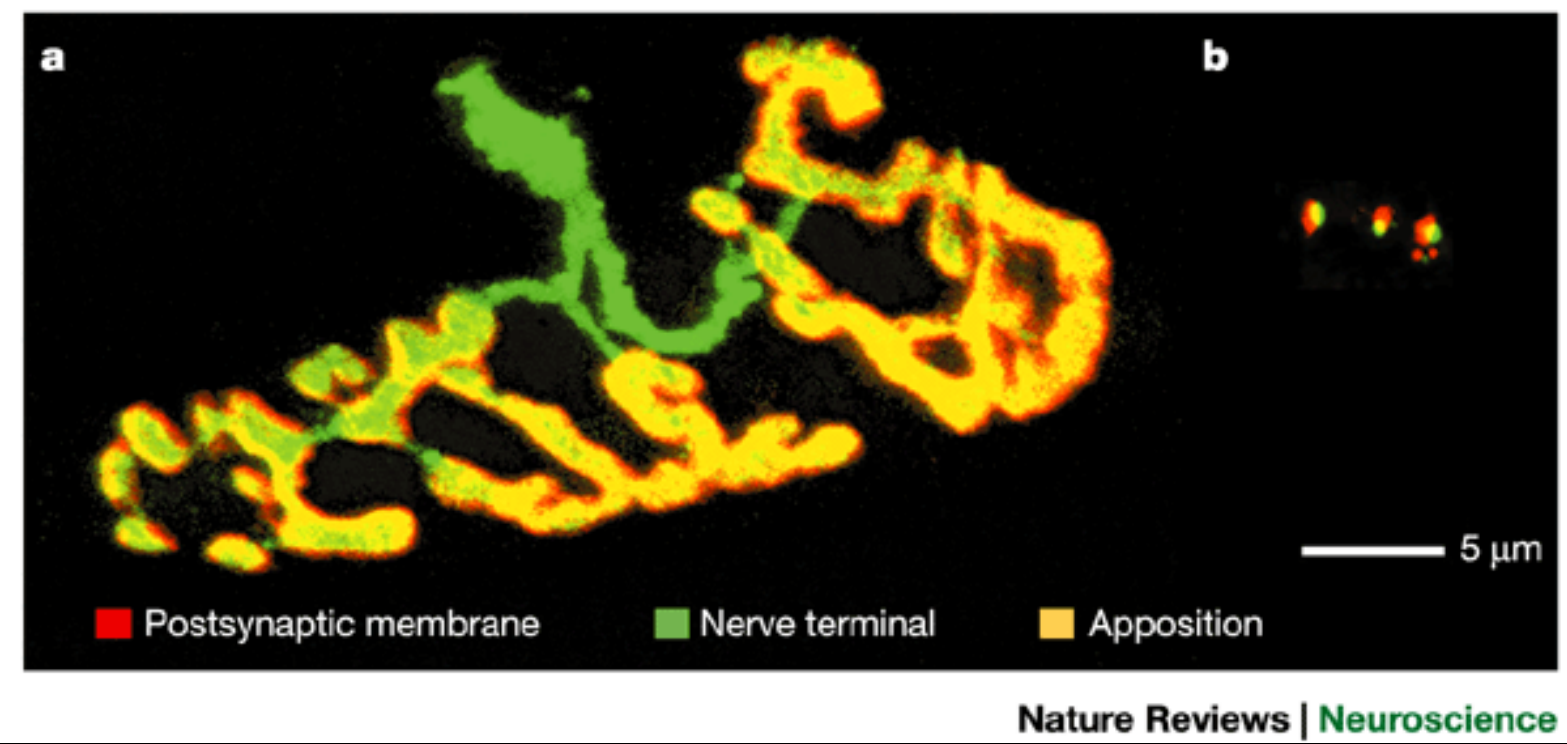
Neuronal Differentiation: CNS Synapse Formation

Comparison of the NMJ and Central Synapses



Sanes and Lichtman 2001

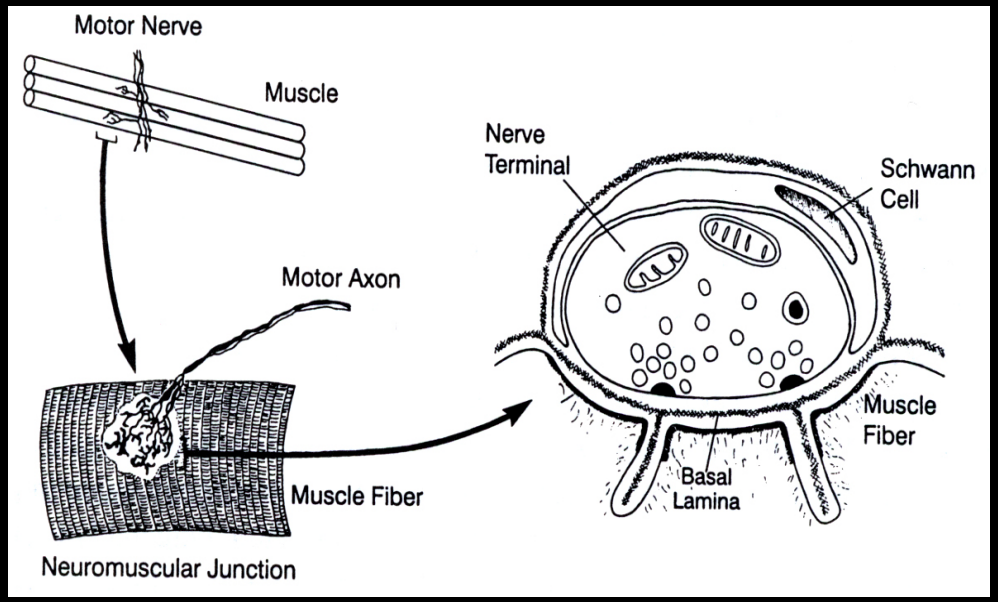
Comparison of the NMJ and Central Synapses

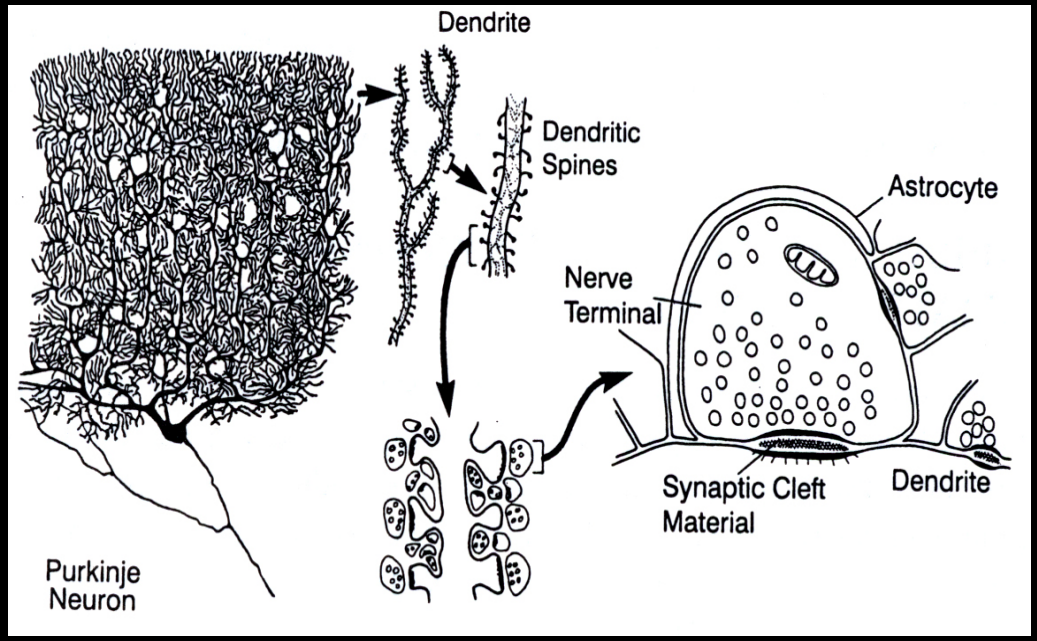
(1) Each neuron in the CNS is innervated by THOUSANDS of synapses whereas each muscle fiber is innervated by a single motor neuron

(2) There is one neurotransmitter at the NMJ, ACh, which acts through a single type of receptor, AChR; in the CNS, there are multiple neurotransmitters and receptors.

(3) There is no basal lamina in the synaptic cleft between CNS neurons

(4) Synaptic transmission at the NMJ is very reliable and always causes the postsynaptic muscle to twitch; in the CNS, many inputs must be INTEGRATED to cause the postsynaptic neuron to fire.





