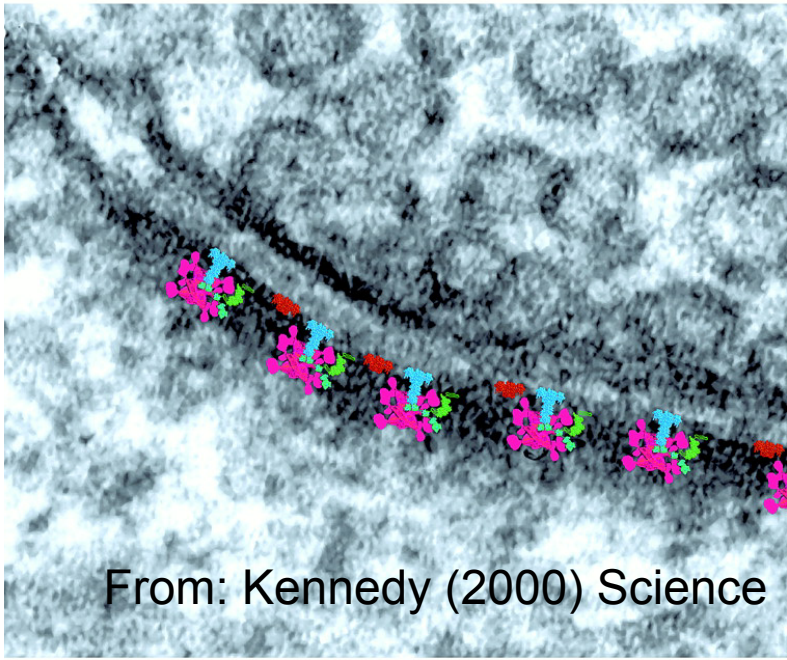
Lecture 7:
Roles for MAGUKS in
Activity-dependent Synaptogenesis

MCP 9.013 ‘04

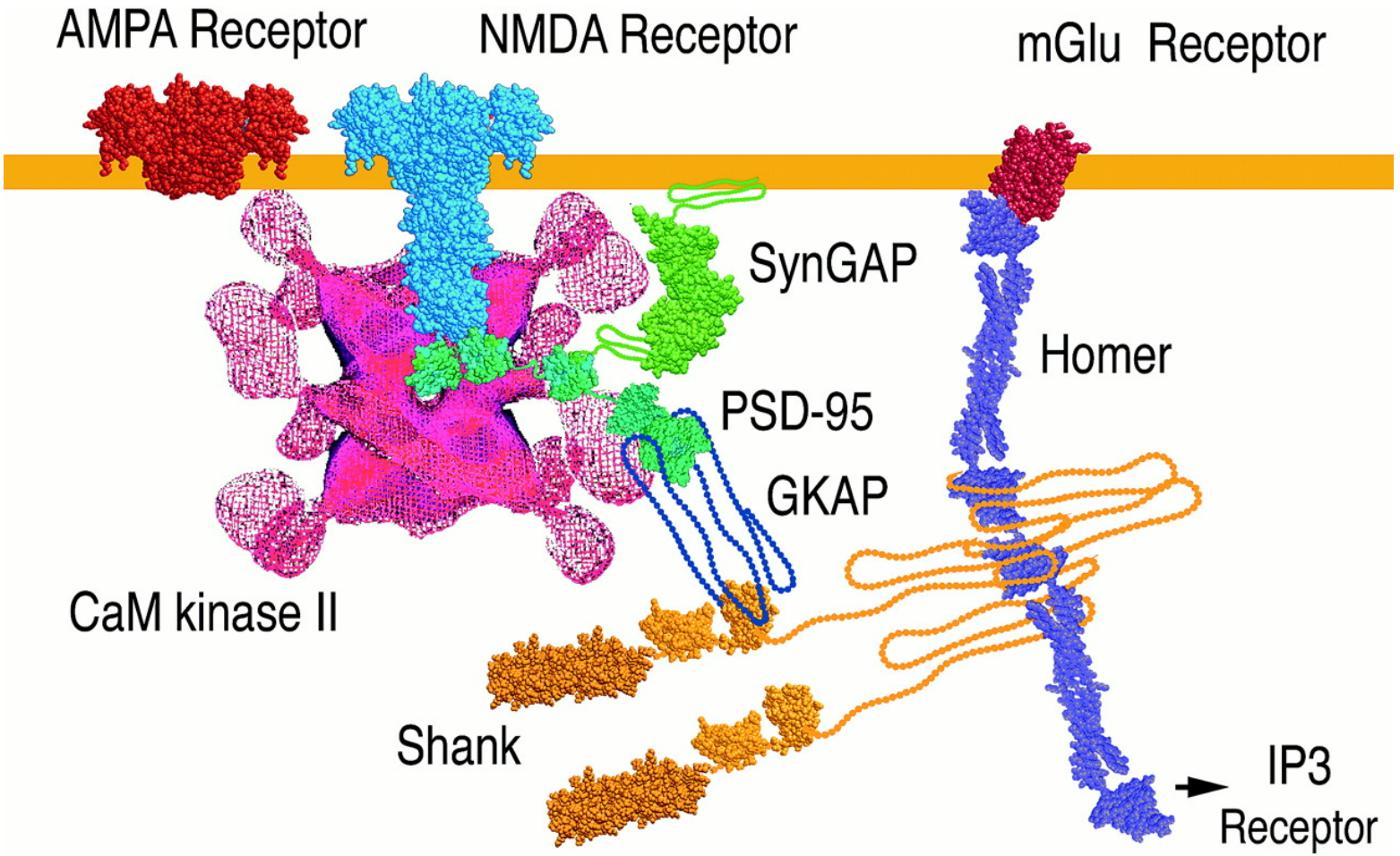

Post-Synaptic Density
(PSD)
.25 μm


PSD site en face
NMDA receptor
10 nm
AMPA receptor
10 nm
CaMKII
20 nm
20 nm
mGluR
5 nm
400 nm


From: Kennedy (2000) Science

MEMBRANE ASSOCIATED GUANYLATE KINASES

MAGUKS are the protein scaffolds of the post-synaptic density



From: Kennedy (2000) Science

Direct Protein Interactions of NMDA Receptor Binding MAGUKS

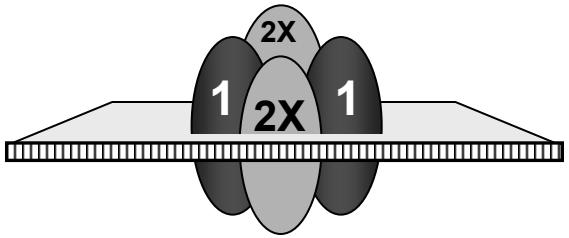
Protein	Bonding Domain on MAGUK	Proposed Function	Source
* NR2A	$PDZ_{1,2}$	Scaffolds NRS to MAGUKS	Korneau et al.'95; Lau et al.'96; Miller et al.'96; Neithammer et al. '96
* NR2B	$PDZ_{1,2}$	Scaffolds NRS to MAGUKS	
*GKAP95/130	GK	Couples MAGUKS to SHANK and dynein light chain	Kim et al.'97;Naisbitt et al.'97;Tuo et al.'9
* Stargazen	PDZ	Stabilizes AMPARs in synaptic membrane	Chen et al. '00
* KA_1	SH_3 + GK	Scaffolds KARs to MAGUKS, block KAR desensitization	Garcia et al. '98;Mehta et al.'01
* $GluR_6$	PDZ	Scaffolds KARs to MAGUKS, block KAR desensitization	Garcia et al. '98
AKAP	SH_3	Scaffolds CaN, PKA, PKC; regulation actin dynamics	College et al. '00; Gomez et al. '00
SynGAP	PDZ	ras GAP	Chen et al. '98; Kimetal. '98
Neuroligin	PDZ_3	Adhesion, binding partner of Neurexin	Irie et al. '97; Bolliger et al. '01, Song et a
$ErbB_4$	$PDZ_{1,2}$	Neuregulin receptor signals to the nucleus	Huang et al. '01
Erbin	PDZ	Increases $ErbB_2$ expression	Huang et al. '01
K^+ channel	PDZ_3	Membrane polarization	Imamura et al. '02
NOS	PDZ	Synthesizes NO, a putative retrograde signal	Craven & Bredt. '02
Fyn	PDZ_3	Tyrosine kinase, phosphorylates NR2A	Tezuka et al. '99
Cript	PDZ_3	Assoc. w/tubulin; relieves GK domain from inhibition of binding	Neithammer et al.'98;Passafaro et al. '99
Ca^{++}-ATPases 2a,4b	PDZ	Maintaining Ca^{++} homeostasis PSD-95 binds both, SAP102 only 4b	Dimaio & Strehler, '01
Kalirin-7	PDZ	Rho family GEP that affects dendrite actin dynamics; inte w/all NR-binding MAGUKS	Penzes et al. '01

fetal and postnatal period: SAP 102

juvenile-adult:PSD-95; in some regions PSD-93

The NMDAR is a tetramer consisting of 2 NR1 subunits and 2 NR2 subunits

$(NR1)_2\ (NR2X)_2$

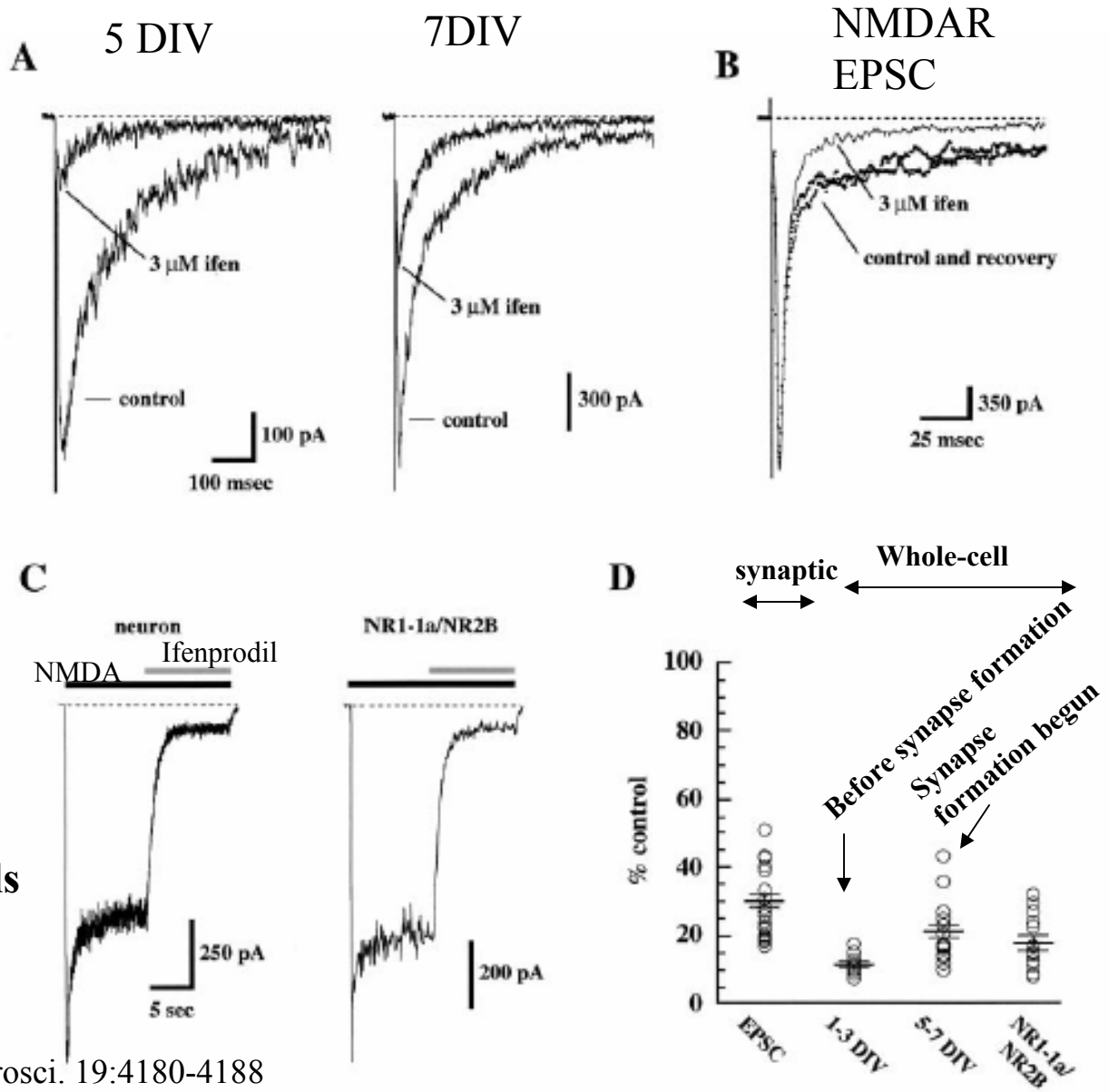


Postnatal Midbrain and forebrain express NR2A & NR2B

	NR2A	NR2B	~~NR2C~~	~~NR2D~~
NR2A	AA	AB	~~AC~~	~~AD~~
NR2B	BA	BB	~~BC~~	~~BD~~
NR2C	~~CA~~	~~CB~~	~~CC~~	~~CD~~
NR2D (embryo)	DA	DB	DC	DD

Developmental decreases with age in NMDAR synaptic current decay times are due to the loss of NMDARs enriched in the NR2B subunit.

Studies on hippocampal autaptic synapses in isolated island cultures

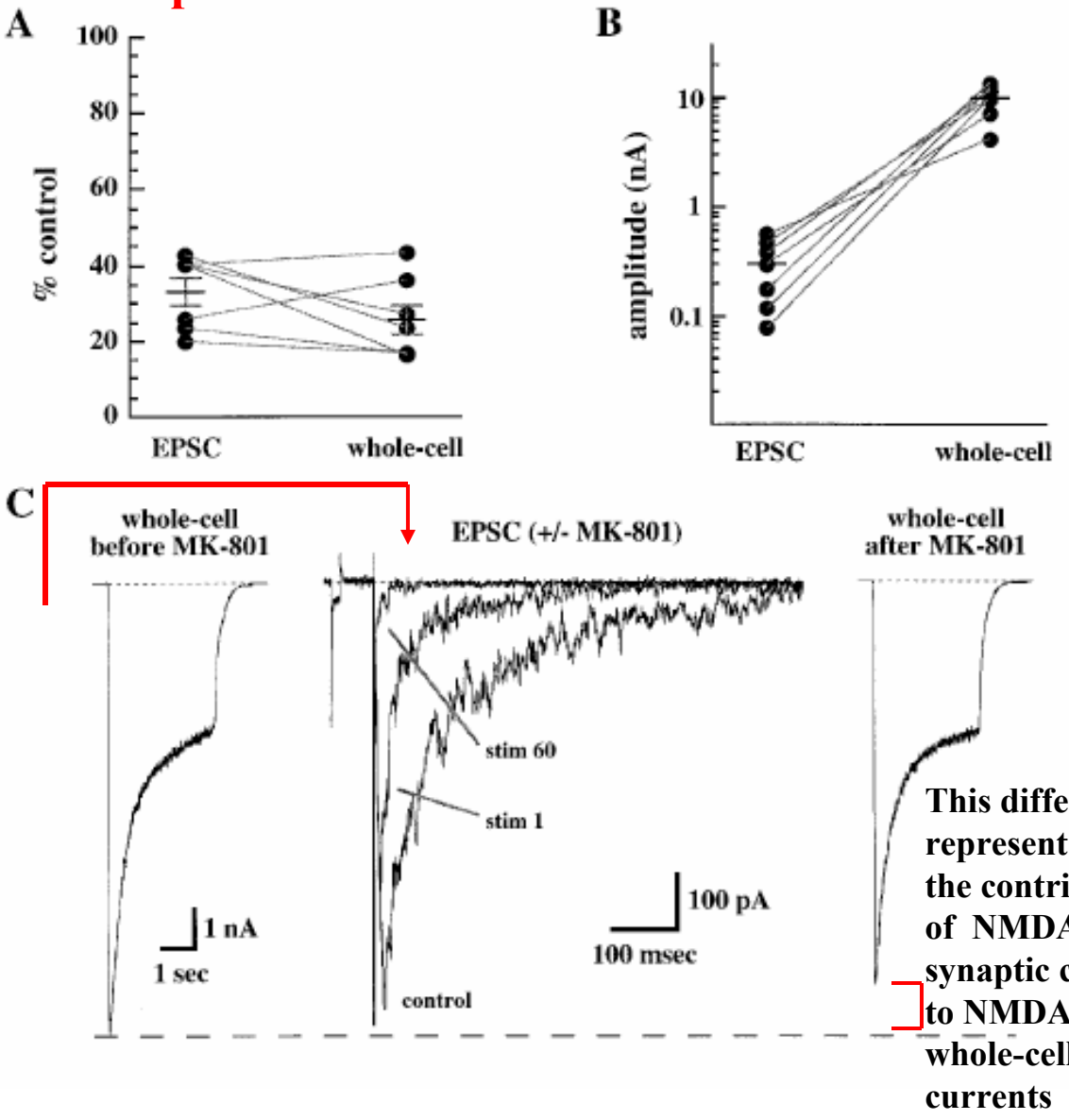


Experiments testing the efficacy of ifenprodil against 2NR1: 2NR2B receptors Transfected into HEK293 cells

Tovar and Westbrook, (1999) J. Neurosci. 19:4180-4188

Extrasynaptic NMDARs out-number synaptic NMDARs by ~ 3 to 1 in ≤ 7 DIV neurons. Use of autapses allows synaptic currents and whole cell currents of the same cell to be compared.

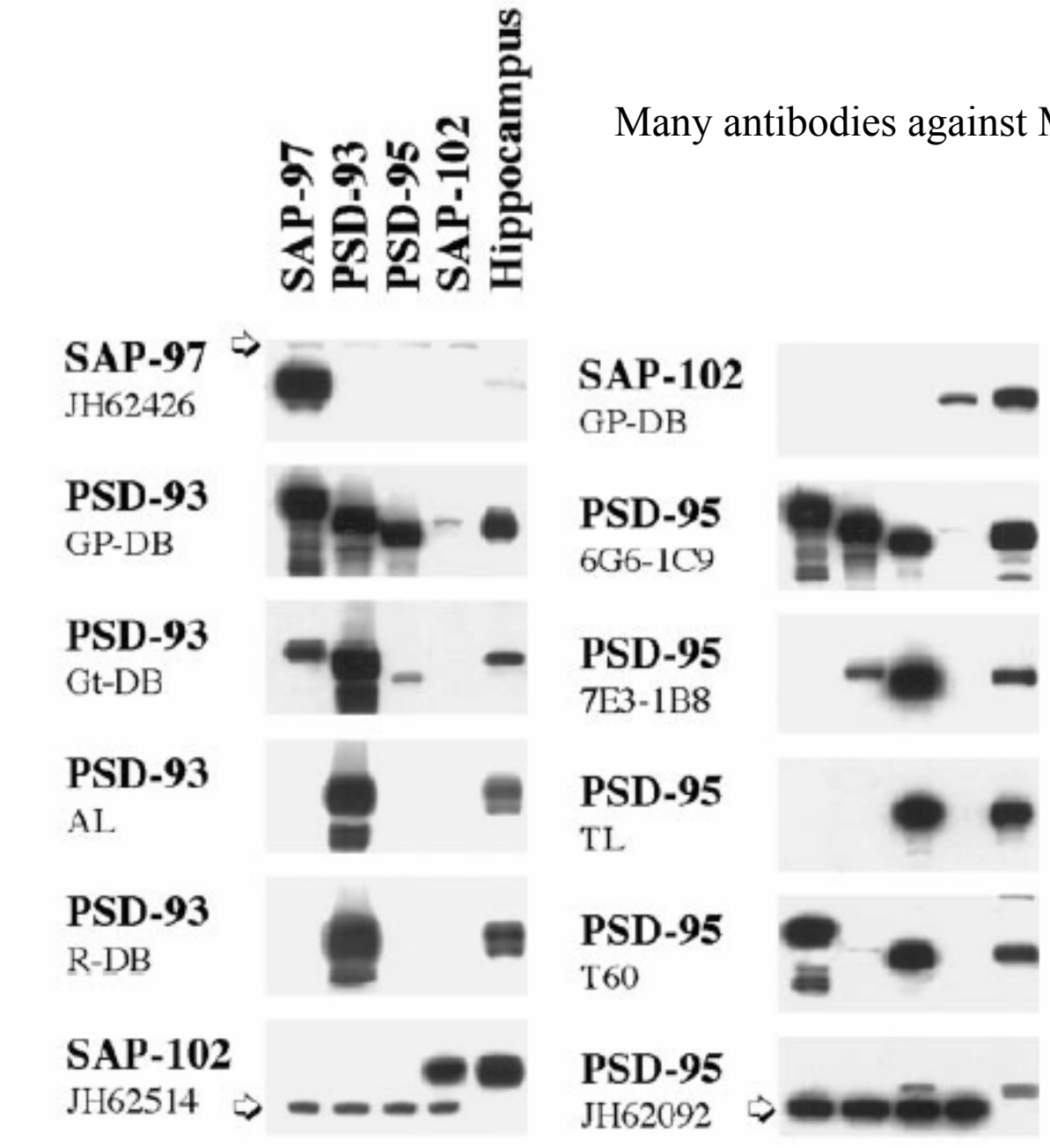
EPSCs are less sensitive than whole cell currents to ifenprodil and are smaller than whole cell currents.



MK801 is a non-competitive NMDAR channel blocker. Therefore, it can be used to specifically block synaptic NMDAR currents

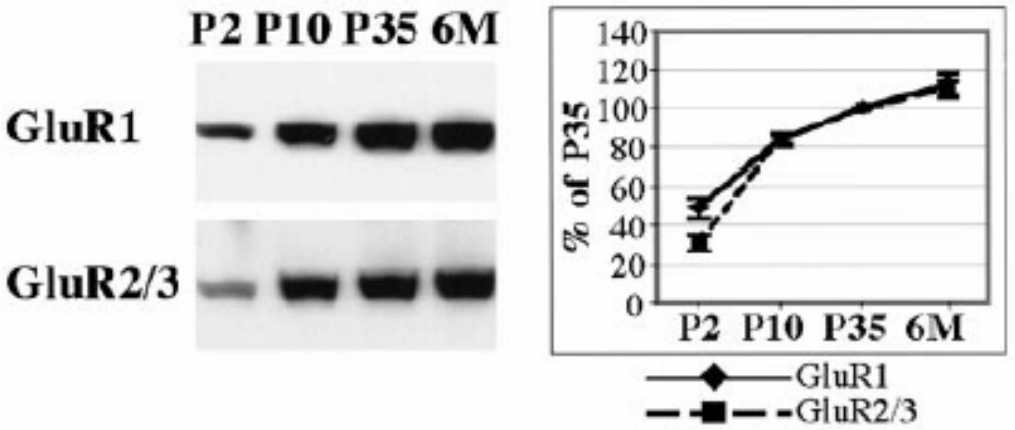
This difference represents the contribution of NMDAR synaptic current to NMDAR whole-cell currents

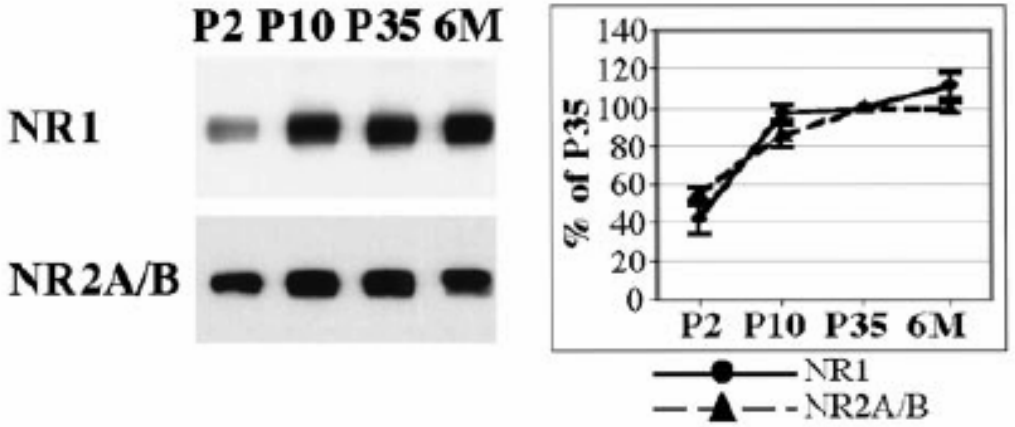
Many antibodies against MAGUKS are not specific



Sans, N. et al., (2000) J.Neurosci. 20, 1260-1271.

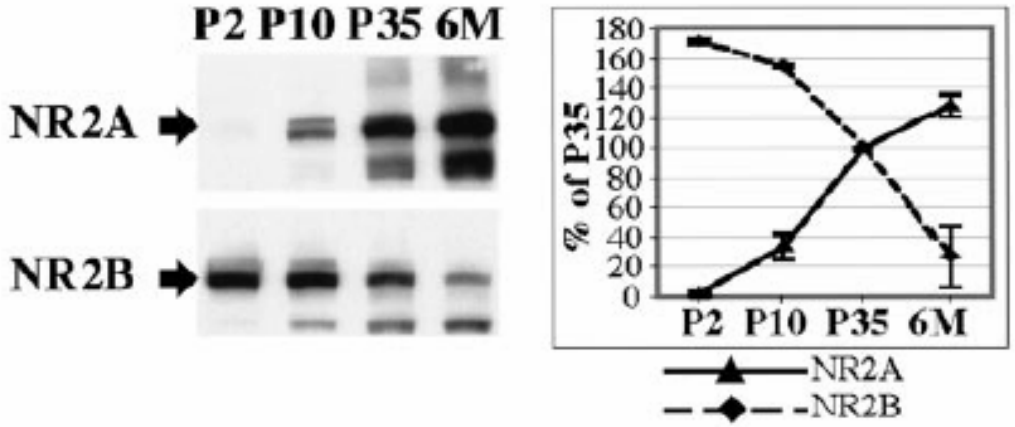
In hippocampus, as in many other brain regions as development proceeds, the number of glutamate receptors increase but the type of NMDA receptor changes.