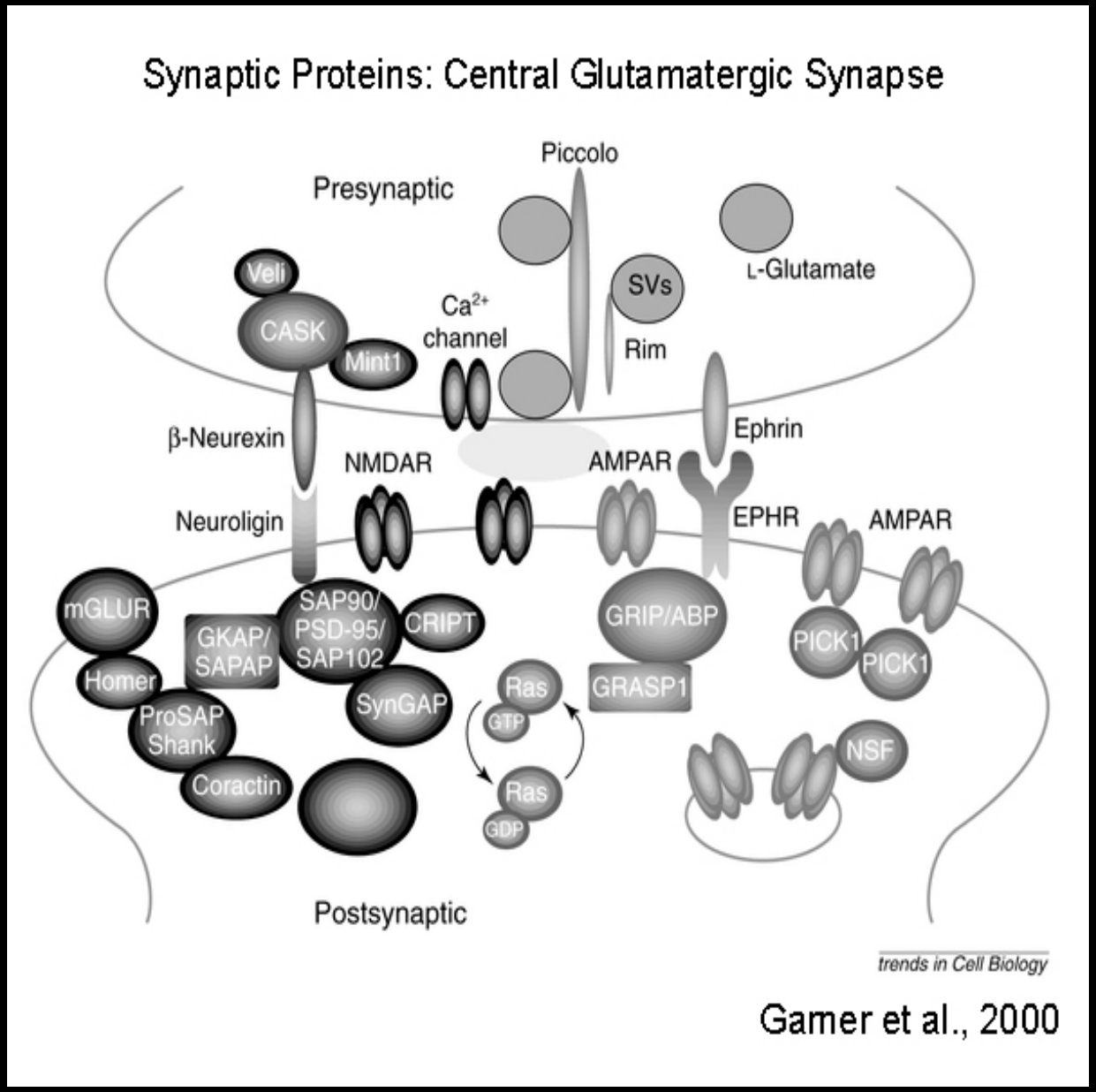
Sanes and Lichtman 1999

There are many proteins that comprise a glutamatergic synapse in the CNS

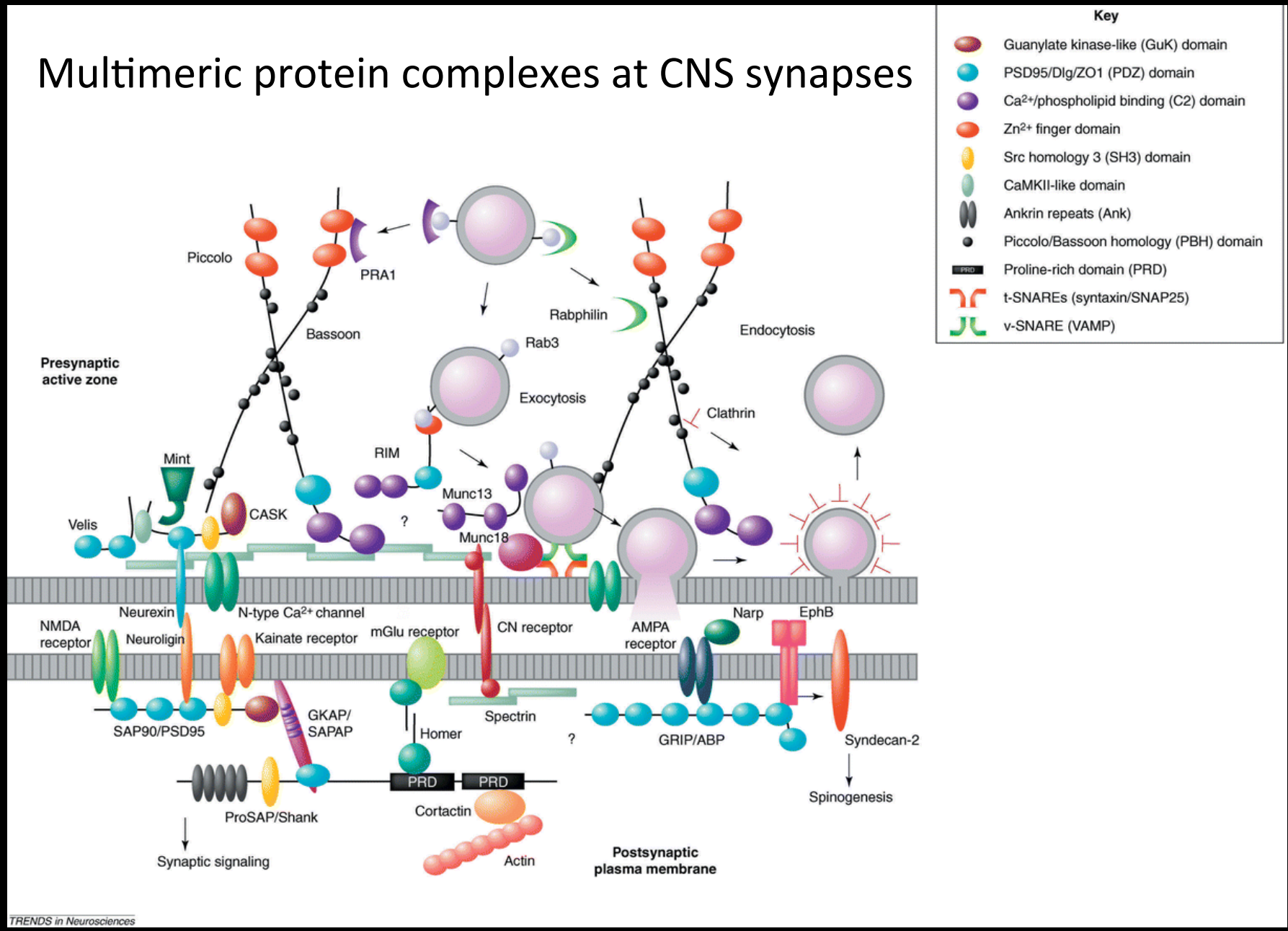
Glutamate Receptors:
(1) AMPA receptors
(2) Kainate receptors
(3) NMDA receptors

Postsynaptic scaffolding proteins:
(1) PSD-95: NMDARs
(2) GRIP: AMPARs
**bind multiple proteins and serve as a scaffold for the synapse that links receptors with the cytoskeleton

Presynaptic proteins:
(1) Synaptic vesicle proteins (release)
(2) Scaffolding proteins

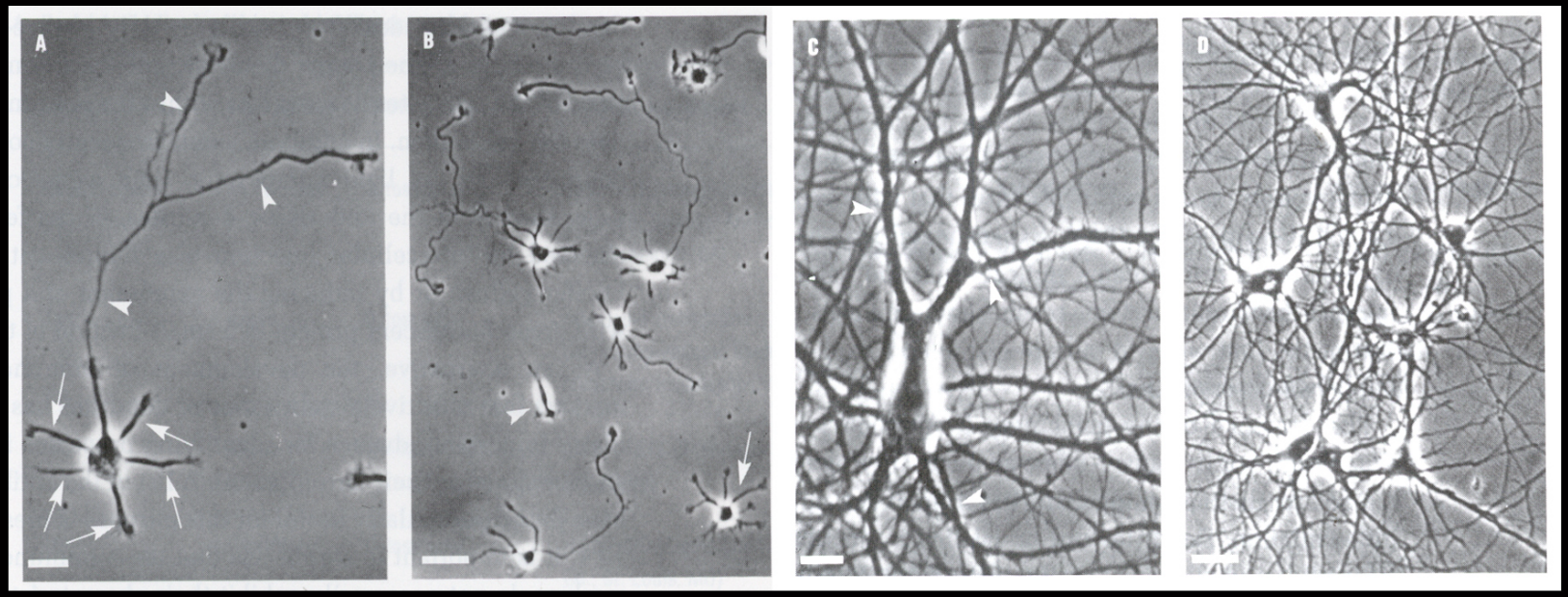


Multimeric protein complexes at CNS synapses



Garner 2002

Approaches to study synapse formation in the CNS: Dissociated neuronal cultures



3 div

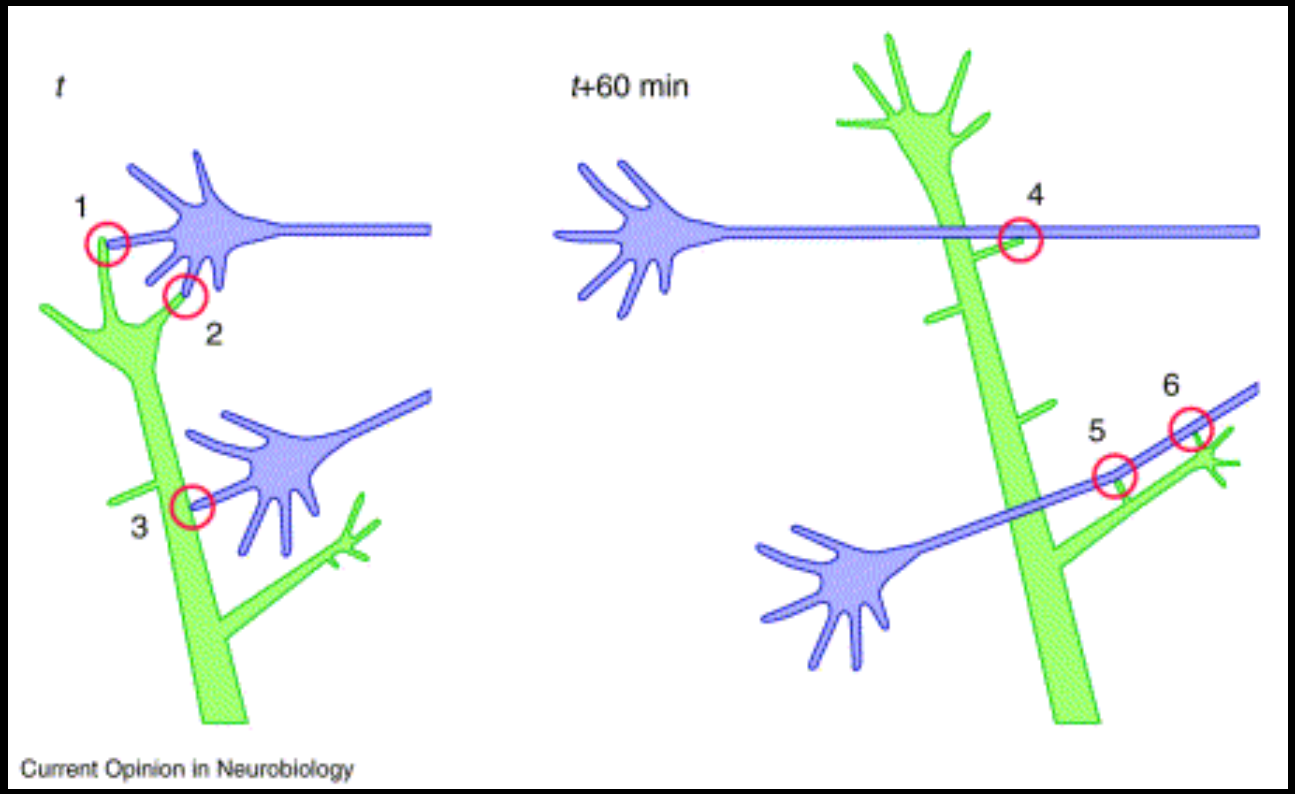
Scale bar = 10μm

21 div

Scale bar = 50μm

Banker and Goslin, 2000

The sequence of molecular and morphological events in synapse assembly and maturation.