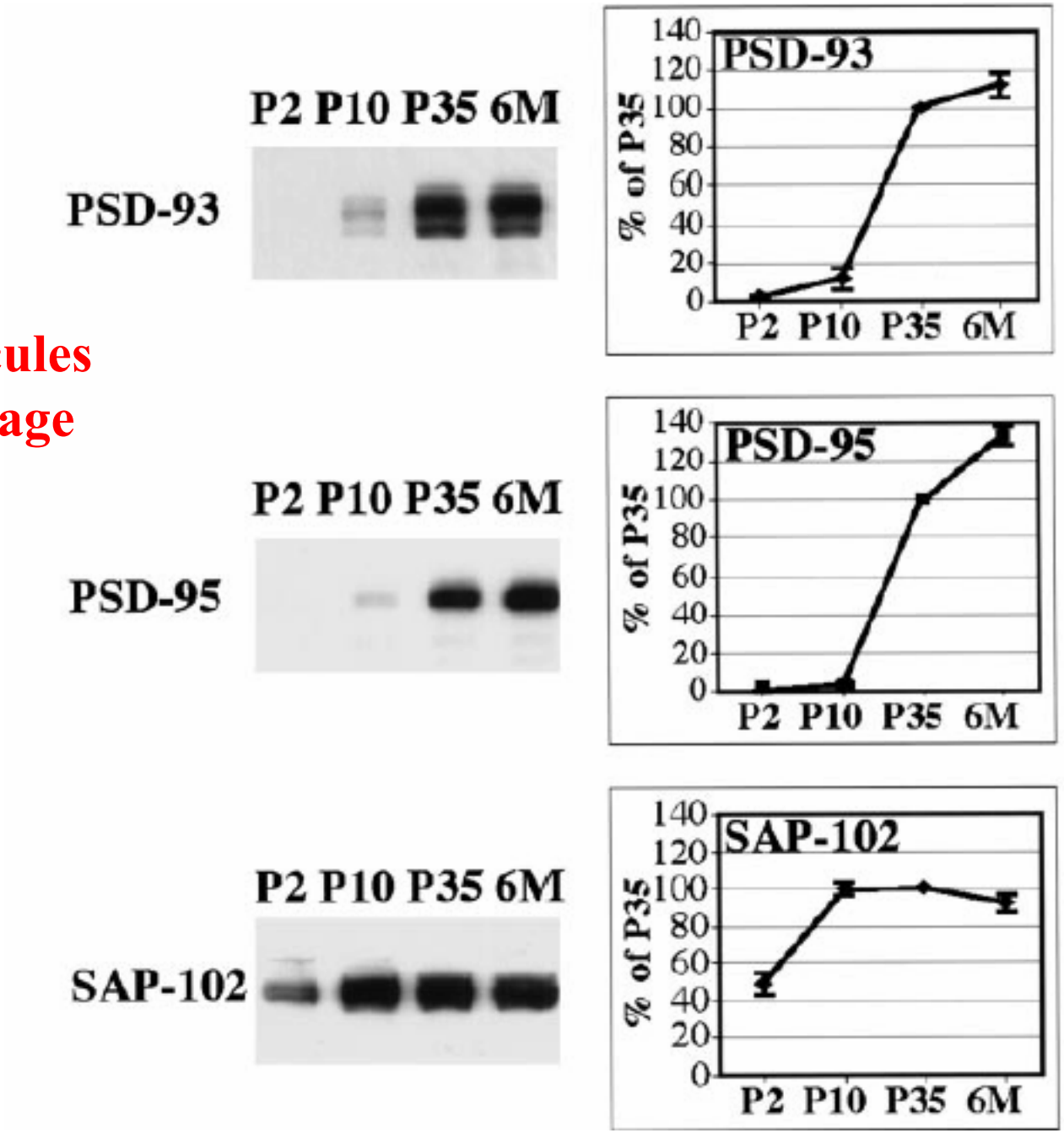


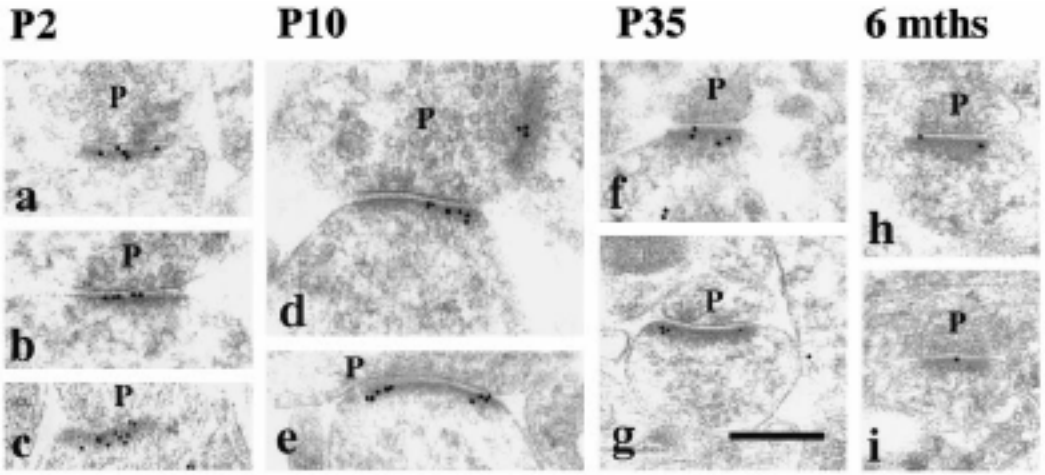
Sequence

2NR1:2NR2B to
2NR1:1NR2B:1NR2A to
2NR1:2NR2B



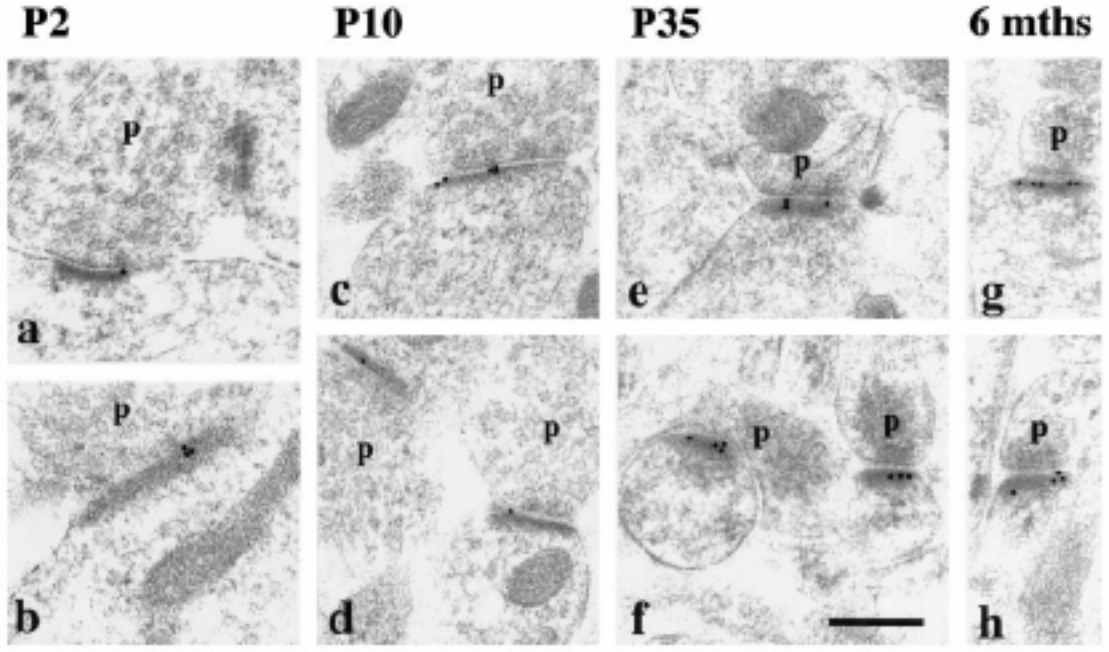
The NMDAR scaffolding molecules also change with age





SAP- 102 is synaptic (as well as extra-synaptic) in neonates and becomes progressively perisynaptic (extrasynaptic) with age.

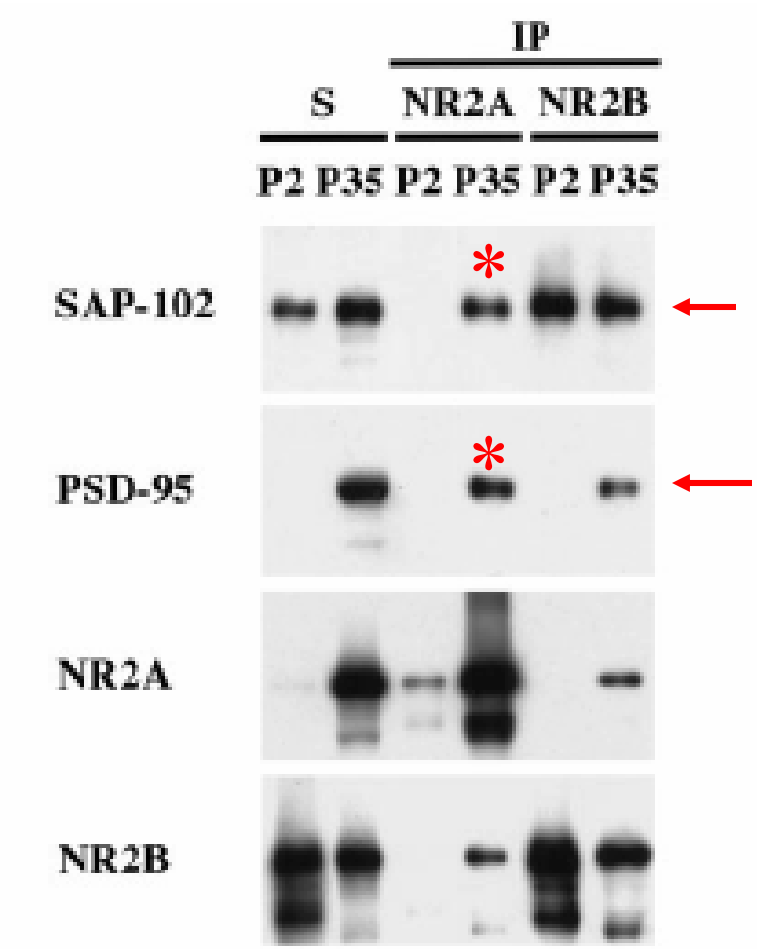
PSD-95 is expressed at low levels in neonates and increases at synapses with age.



NR2B always immunoprecipitates more SAP-102 than PSD-95.

NR2A immunoprecipitates more PSD-95 than SAP-102.

PSD-95 and NR2A are not at immunoblot detectable levels in the neonate.



Synaptic Activity Controls:

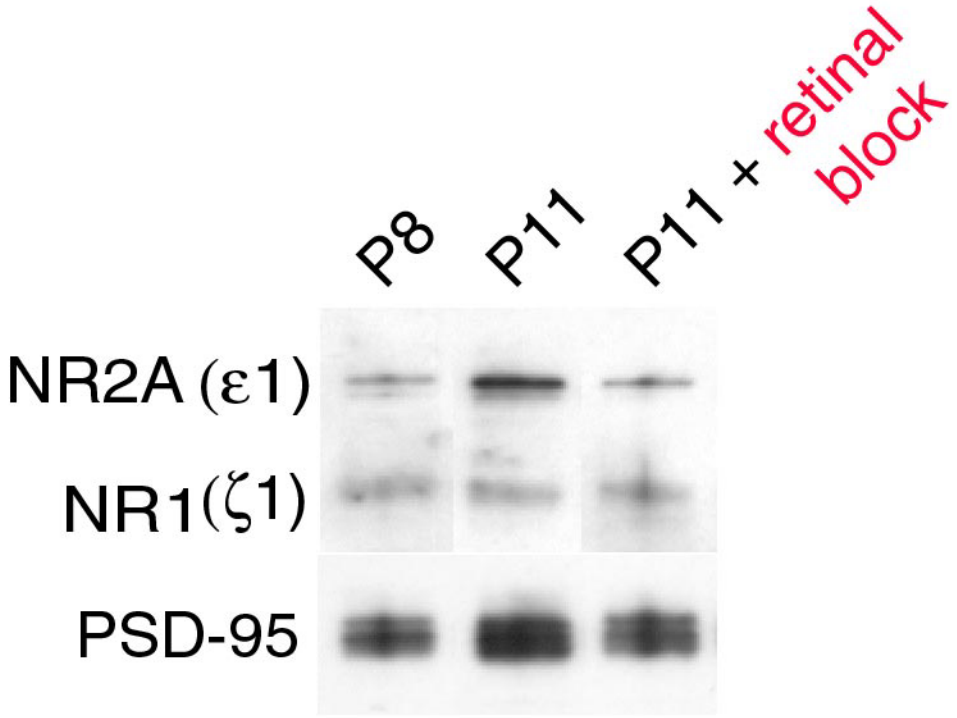
1. **The levels of NR2B transcription (e.g. increased activity, decreased NR2B transcript).**

2. **The transport of PSD-95 to the synapse.**

3. **Possibly the local synaptic translation of NR2A.**

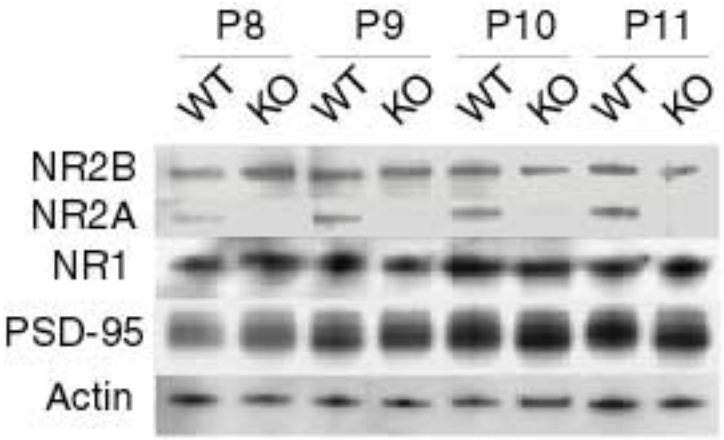
In Wildtype Mice Both NR2A and PSD-95 Become Enriched In the Synaptoneurosome (Dendritic) Fraction In the P8-P11 Interval

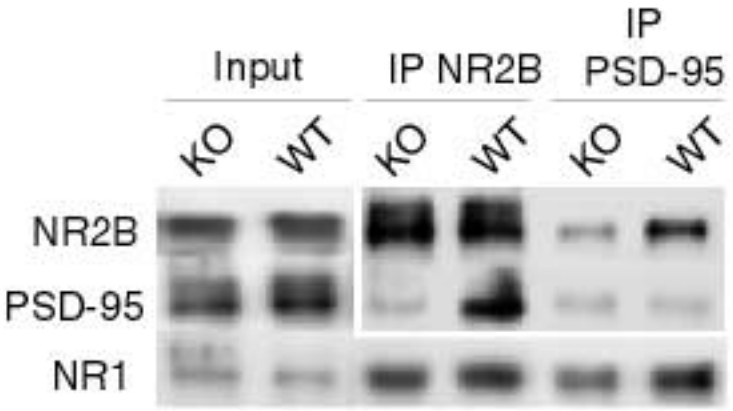
Tissue from the superficial visual layers of the superior colliculus (sSC)



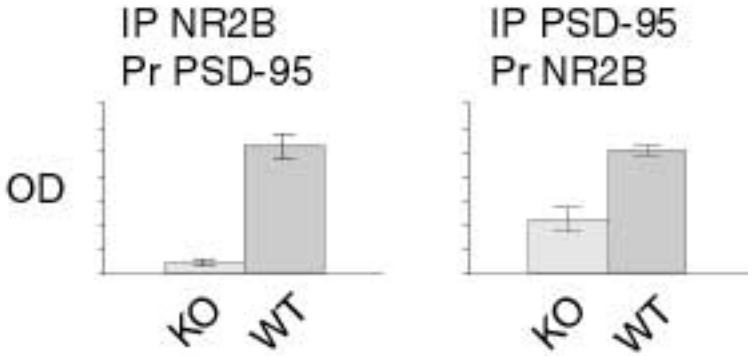
This increase is eliminated when the photoreceptor to bipolar to ganglion cell pathway is blocked

Townsend, M et al., (2003) PNAS 89:10593-10597

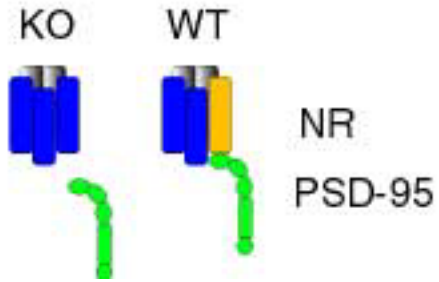




PSD-95 increases in NR2A KO synaptoneurosome fractions.

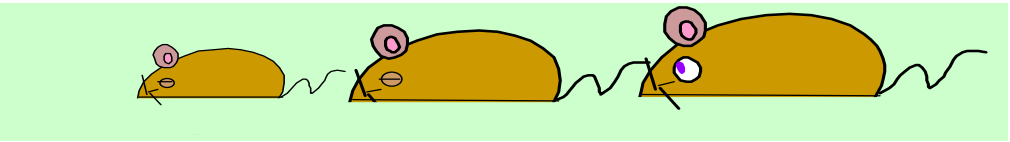


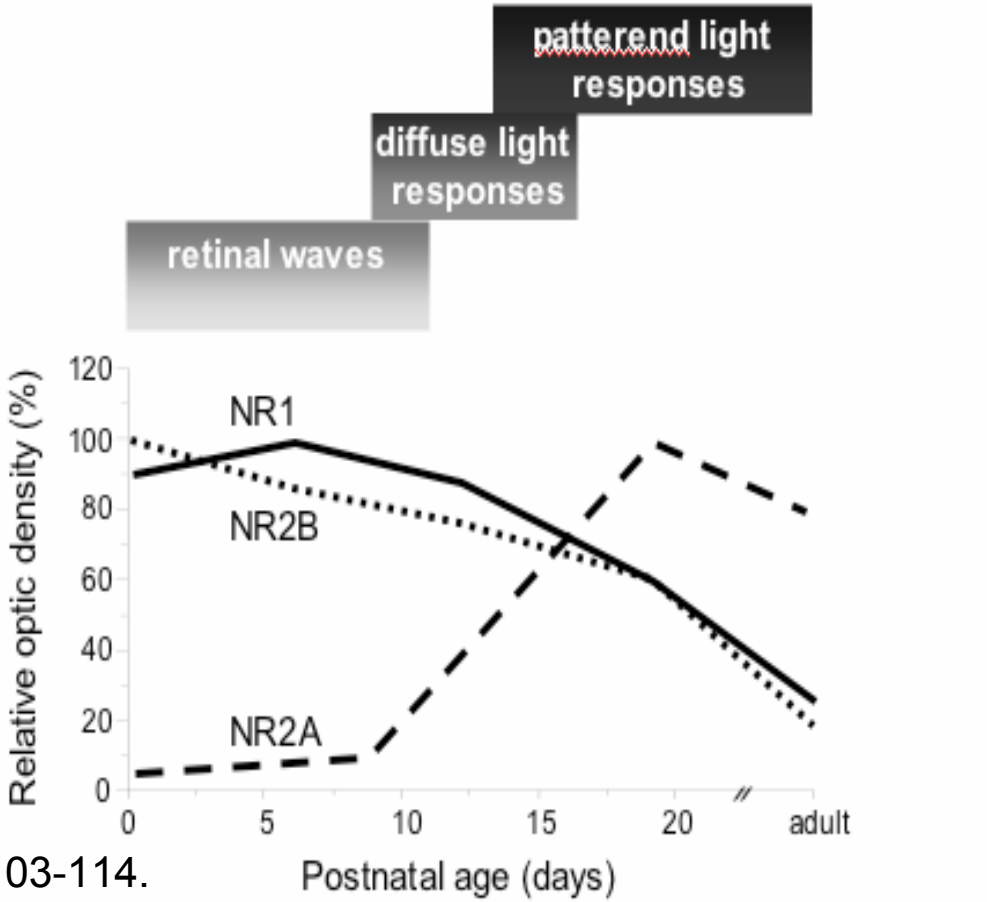
However, this PSD-95 does not bind NMDA receptors effectively.



Townsend, M et al., (2003)PNAS.

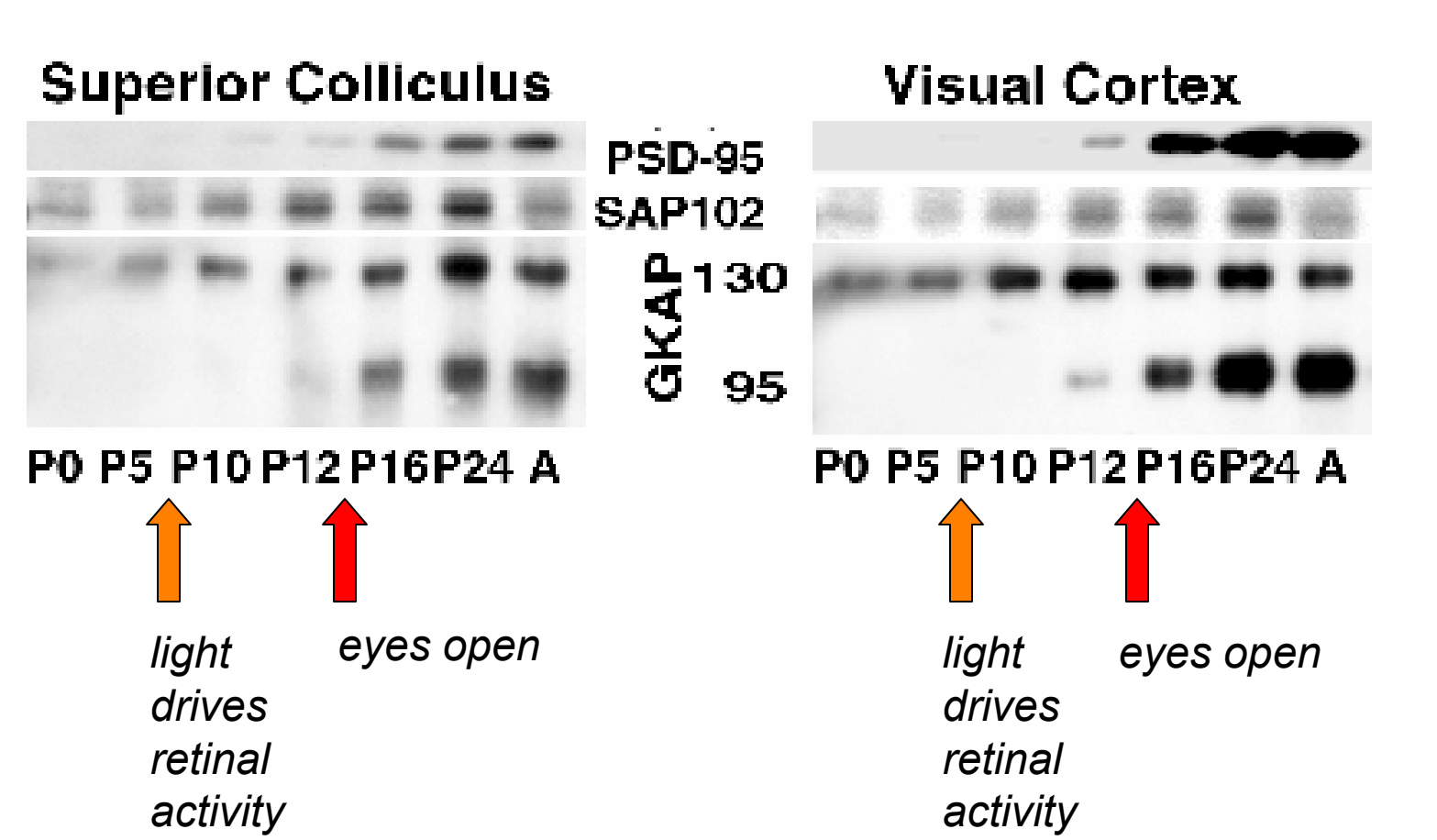
The normal development of the retina provides natural predictable changes in the amount and patterning of activity to central visual neurons. Post-synaptic responses of visual neurons can therefore be studied after known in vivo stimulation.