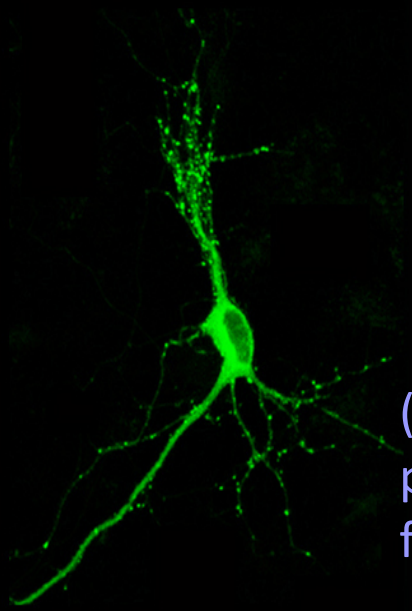


Initiation of synaptogenic contacts by axons and dendrites

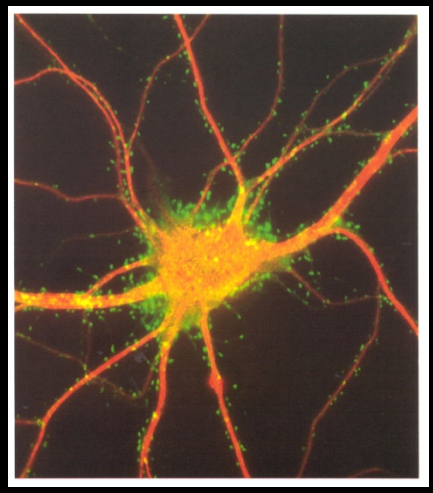
Ziv and Garner review, Curr. Opin. Neurobiol. 2001

Recruitment of the "core components" of CNS synapses

Axon terminal: synaptic vesicles and active zone proteins
Postsynaptic dendrite: neurotransmitter receptors



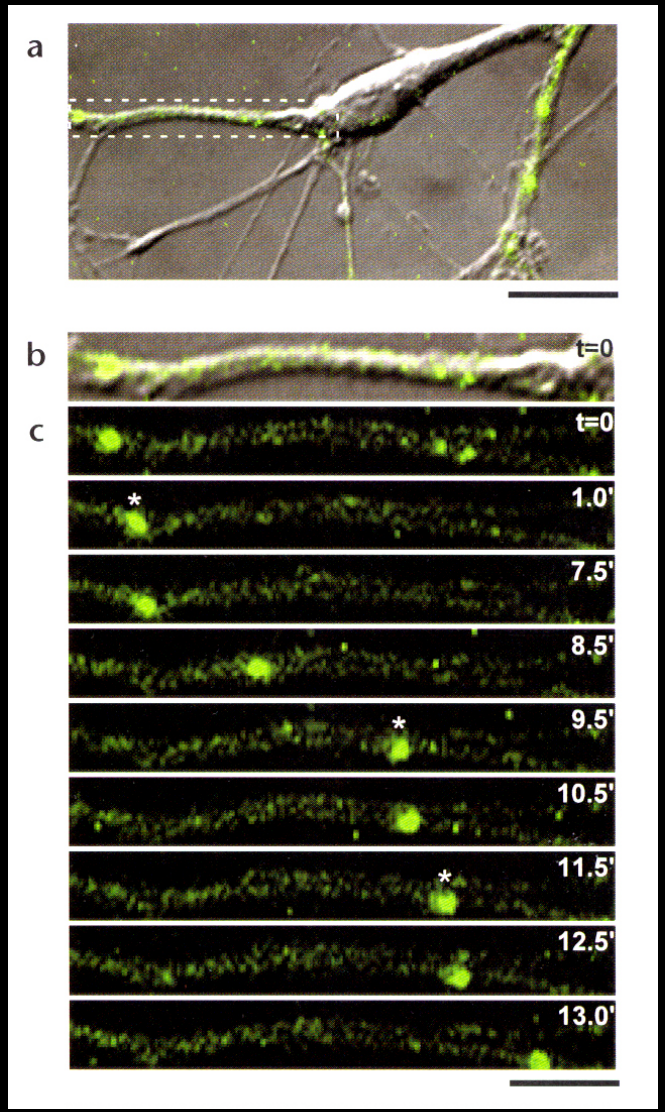
(1) Immunocytochemistry of dissociated neurons at successive stages in culture



(2) Imaging GFP-tagged synaptic proteins in real-time as synapses form

Imaging time-course of recruitment of synaptic proteins to new synaptic contacts

- Smith group



VAMP-GFP clusters are highly mobile within axons prior to synapse formation

-Intensity of VAMP-GFP indicates that there must be multiple synaptic vesicles in each cluster, implying that these clusters are TRANSPORT PACKETS

-Transport packets are dynamic within axons

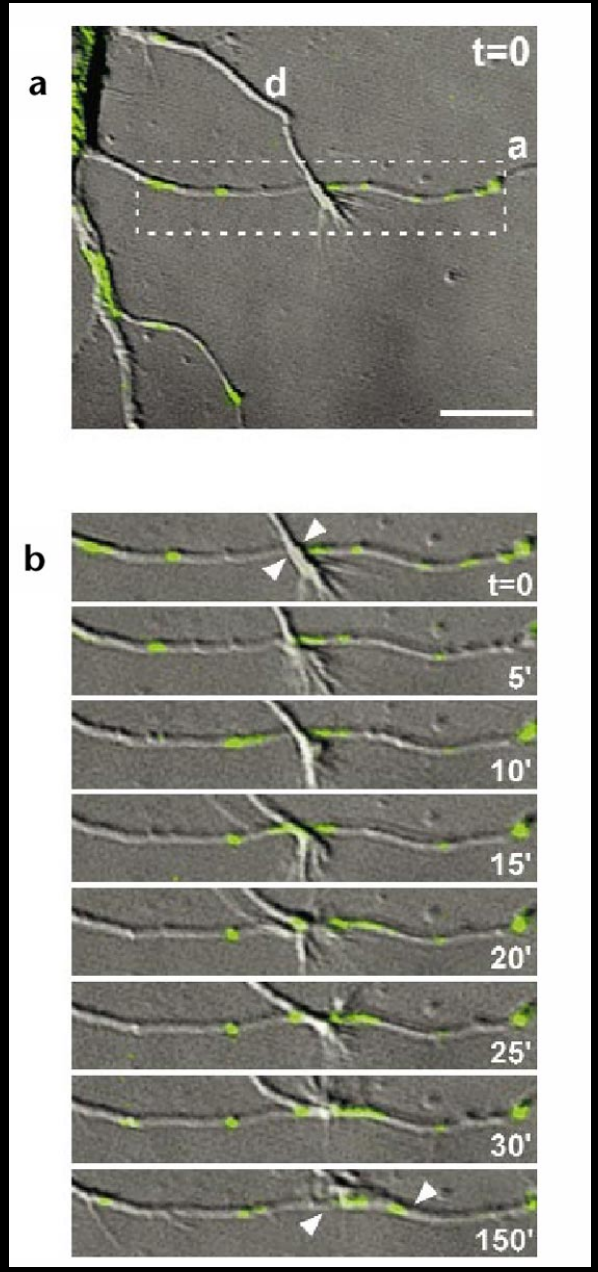
Ahmari et al. 2000

Imaging time-course of recruitment of synaptic proteins to new synaptic contacts

- Smith group

VAMP-GFP-containing transport packets are stabilized specifically at new sites of axon and dendrite contact

**within less than 1 hour, evoked vesicle recycling occurs at these young synapses



Ahmari et al. 2000

Imaging time-course of recruitment of synaptic proteins to new synaptic contacts

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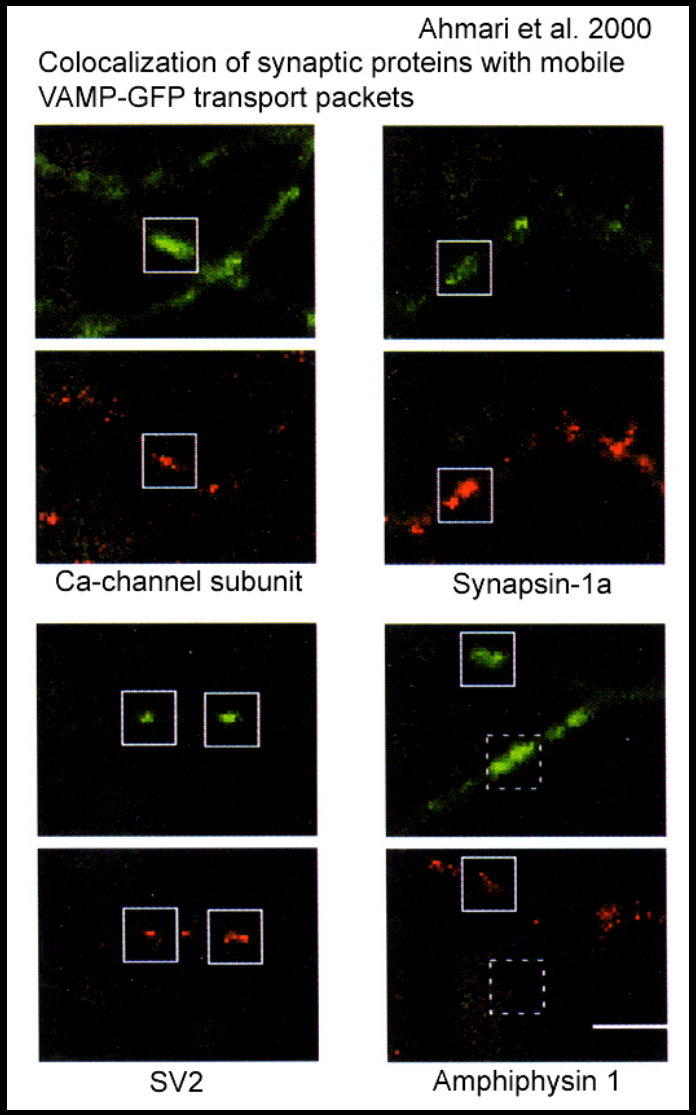


Major components of the presynaptic active zone are transported along developing axons as discrete transport packets

-assessed by immunocytochemistry with antibodies to other proteins found in the presynaptic terminal and colocalization with VAMP-GFP clusters:

(1) Ca-channels
(2) Synapsin (synaptic vesicle protein)
(3) SV2 (synaptic vesicle protein)
(4) Amphiphysin 1 (involved in endocytosis)