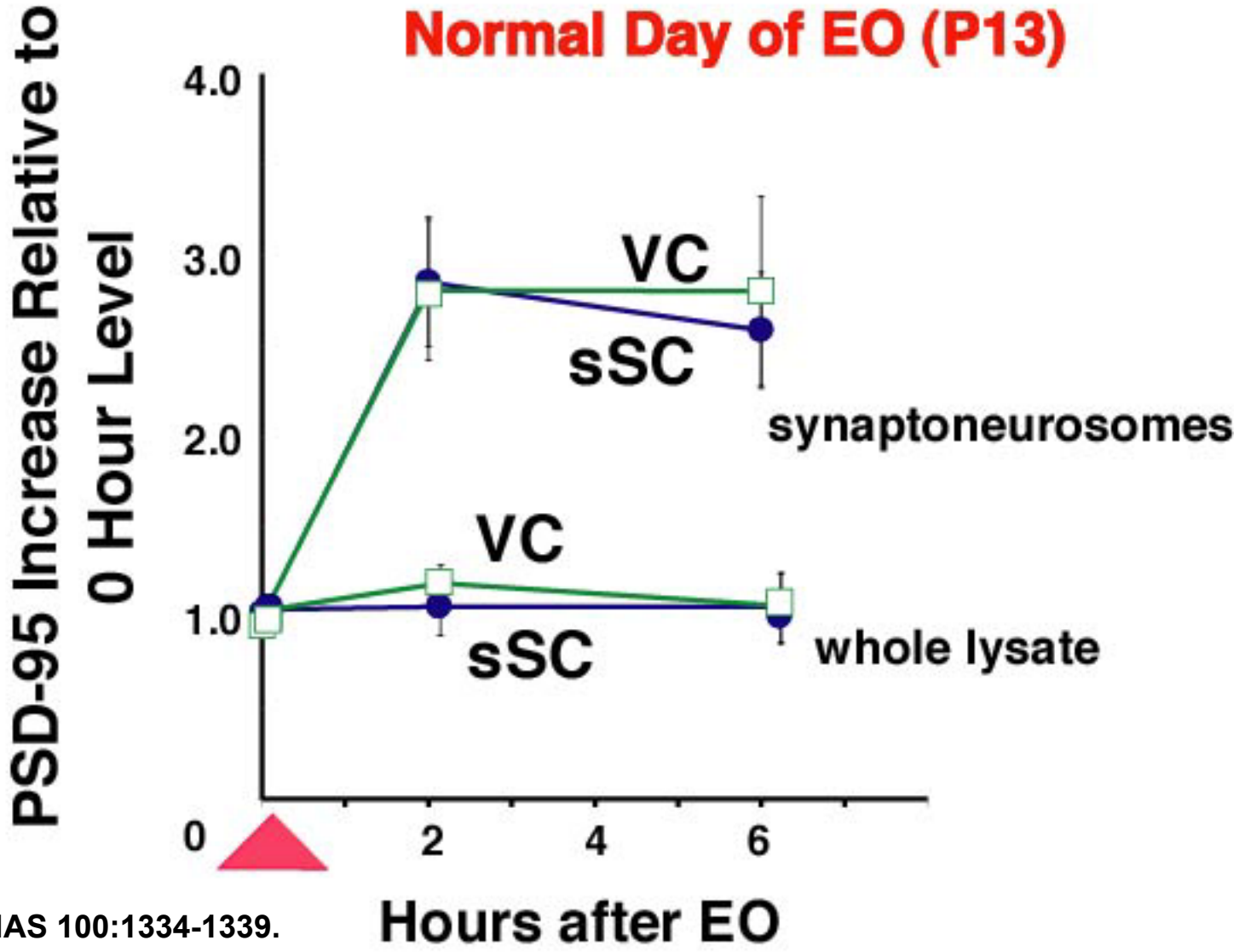
Shi et al.,(1997) Neuron 28:103-114.

Western blots of dendritic fractions (synaptoneurosmes) from the visual layers of the superior colliculus & visual cortex reveal changes in PSD proteins with age.



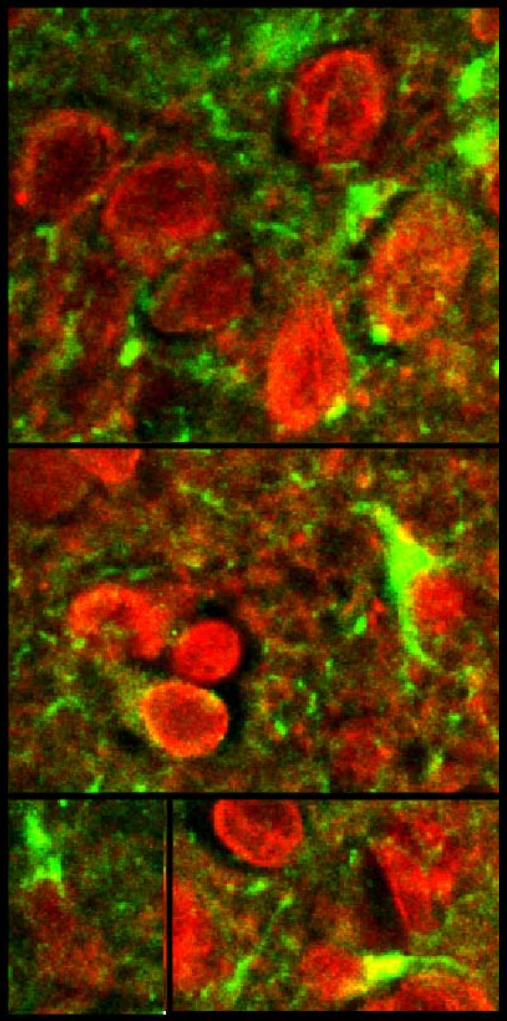
Yoshii et al.,2003

PSD-95 Increases In Dendritic Fractions Within Hours of Controlled Eye-Opening



Yoshi et al, (2003) PNAS 100:1334-1339.

PSD-95 Is Concentrated in Cell Bodies Before Eye-Opening

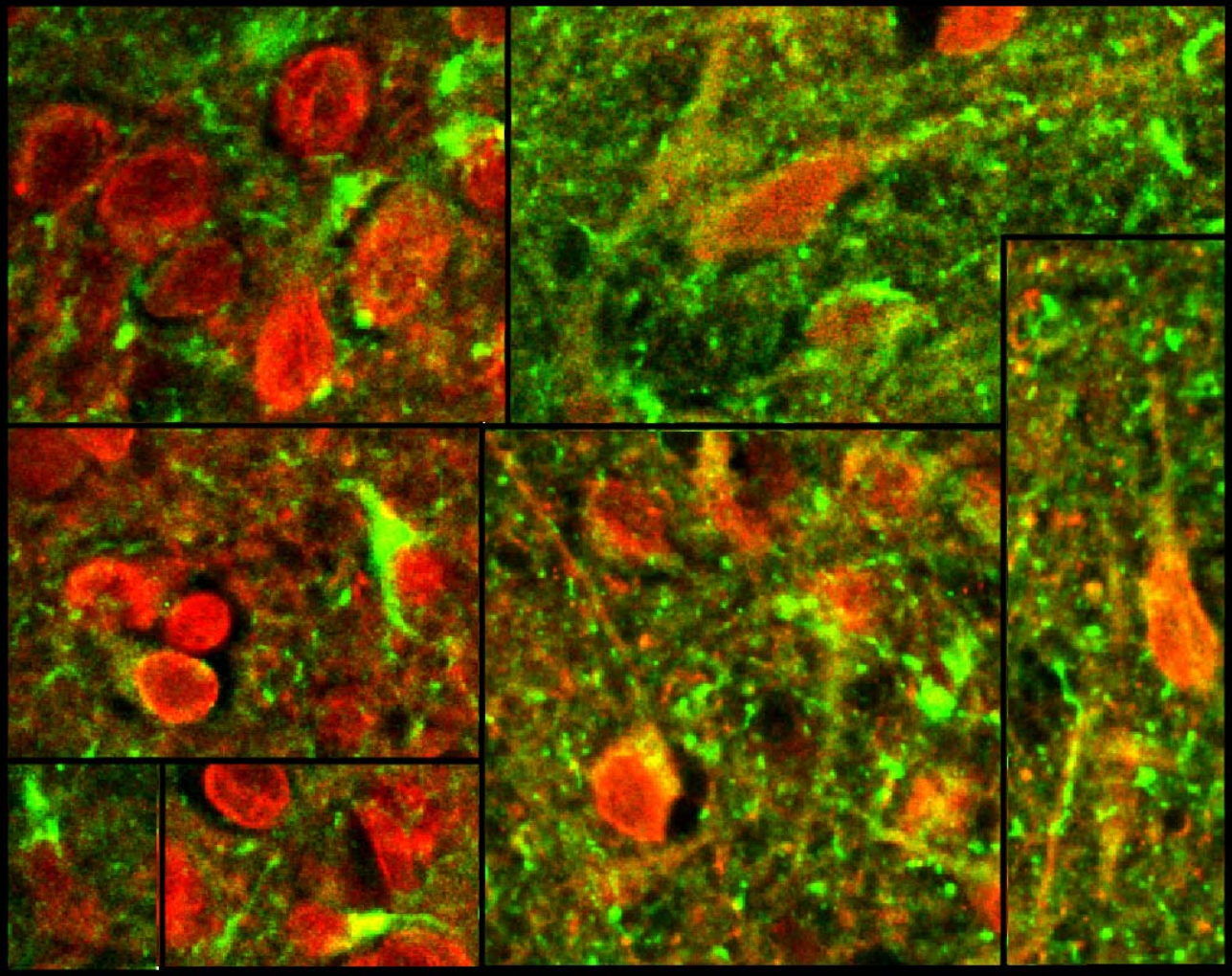


BEO

Green: PSD-95
Red: MAP2

(Yoshii et al. 2003, PNAS)

PSD-95 is Redistributed to Synapses after Eye-Opening



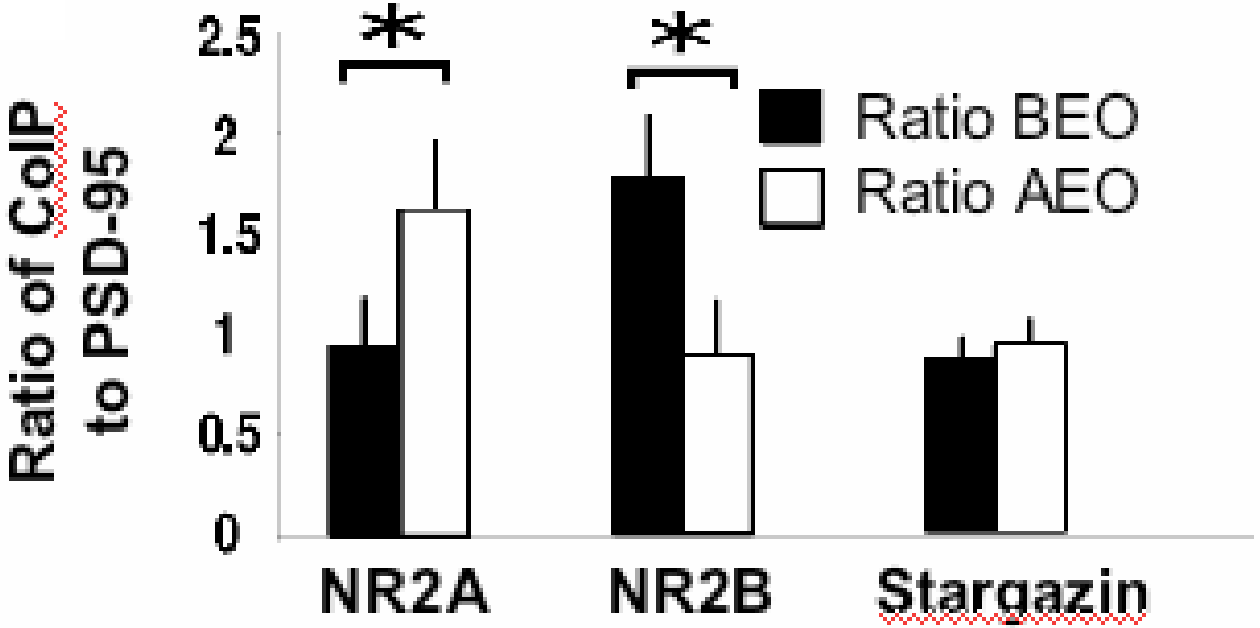
Green: PSD-95
Red: MAP2

BEO

6hrs AEO

(Yoshii et al. 2003, PNAS)

Eye-opening produces a switch in the ratios of the NR2B and the NR2A subunits associated with PSD-95 in dendritic fractions.