**Suggests that many presynaptic proteins may be formed and transported to synapses together in transport packets

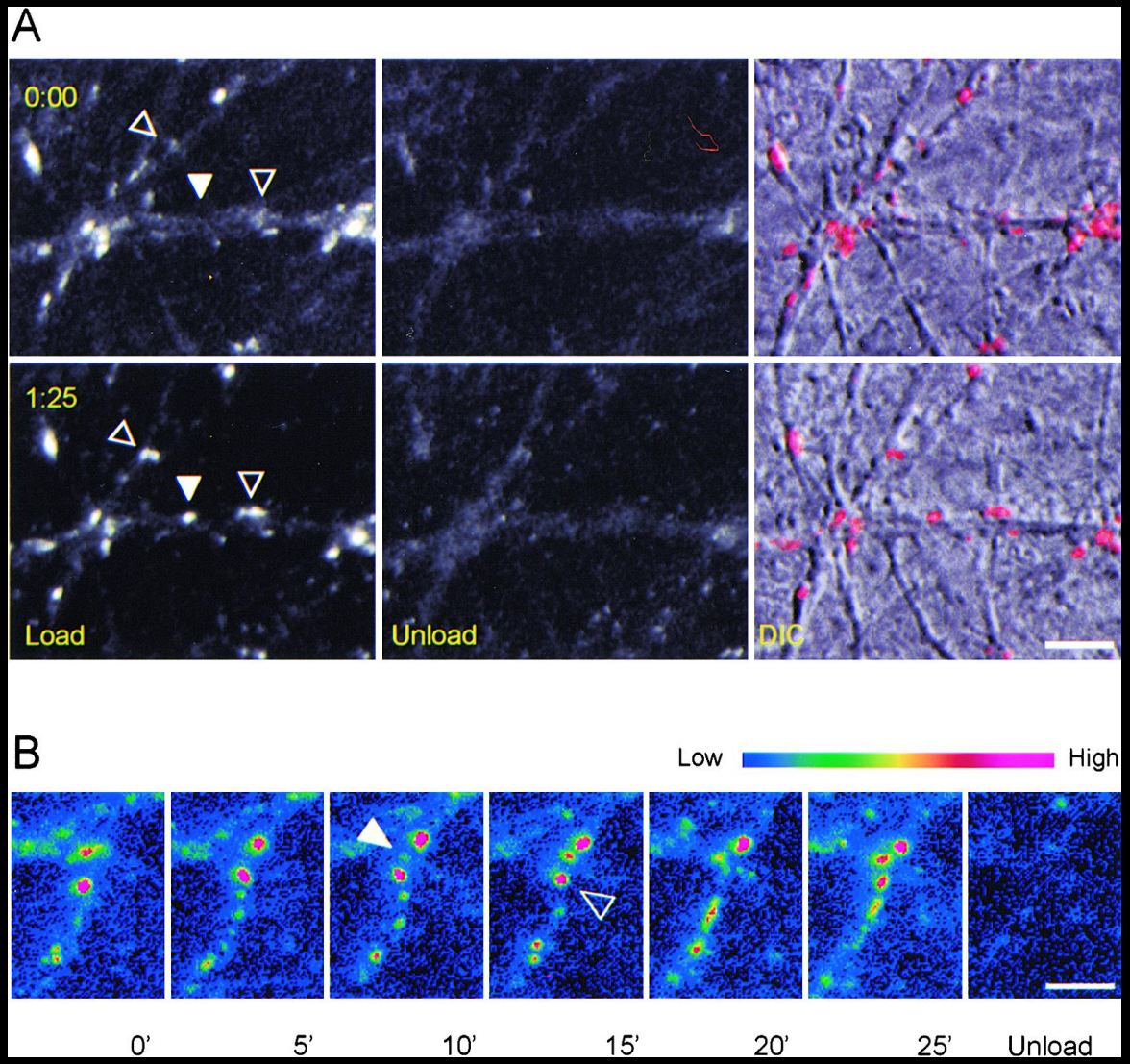
Ahmari et al. 2000

Imaging time-course of recruitment of synaptic proteins to new synaptic contacts

- Garner & Ziv groups

Appearance of Apparently New Presynaptic Bouton Detected by Labeling with FM 4-64

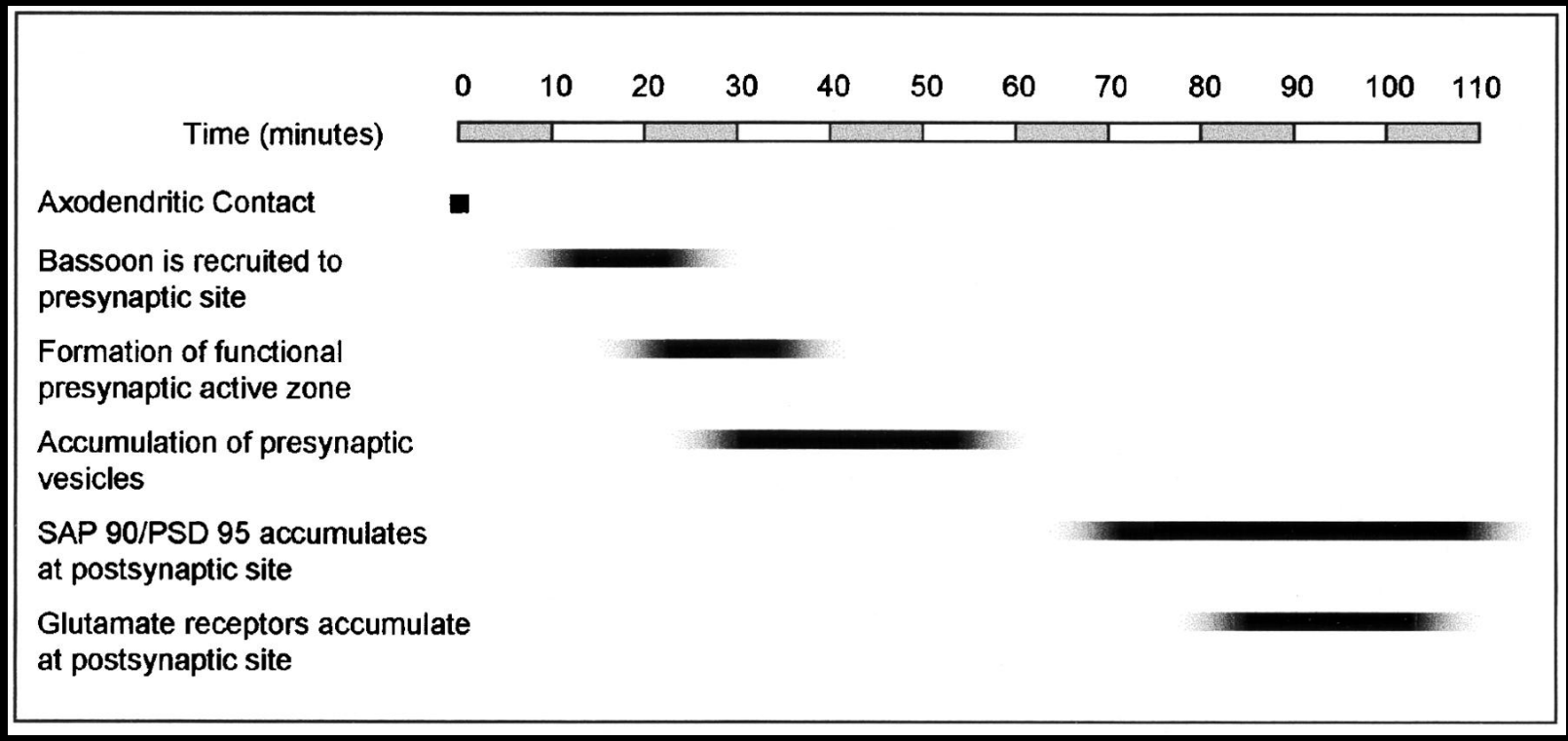
After imaging new spots, cells were fixed and retrospectively immunostained for various synaptic proteins



Friedman et al. 2000

Imaging time-course of recruitment of synaptic proteins to new synaptic contacts

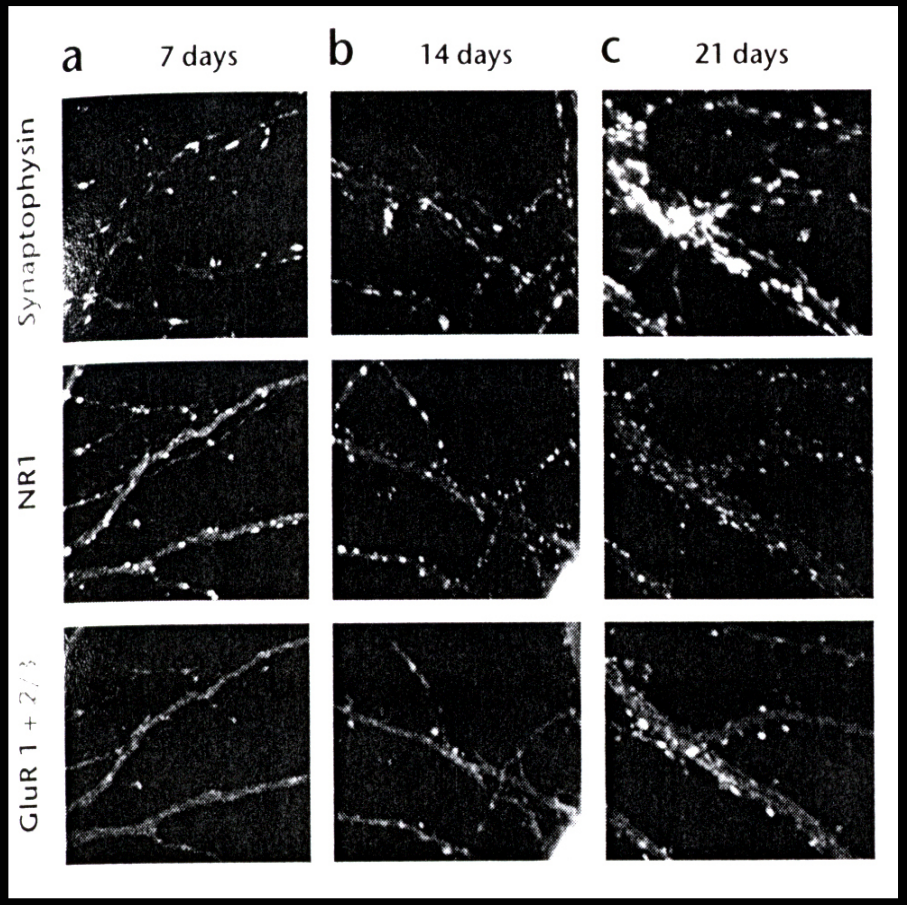
- Garner & Ziv groups

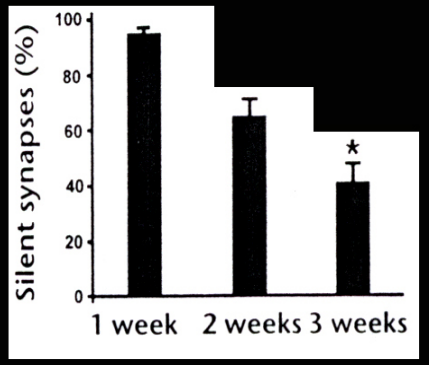


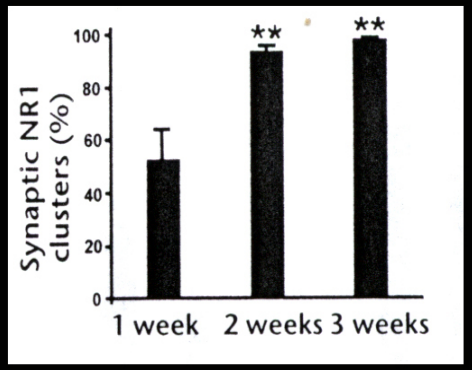
Friedman et al. 2000

Model for glutamatergic hippocampal synapse formation based on immunocytochemistry

Almost all synapses are silent early in development and AMPA receptors are gradually inserted with maturity