Yoshii et al.,2003

Over-expression of tagged PSD-95 and either tagged NR2A or NR2B shows co-localization of NR2A and PSD-95 at synapses

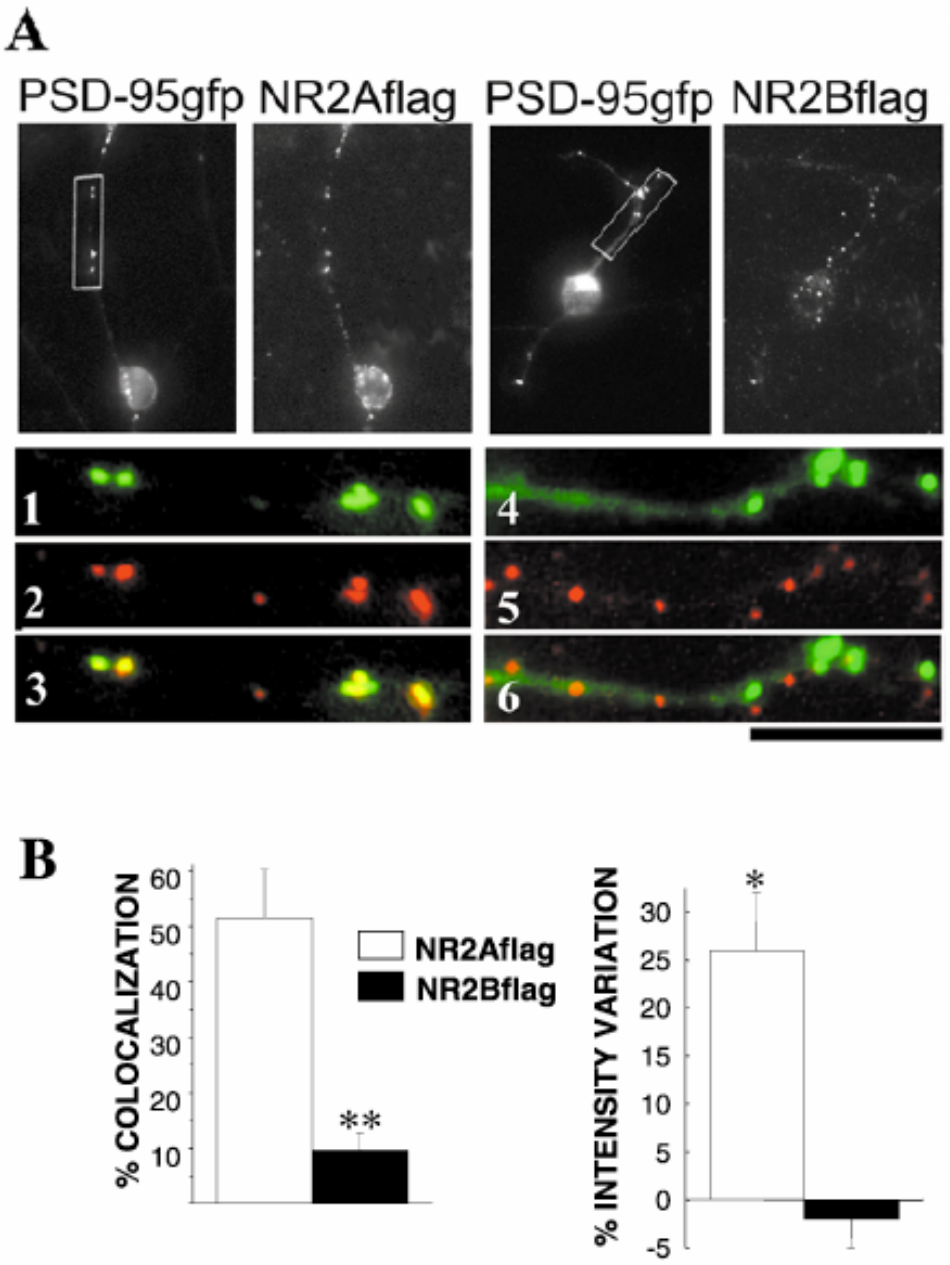
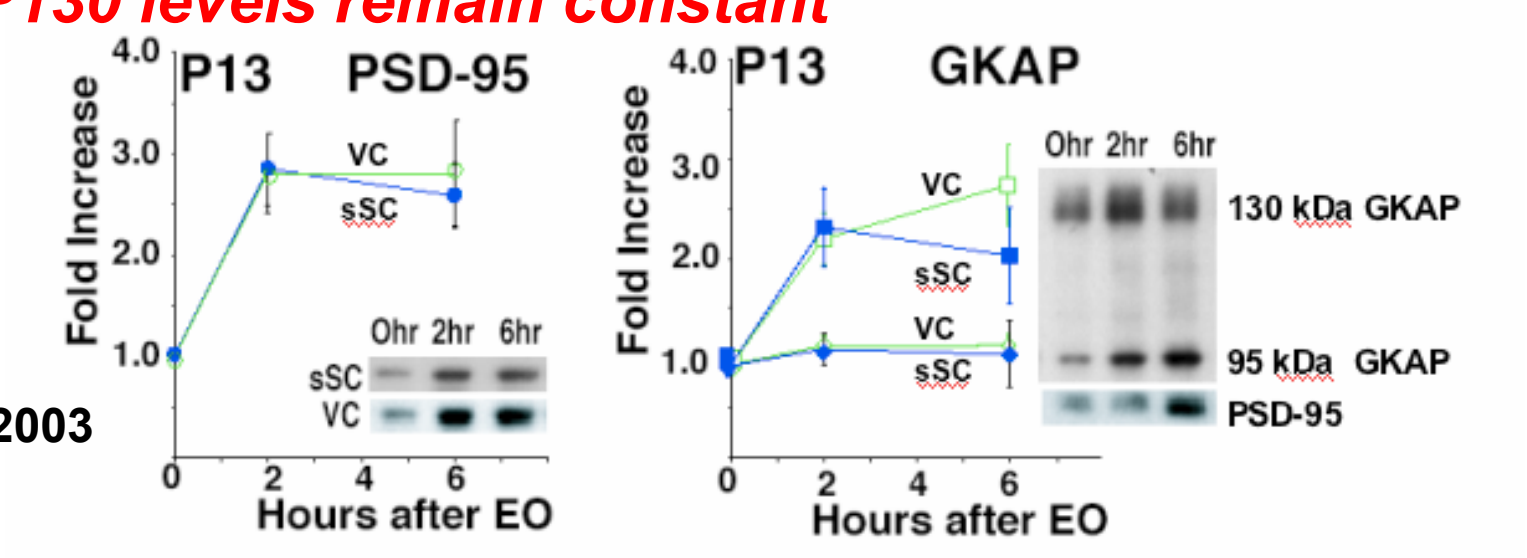


Figure 6. Distinct colocalization of PSD-95gfp with NR2A-flag and NR2B-flag clusters

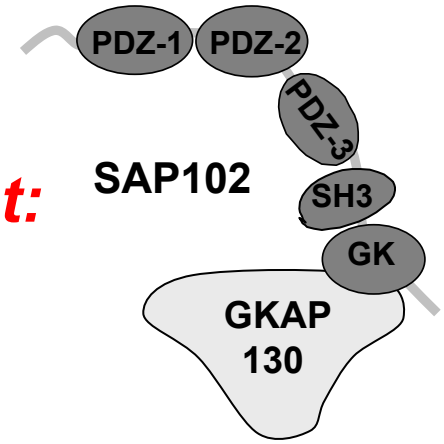
Losi,G et al.,
(2002) J. Physiol.
548:21-29.

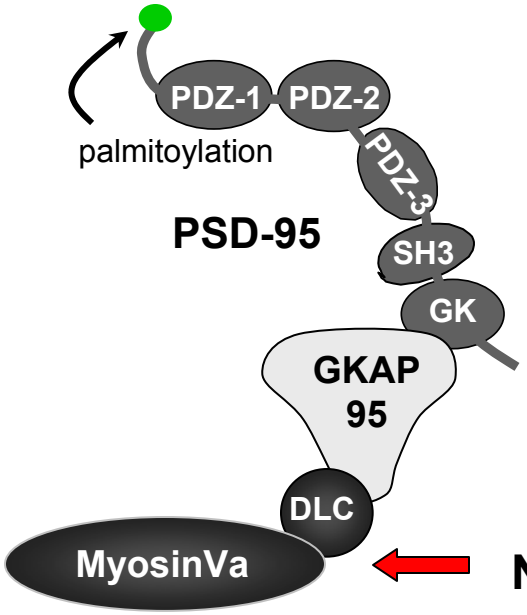
GKAP95 increases at the synapse with PSD-95, while GKAP130 levels remain constant



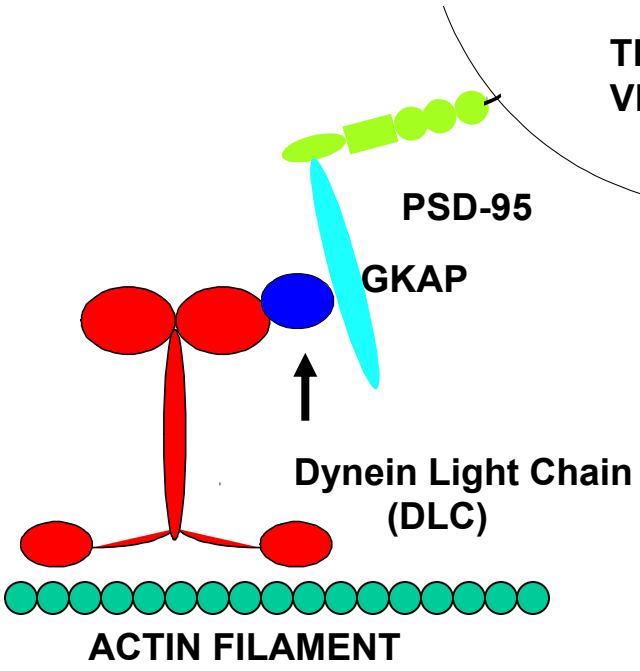
Yoshii et al,2003

data suggest:

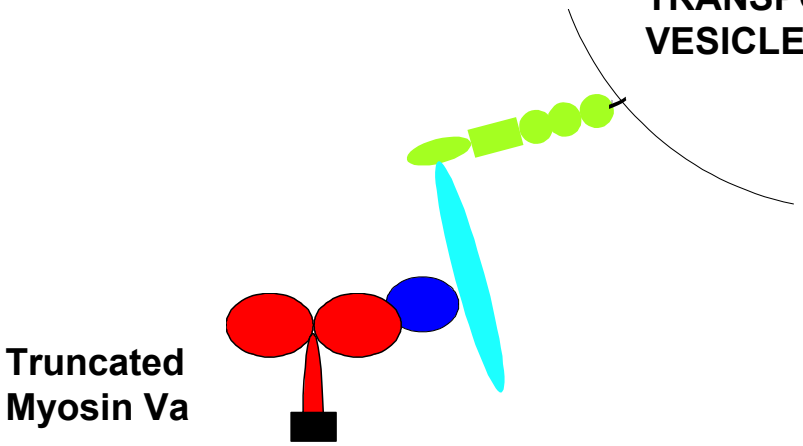




Naisbitt et al, 2000

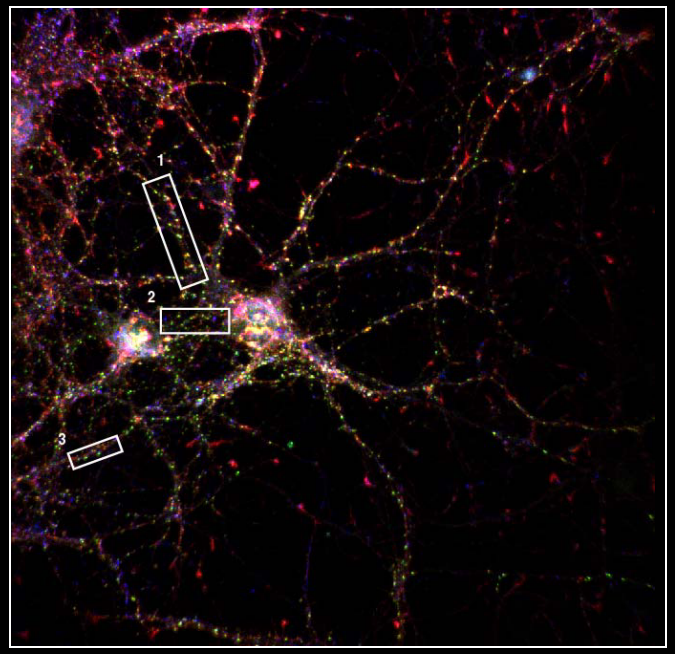


The Flailer mouse has an additional truncated myosin Va expressed only in brain. It is ataxic and has seizures. The truncated myosin Va appears to operate as a dominant negative (Jones et al.,2000)

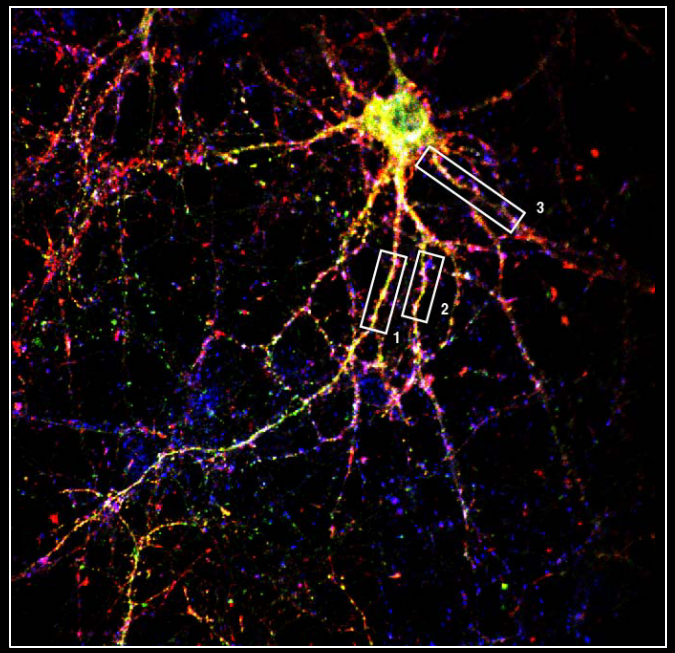


Localizing Synaptic Distribution of PSD-95 (1)

Wild type



Flailer



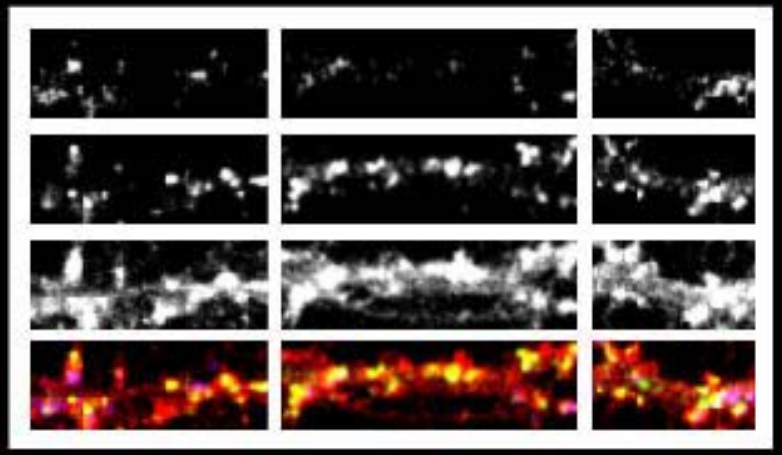
DIV 14

Red:Phalloidin
Green:PSD-95
Blue:Synaptophysin

Localizing Synaptic Distribution of PSD-95 (2)

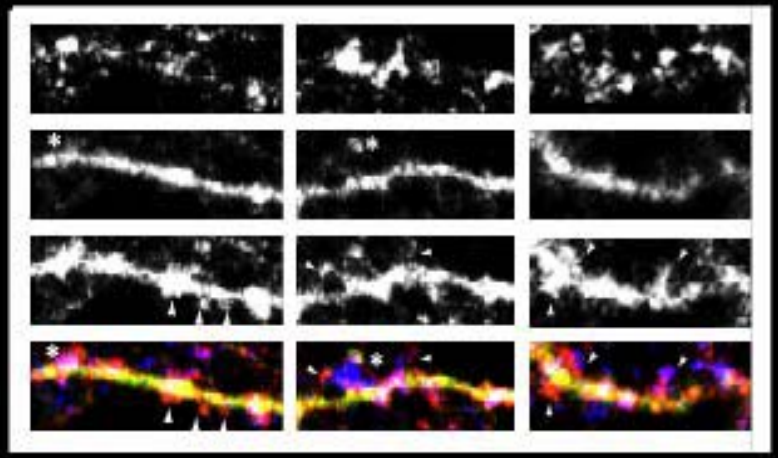
Wild Type

synaptophysin
PSD-95
Phalloidin
Overlay

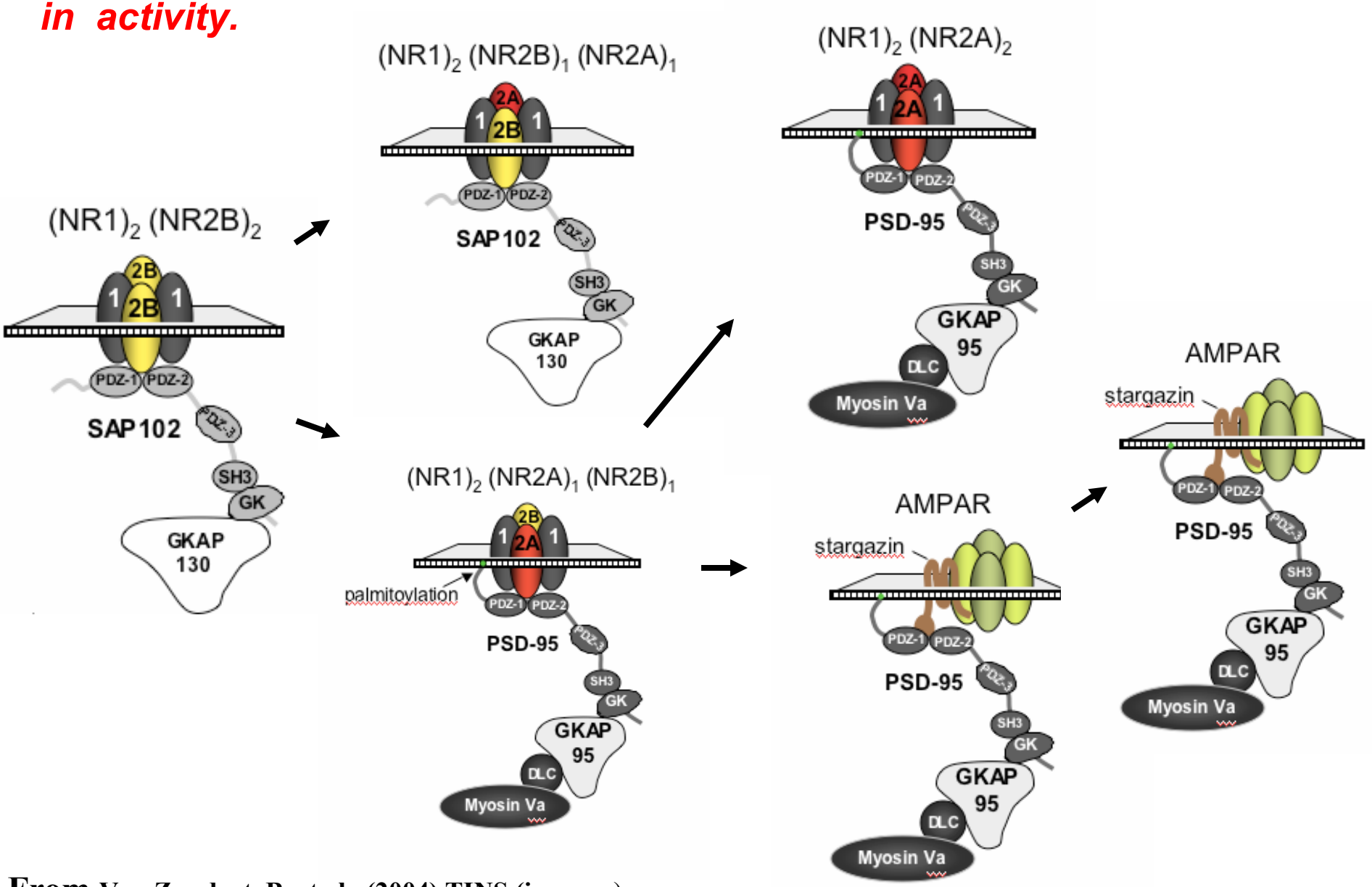


Flailer

synaptophysin
PSD-95
Phalloidin
Overlay



Hypothesis: The entire ionotropic glutamate receptor scaffolding, trafficking,and signaling complex changes with developmental increases in activity.



From Van Zundart, B. et al., (2004) TINS (in press)

Culture hippocampal slices. After ~ 4 days transfect with tagged PSD-95.

Several days later record from an infected cell and a non-infected neighbor.

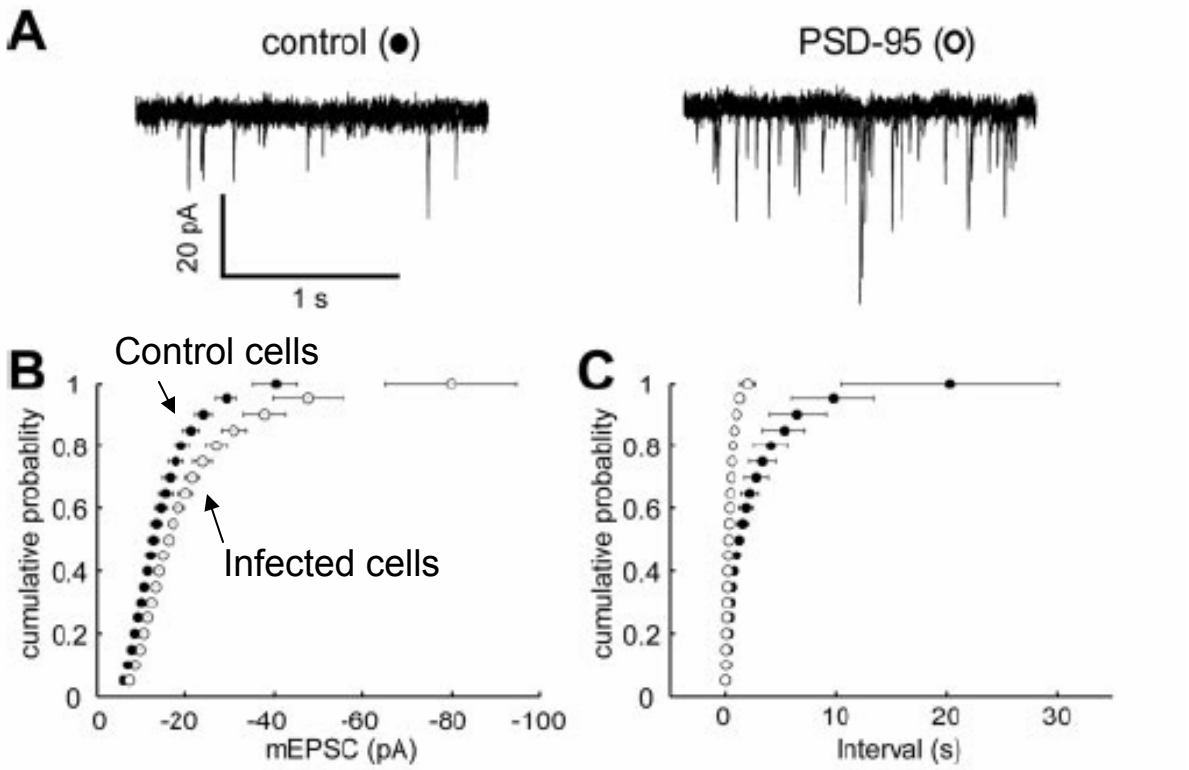
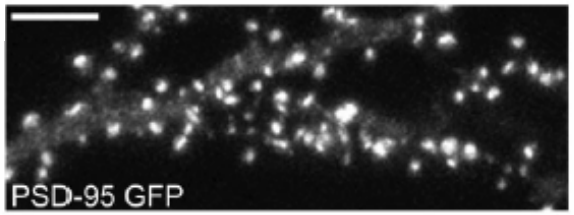


Figure 1. Expression of PSD-95 enhances the amplitude and frequency of mEPSCs. *A*, Five superimposed sample traces showing mEPSCs from a control cell (left) and a PSD-95-expressing cell (right). Note that there are many more events in the PSD-95-expressing cell, and that some of the events are larger than events recorded in a control cell. *B*, Cumulative frequency distributions of the amplitudes of mEPSCs recorded from control cells (closed circles) and PSD-95-expressing cells (open circles) ($n = 7$). *C*, Cumulative frequency distributions of the interevent intervals of mEPSCs recorded in control cells (closed circles) and PSD-95-expressing cells (open circles).

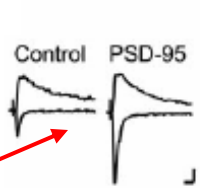
Stein, V., et al., (2003) J. Neurosci. 23, 5503-5506

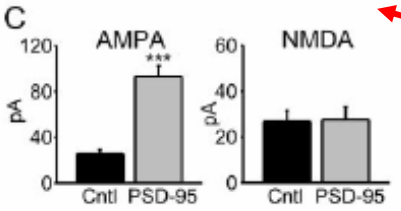
A New Function For PSD-95 via the Stargazin Family of Molecules

PSD-95 over-expression



Enlarged AMPAR currents at synapses

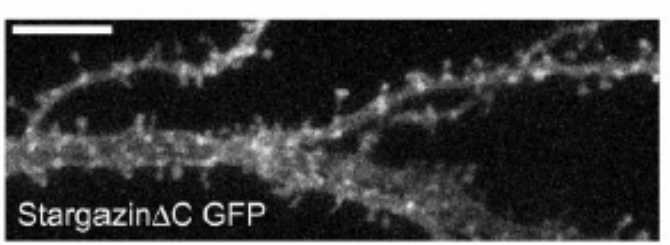


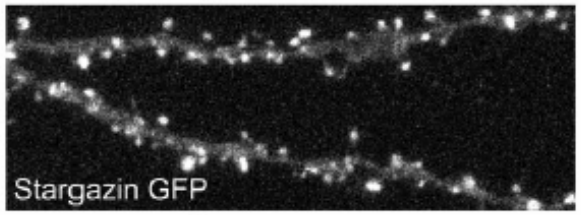


Both PSD-95 & Stargazin are localized at synaptic puncta

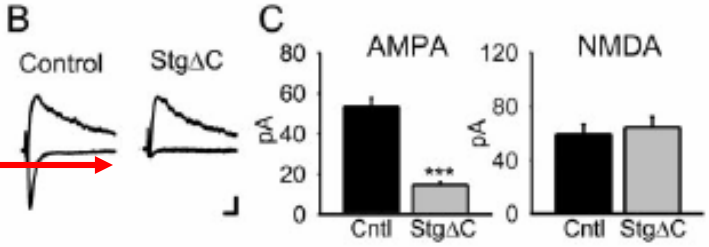
Removal of the c-terminal of stargazin (stargazinΔC) blocks its binding to PSD-95.

stargazinΔC localization is more diffuse

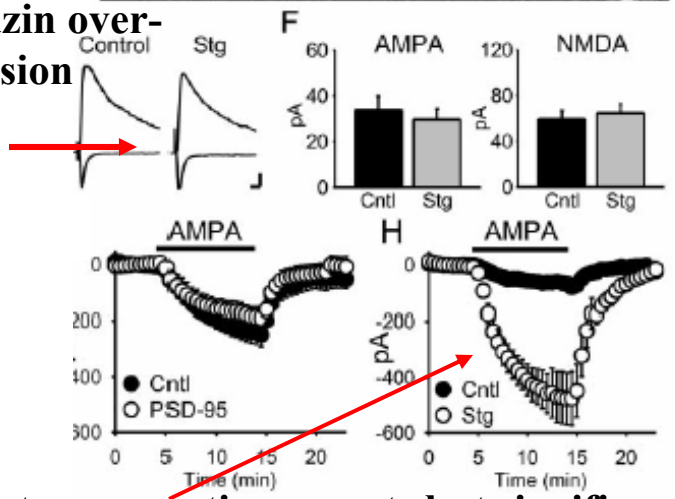




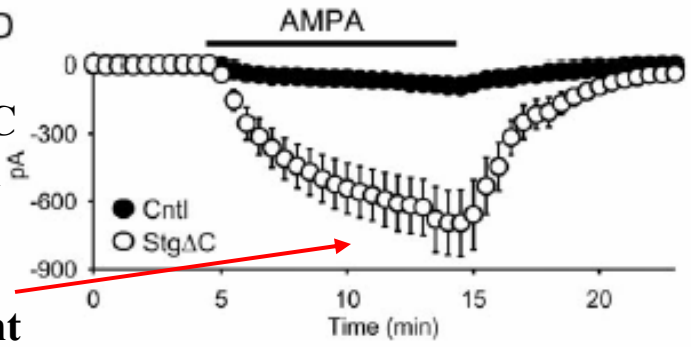
stargazinΔC reduces AMPAR synaptic current



Stargazin over-expression



But stargazinΔC over-expression still increases extra-synaptic AMPAR current



No effect on synaptic currents but significant increases in extra-synaptic AMPAR current.