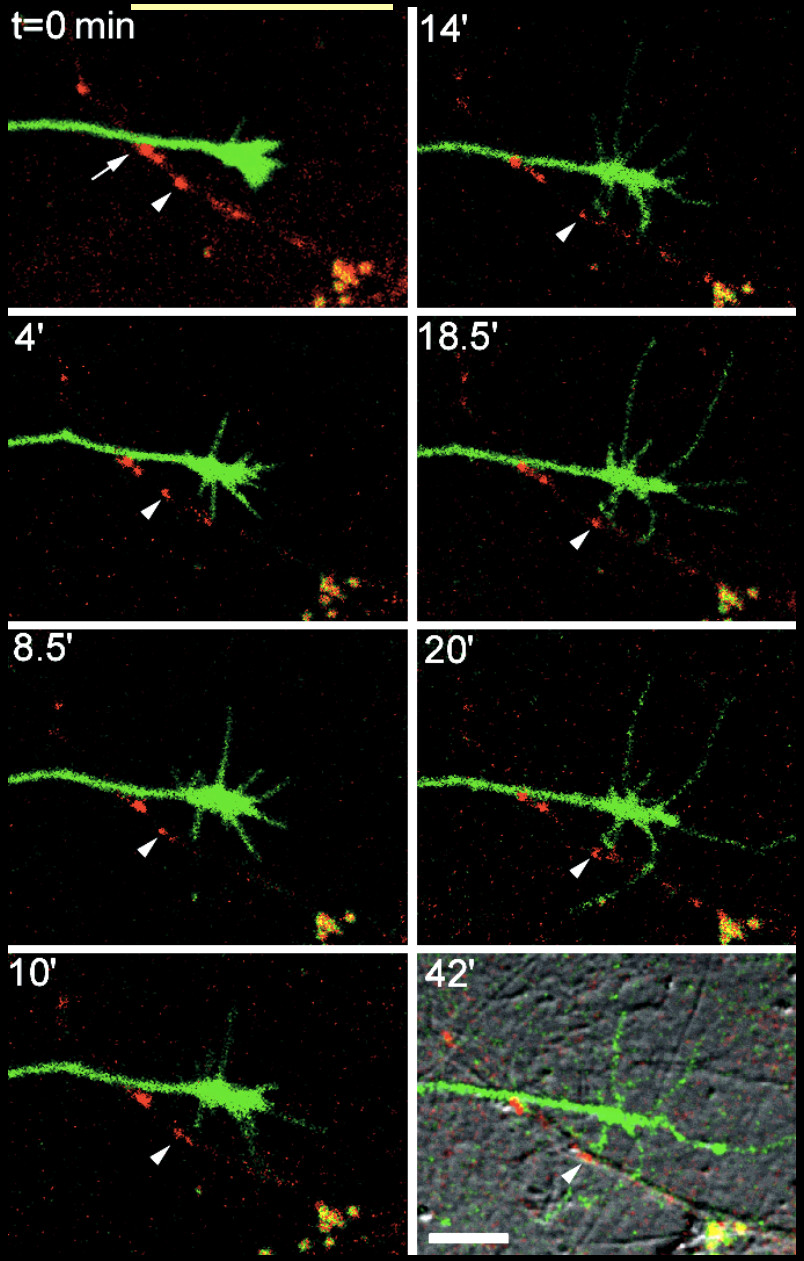


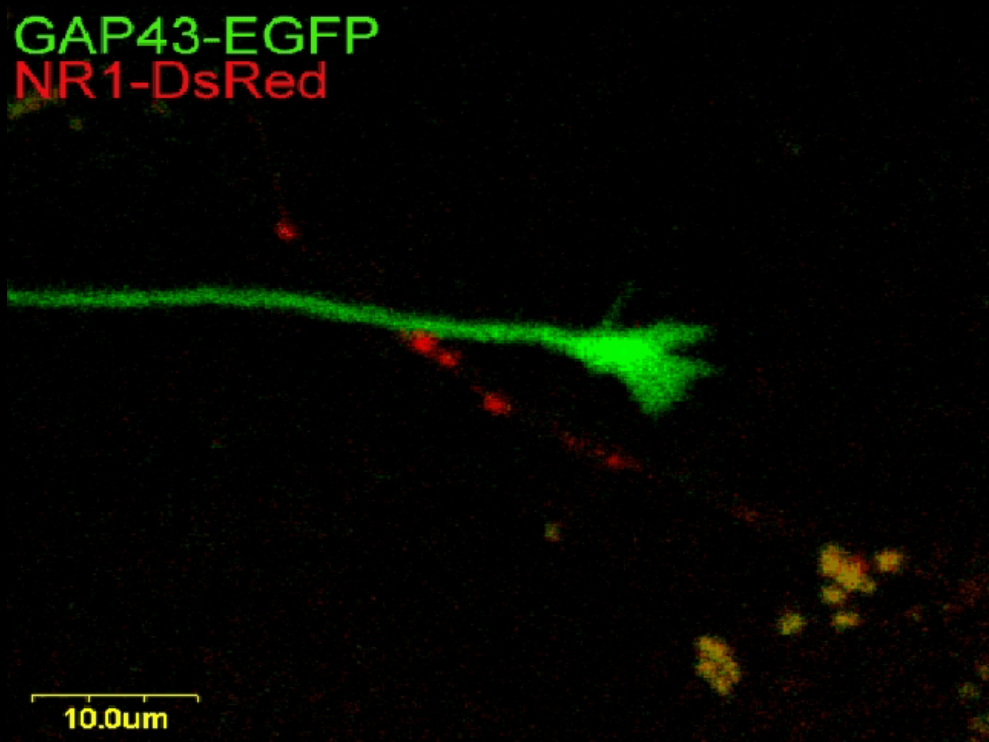


Liao et al., 1999

NMDA receptors precede the recruitment of AMPA receptors by weeks

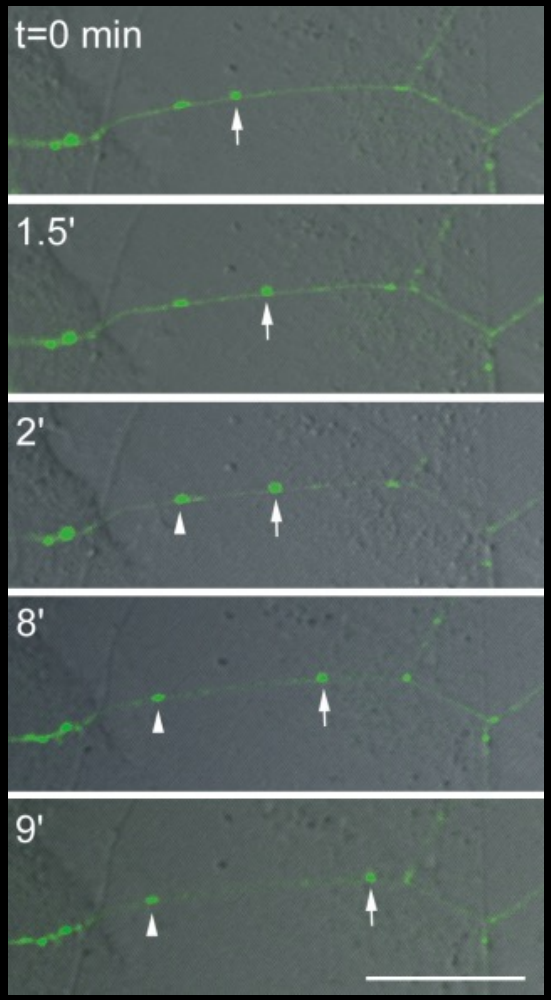
NMDARs can be recruited to sites of contact within minutes of contact with an axonal growth cone



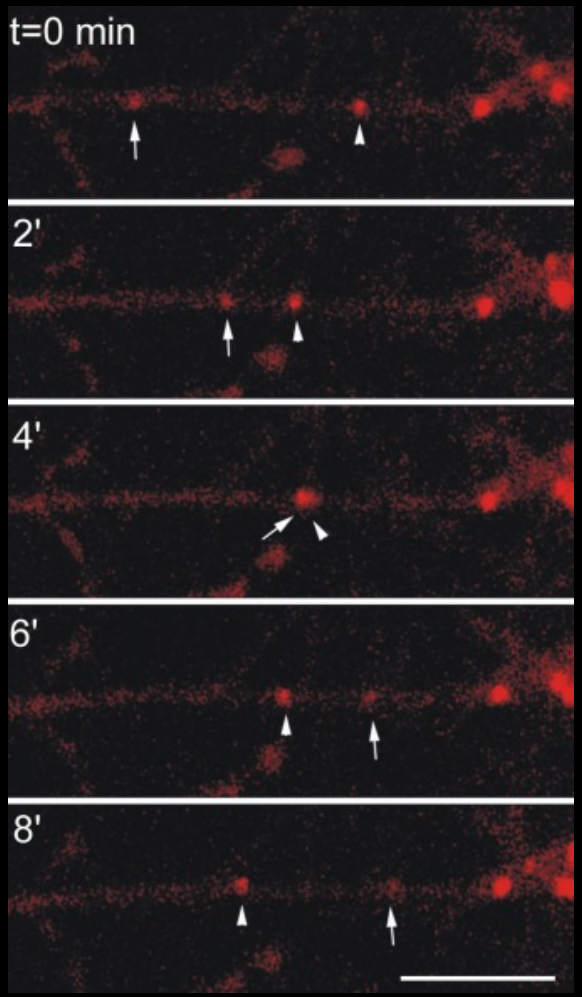


Washbourne et al. 2002

NR1 clusters are highly mobile in dendrites of cortical neurons



NR1-EGFP



NR1-DsRed

**average speed
= 6 μm/min

Washbourne et al. 2002

Recruitment of other synaptic proteins to sites of NMDAR recruitment

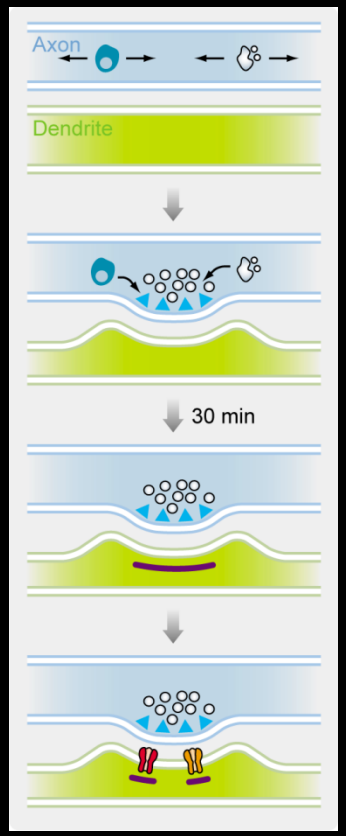
-using retrospective immunostaining

(1) Synaptic vesicles: similar time-course as NMDARs (within 7 min)

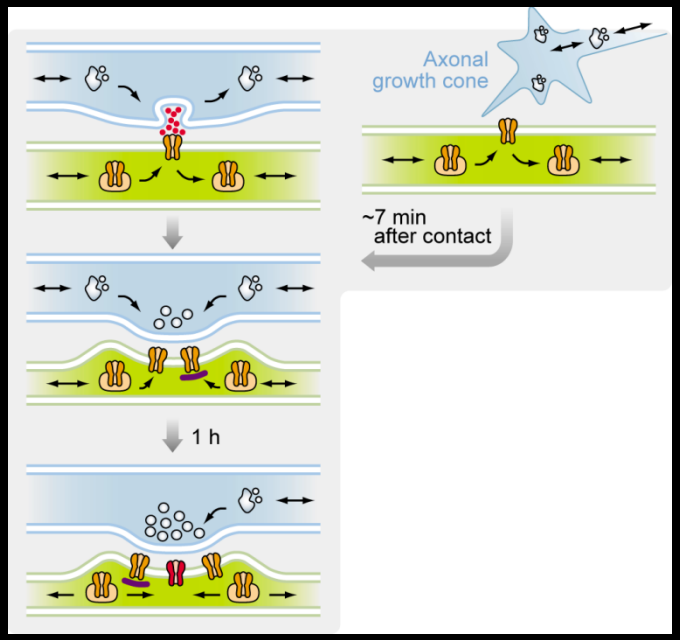
(2) AMPARs: never present within 1 hour of NMDAR recruitment, but always present after 1 hour (McAllister) or weeks later (Ziv)

(3) PSD-95: variable time-course

Current views of the hierarchy and time-course of CNS synaptogenesis

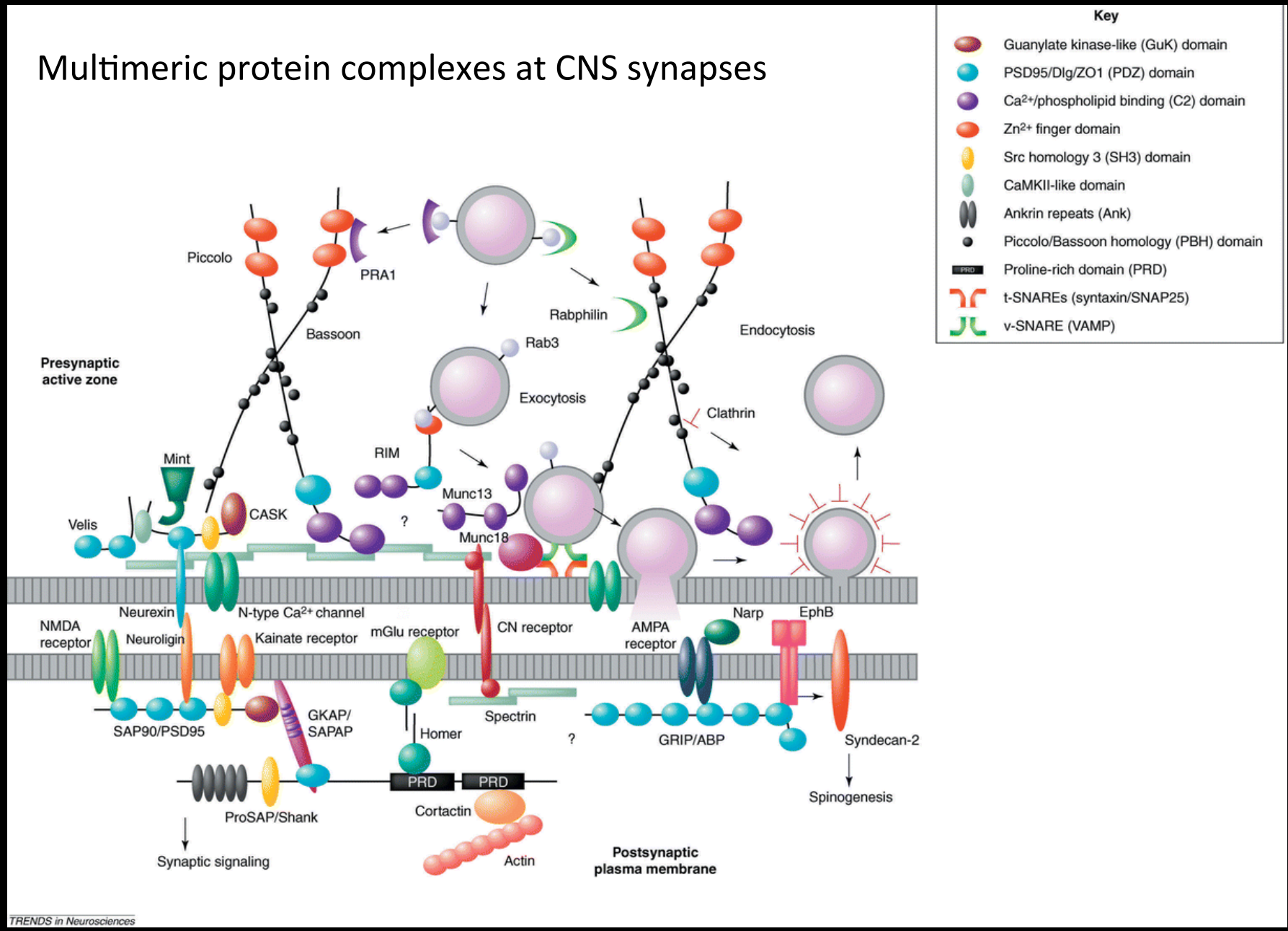


Garner and Craig



McAllister

Multimeric protein complexes at CNS synapses



Garner 2002

Molecules that induce and/or regulate synapse formation

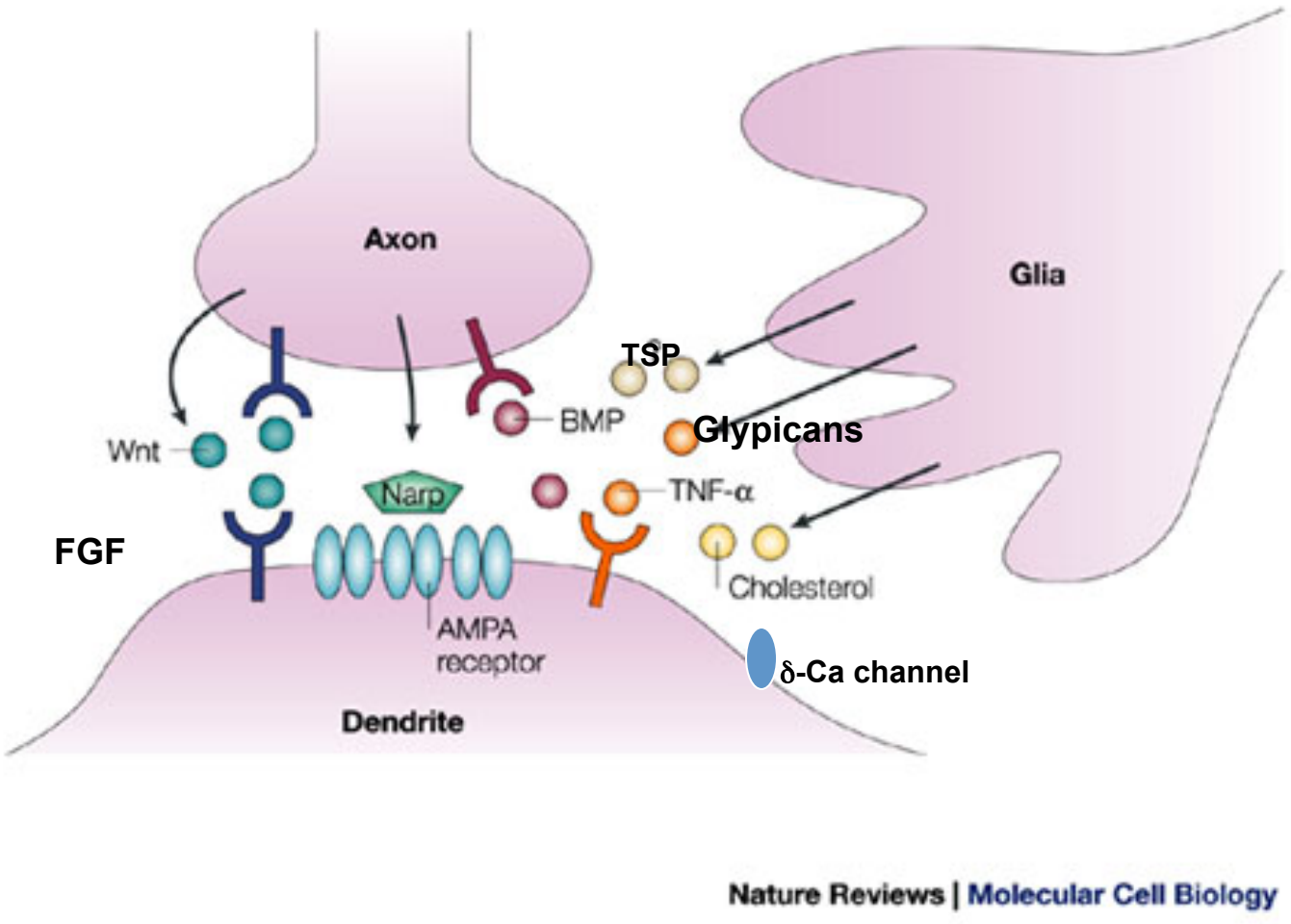
(1) Diffusible molecules
- (1) Narp
- (2) Wnt
- (3) BMP (Drosophila: homeostasis)
- (2) Neurotrophins ?
- (3) Neurotransmitter ??

(2) Glia
- (1) Diffusible
- (2) Contact-mediated
- (3) Temporary synaptic target

(3) Trans-synaptic adhesion molecules
- (1) Cadherins
- (2) Neuroligin/neurexin
- (3) SynCAM
- (4) ephrin B/EphB
- (5) NCAM
- (6) LRRTMs

**Important distinction between instructive versus permissive effects

Secreted factors in synapse development



Sheng review, Nat. Rev. Mol. Cell Biol. 2003

Diffusible molecules: Neurotransmitters

**not likely to be required for synapse formation as synapses can form in the absence of SV release

-Munc-13 KO (Varoqueaux et al. 2002)

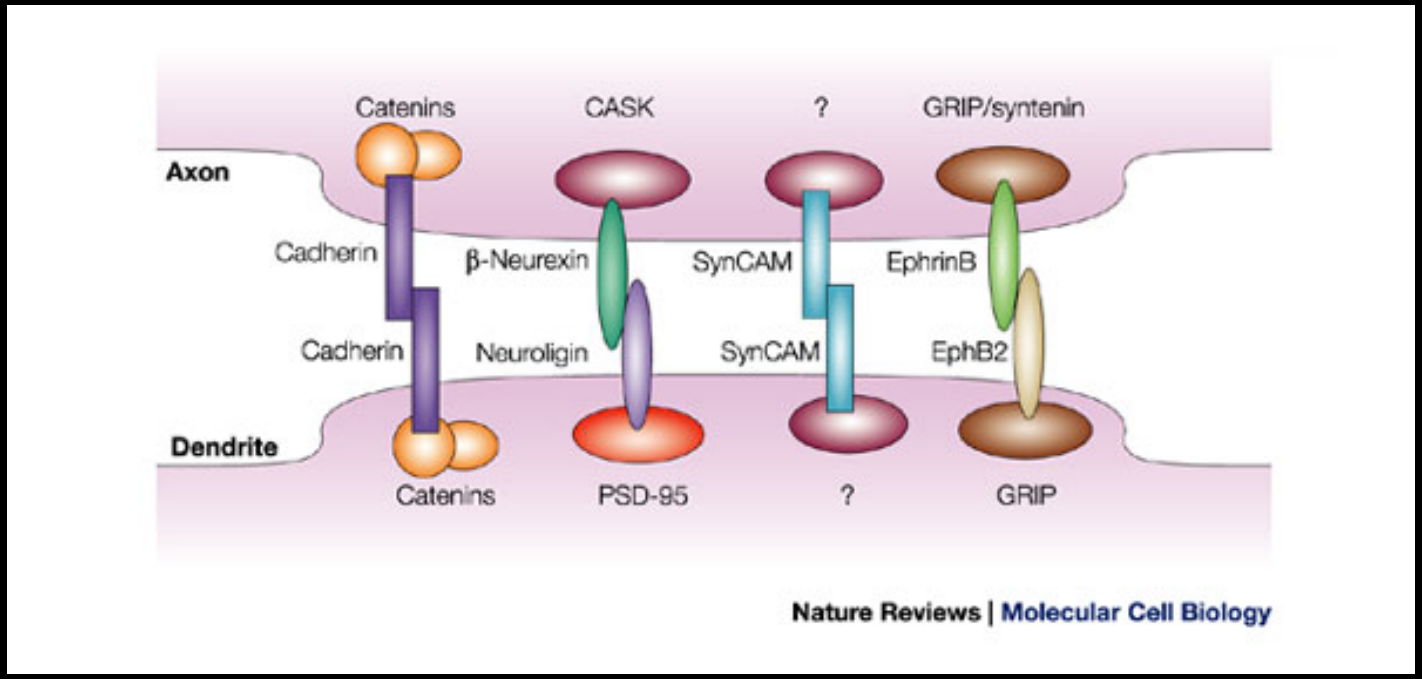
-Munc-18 KO (Verhage et al. 2000)

**activity may still play a role in modulating filopodial dynamics and thus, the likelihood of synapse formation or the location of synapse formation