Schnell, E., et al., (2002) PNAS 99:13902-13907

What do these results imply about the function of PSD-95 ?

That PSD-95 over-expression produces a significant increase in AMPAR but not NMDAR currents at the synapse.

What do these results imply about the function of stargazin?

That stargazin facilitates AMPAR expression on the surface of neurons.

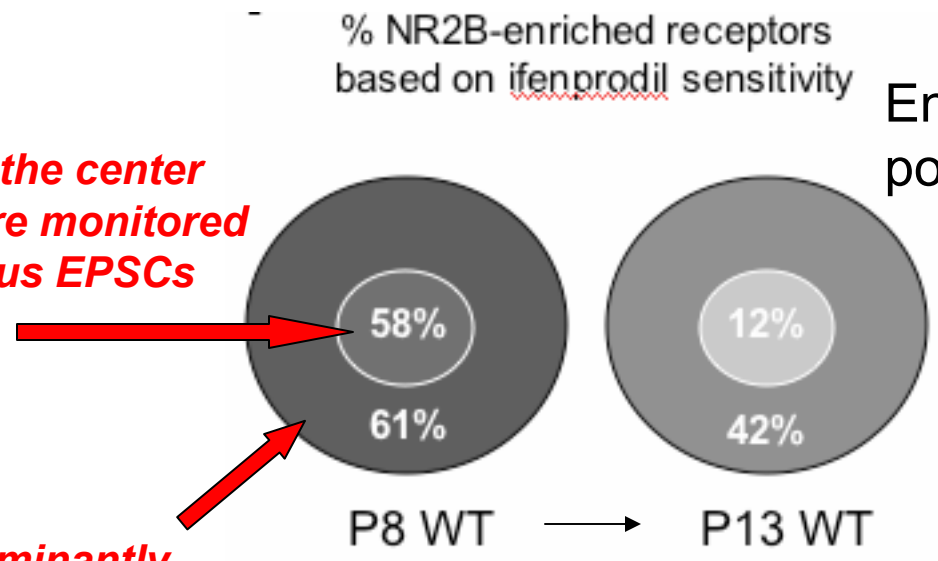
What does the truncated stargazinΔC result imply about the function of the stargazen C terminus?

That the C-terminus is not necessary for stargazin to increase the surface expression of AMPARs but that it is necessary for clustering AMPARs at the synapse.

The C-terminus of Stargazin is a PDZ binding domain. Therefore what do these experiments suggest about the interaction of PSD-95 and Stargazin?

That PSD-95 binds Stargazin through its PDZ-domains and localizes it and the associated AMPARs to the synapse.

Ifenprodil blocks current through NR2B-rich NMDARs. Therefore, it can be used to determine how much of the NMDAR current is carried by NR2B-rich NMDARs.



En face views of the post-synaptic surface

Responses from the center of the synapse are monitored using spontaneous EPSCs

Responses predominantly from extrasynaptic NMDARs are monitored using evoked EPSCs.

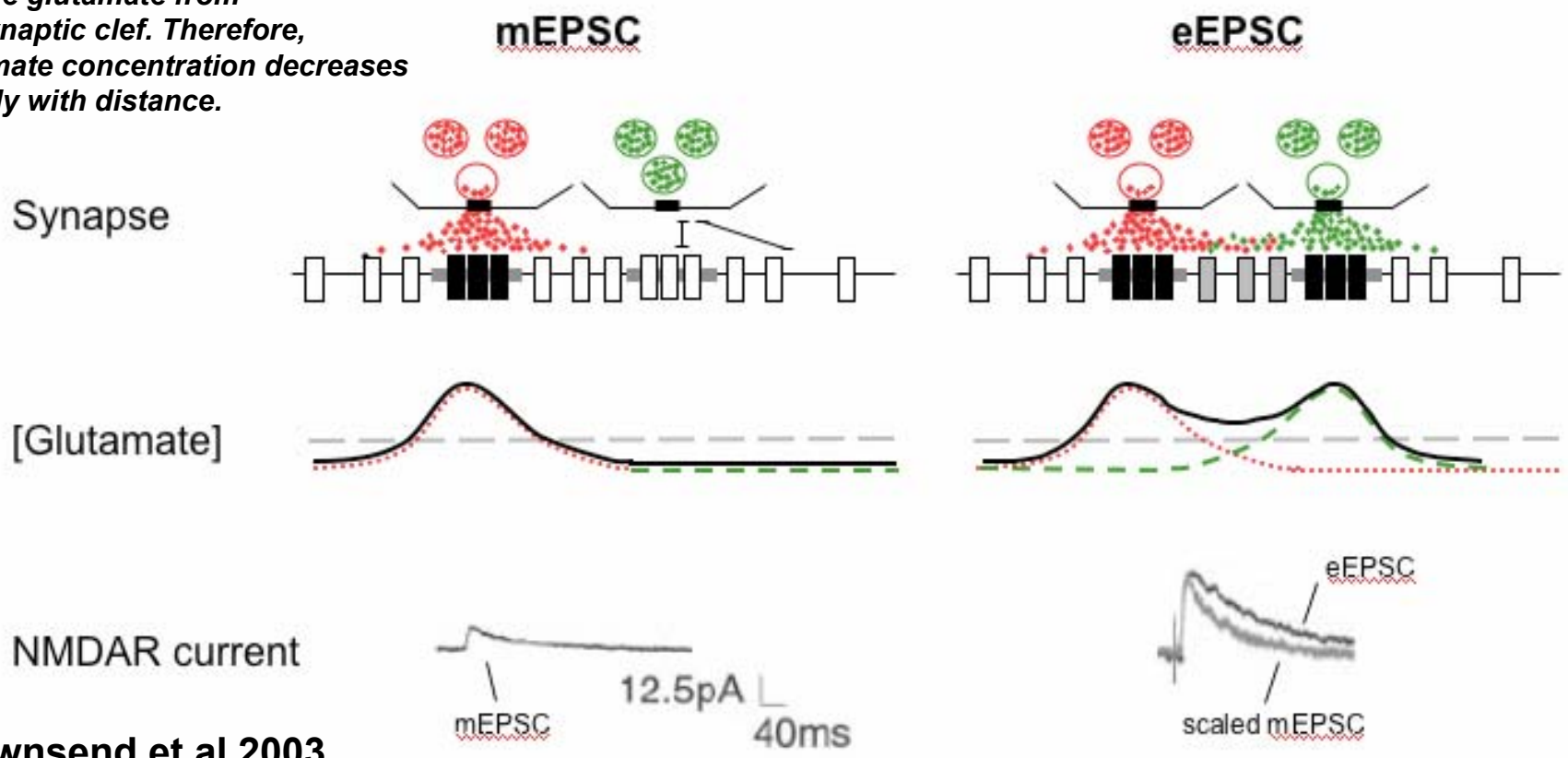
1. The proportion of current carried by NR2B- rich receptors decreases with age.

2. The decrease in the NR2B- rich receptor contibution is much faster at the synaptic center than in the extrasynaptic domain.

Townsend et al, 2003

Extrasynaptic NMDAR receptors participate in evoked currents but not in most miniature synaptic currents

Glutamate transporters remove glutamate from the synaptic clef. Therefore, glutamate concentration decreases rapidly with distance.

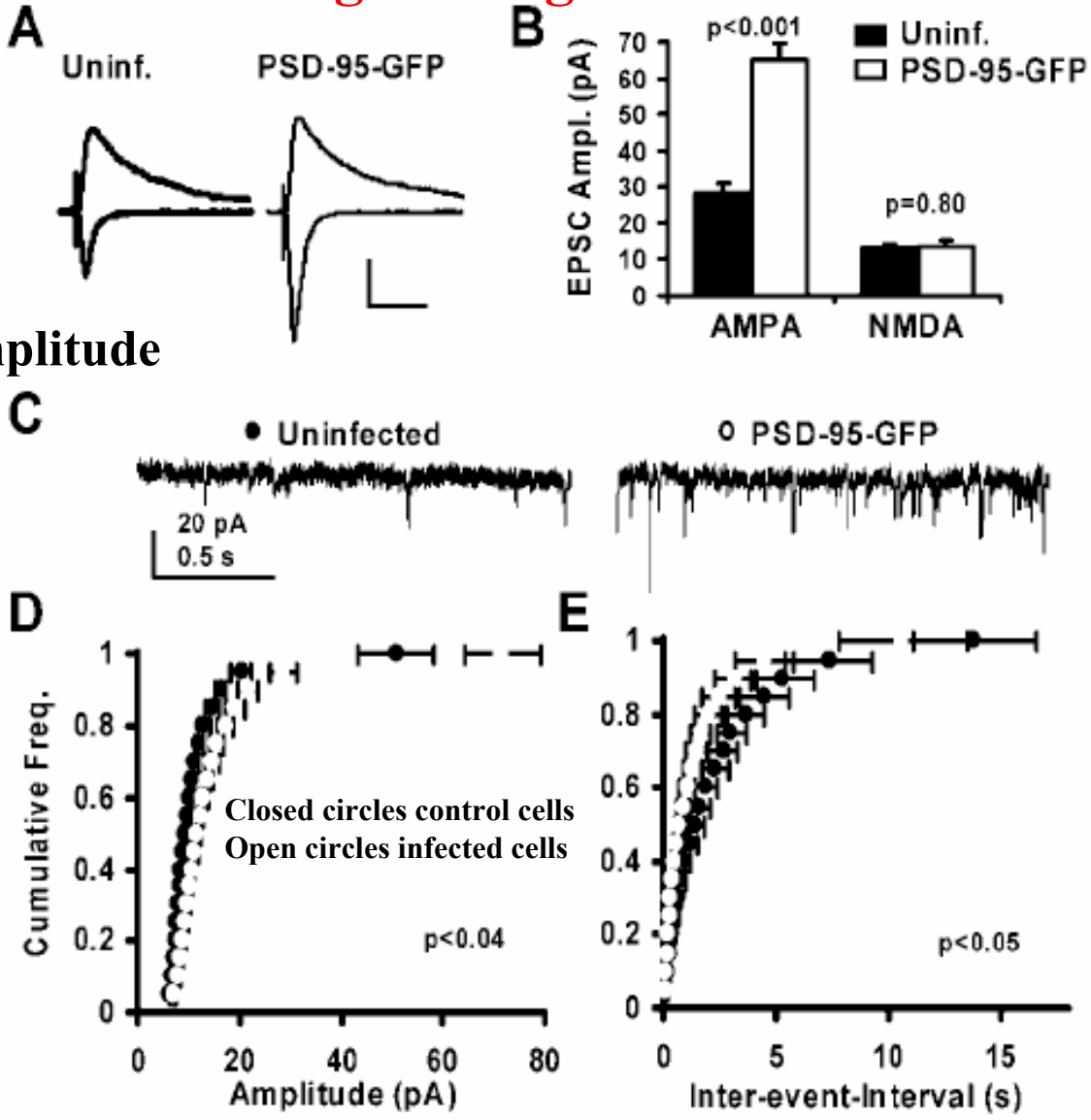


Townsend et al,2003

[glutamate] released from active zone 1
[glutamate] released from active zone 2
[glutamate] in cleft
[glutamate] threshold for NMDAR activation
non-activated NMDAR
activated NMDAR: located in subsynaptic membrane
activated NMDAR: located in peri/extrasynaptic membrane
glutamate vesicle
active zone
PSD

Hippocampal slice cultures comparing mEPSCs in neurons overexpressing PSD-95 and neighboring uninfected neurons

The frequency and amplitude of AMPAR responses increase



Stein,V., et al., (2003) 23:5503-5506