Diffusible molecules: Neurotrophins

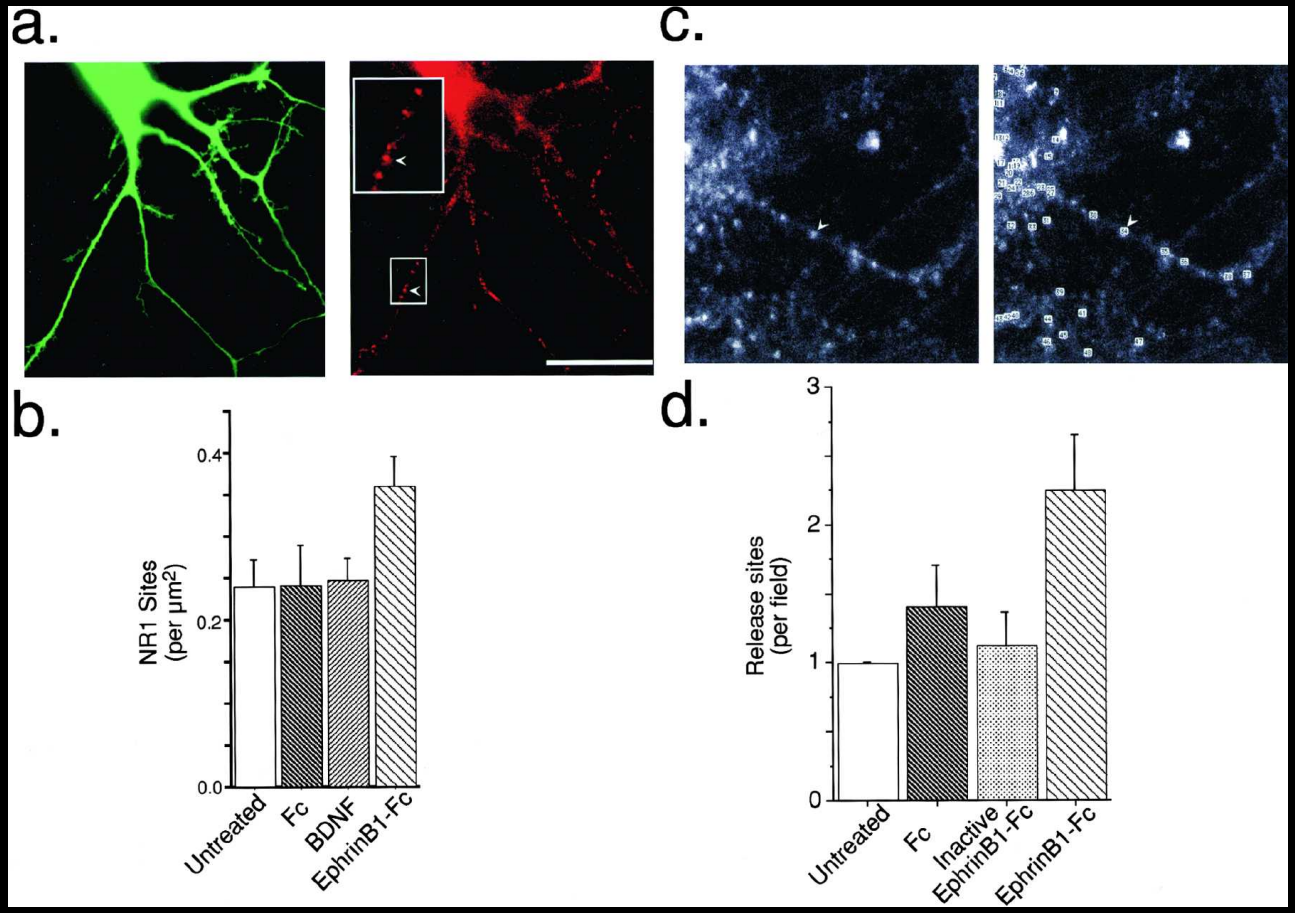
(1) BDNF is thought to be released at synapses and TrkB can be found at synapses
(2) BDNF increases synaptic transmission and the number of docked SVs
(3) BDNF increases the number of SV clusters in retinal axons in Xenopus in vivo (Cohen-Cory 2001)
(4) BDNF increases excitatory synapse density on hippocampal neurons indirectly by first increasing the density of inhibitory synapses (Elmariah et al. 2004)
(5) Conditional knockouts suggest that BDNF influences glutamatergic synapse formation by acting both pre- and postsynaptically
(6) FGF has recently been shown to play a major role in synapse maturation/formation in the hippocampus (H. Umemori) – different FGF receptors may play distinct roles at excitatory or inhibitory synapses.

Trans-synaptic protein interactions that have been implicated in synaptic contact/adhesion and synapse development



Sheng review, Nat. Rev. Mol. Cell Biol. 2003

Trans-synaptic molecules: EphrinBs/ EphB receptors



-EphB2 interacts physically with NMDARs and enhances their channel activity, and induces their clustering

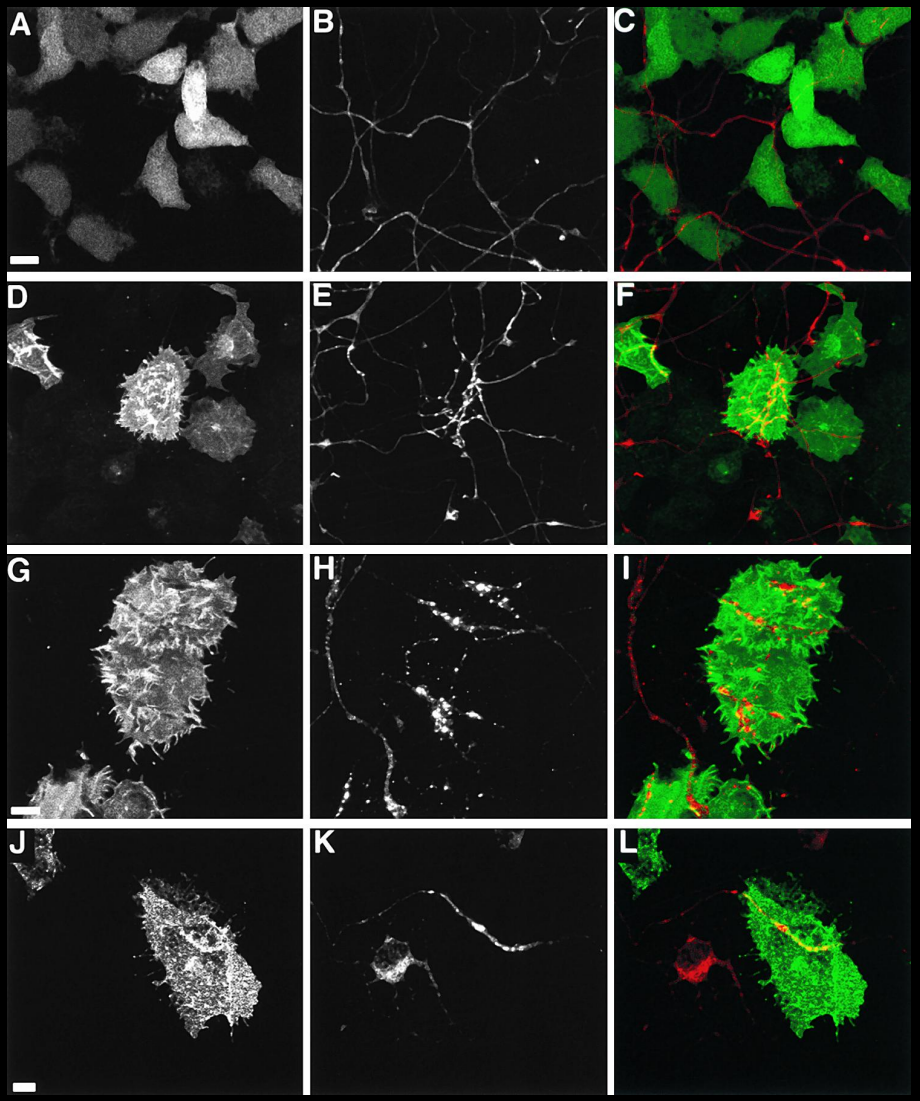
**EphB2-KO mice show deficits in activity-dependent plasticity but have normal synapse density and structure

Dalva et al. 2000

Trans-synaptic molecules: Neurexins/neuroligin

Neuroligins = postsynaptic membrane proteins that bind thru their extracellular domains to presynaptic beta-neurexins
-cytoplasmic domain of neuroligin binds to PSD-95; and neurexin binds to CASK presynaptically

**one of ~5 molecules shown to be sufficient to induce presynaptic differentiation

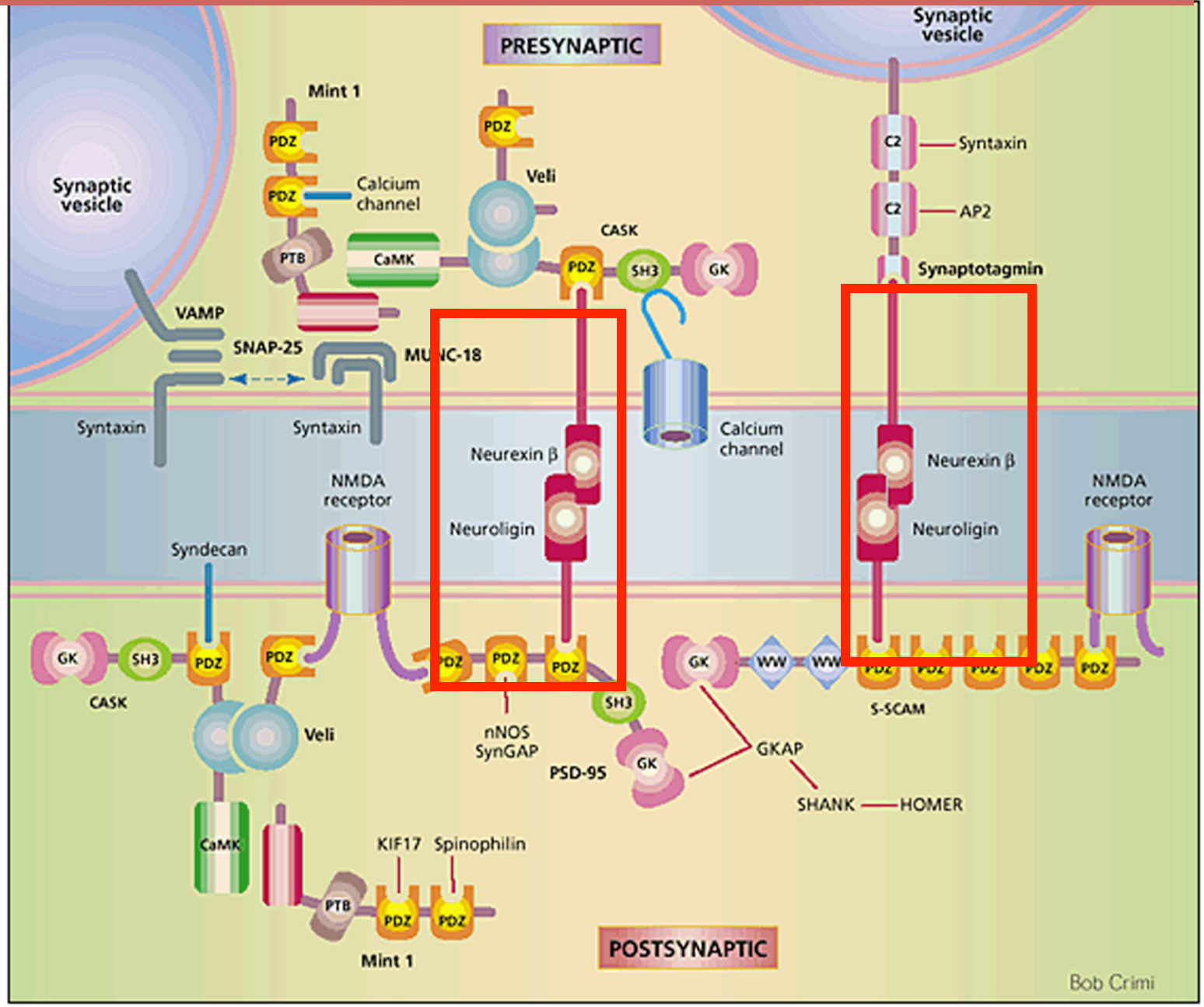


Neuroligin Expression in HEK293 Cells Causes Accumulation of Synapsin in Axons

-soluble beta-neurexin recombinant protein inhibited synapse formation in culture

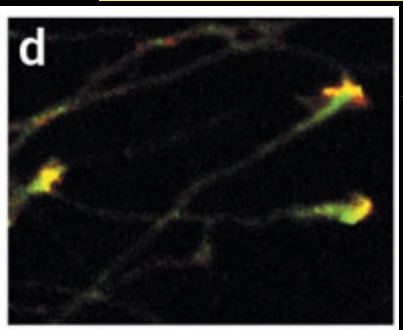
Scheiffele et al. 2000

Neurligins and Neurexins are Heterotypic Cell Adhesion Molecules
Specifically expressed at Glutamatergic and GABAergic synapses

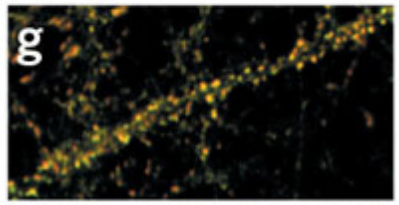


Neurexinß is at presynaptic membranes; Neuroligin in postsynaptic membranes induces presynaptic differentiation.

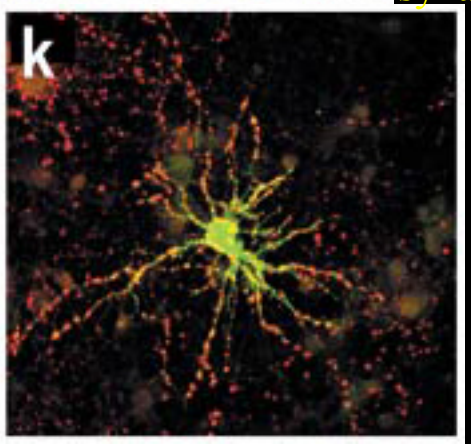
Neurexin is Presynaptic:



Pontine explant
Neurexin (green) goes into growth cones (red = filamentous axin)

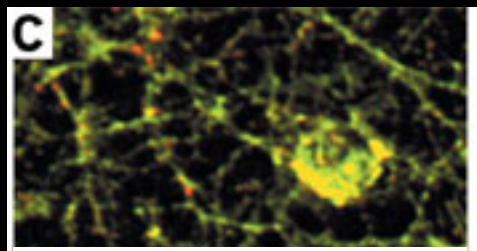


Dissociated hippocampus
Neurexin (green) at synapses (red = synaptobrevin)

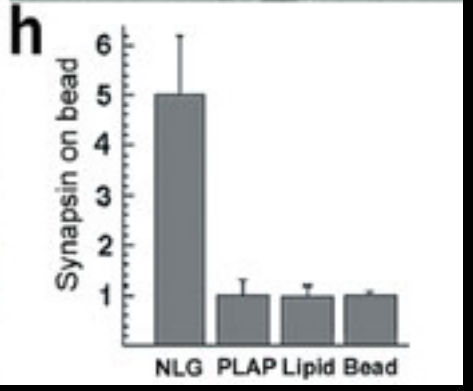


hippocampal neurons in culture
Neurexin (green) at synapses (red = synapsin)

Postsynaptic Neuroligin Induces:



Presynaptic Neurexin



Neuroligin-AChE-ectodomain/membrane coated beads induce presynaptic differentiation in neurites of co-cultured hippocampal or cerebellar neurons

Dean et al. (2003) Nat Neurosci. *6*, 708-16

What role do neuroligins and neurexins play at excitatory and inhibitory synapses?

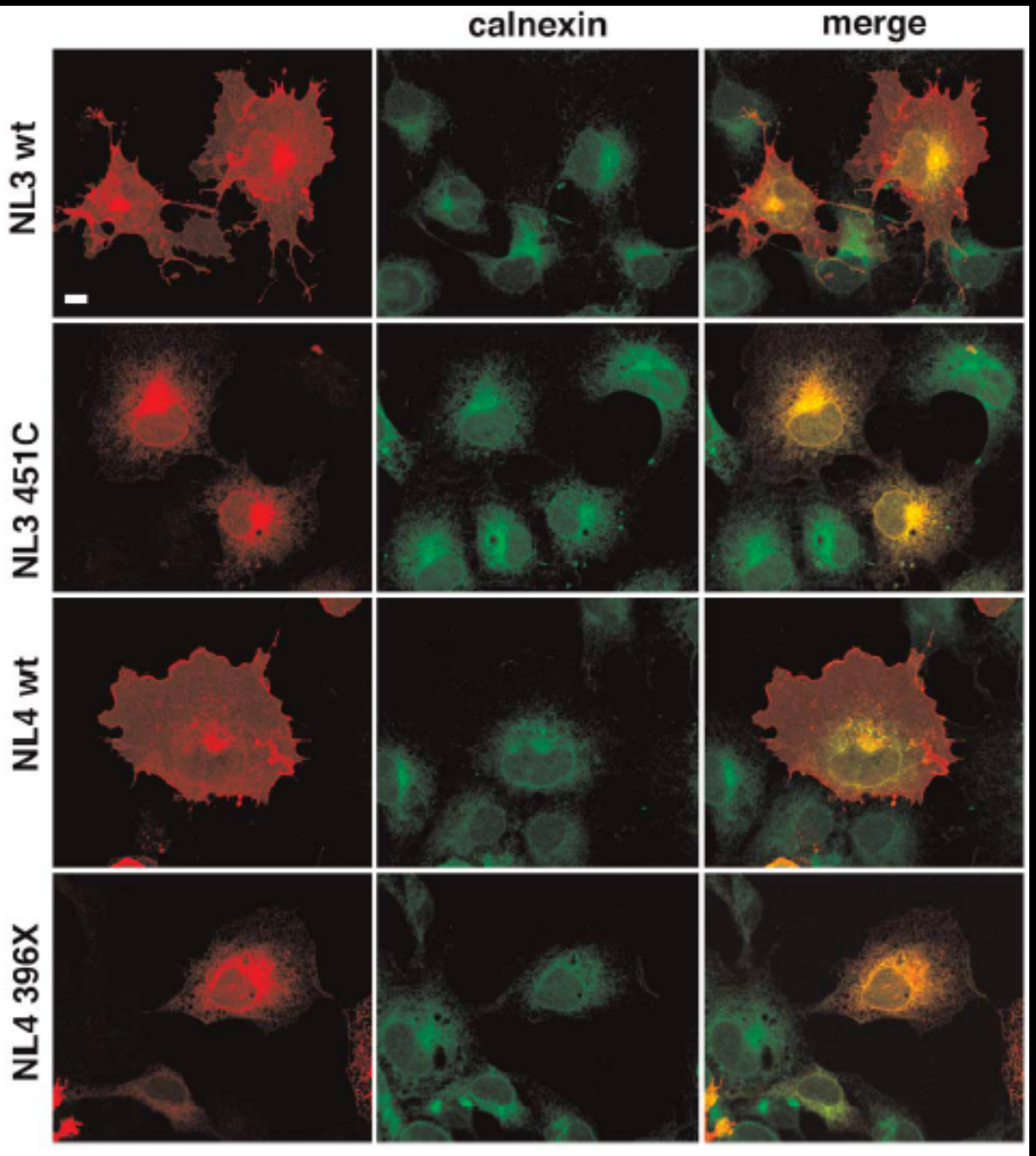
Neuroligin and Autism

Autism is a devastating disease.

- Disrupts behavioral development in young kids
- Affects 1 in 200-1000 kids
- Incurable, "palliative" treatment only modestly effective
- For a long time nobody has had any clue what causes it
- At one time "bad mothering" was blamed (unfortunately by some Psychiatrists)
- Neuropathology all over the map – mostly mild – little consensus among researchers.

Hint: This stuff really matters to basic science. It's *why* taxpayers and foundations support biological research.

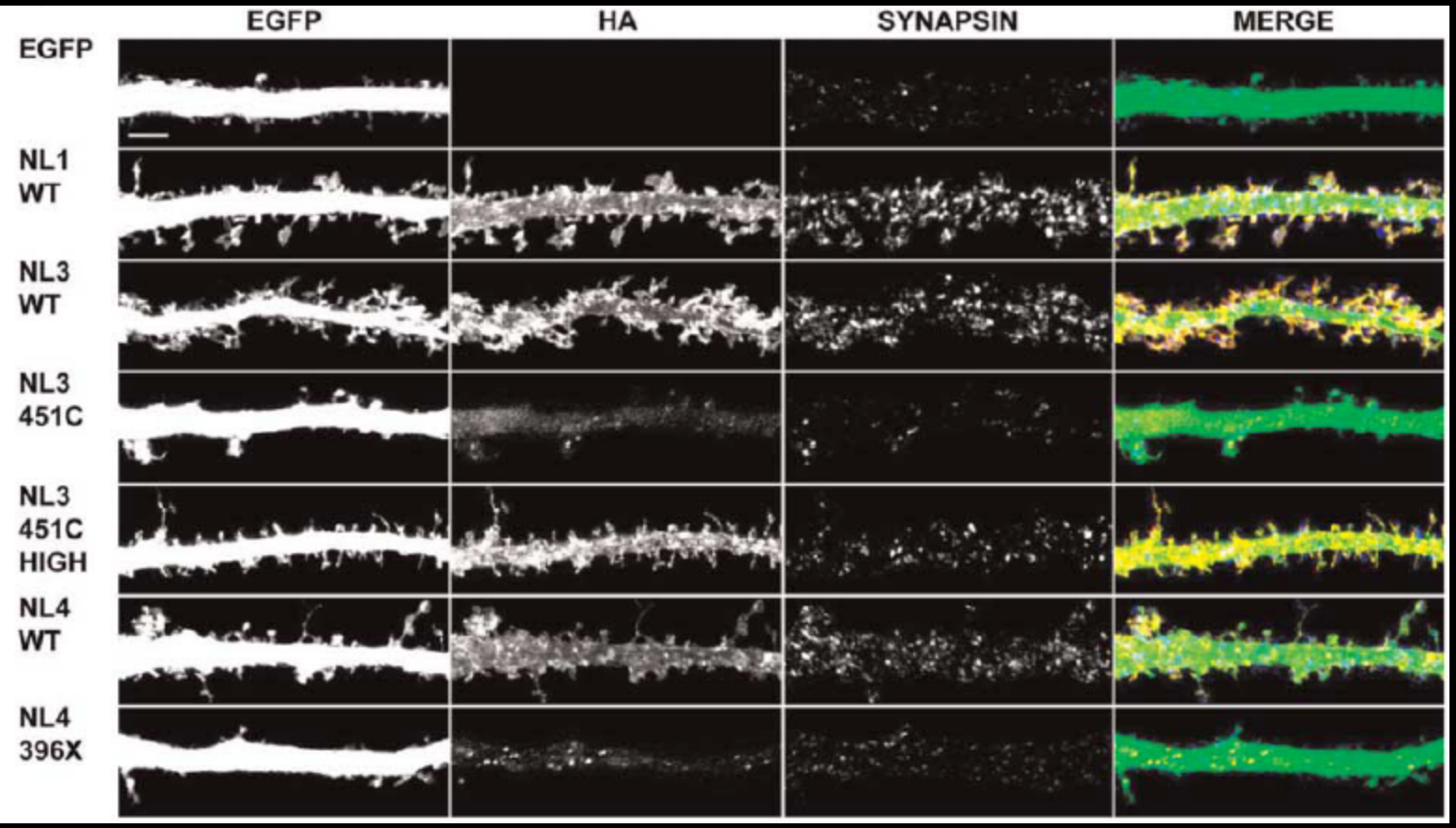
Mutant forms of NLGN associated with autism are inefficiently transported to the cell surface.



COS cells transfected with wildtype and mutant forms of Neuroligins. Calnexin marks the ER.

– Chih et al (2004) Hum Mol Gen *13*, 1471-7

Mutant forms of NLGN found in autism do not promote synaptogenesis when expressed in hippocampal neurons in primary culture.



Dendrites of dissociated rat hippocampal cells transfected with HA-tagged forms of NLGN genes. Synapsin marks presynaptic membrane (clusters of presynaptic vesicles).

Chih et al (2004) Hum Mol Gen *13*, 1471-7

Neuroligin/Autism

Caveats:

Constitutive Neuroligin KO mice have "normal" synapse ***formation*** (but abnormal synapse ***electrophysiology.*** (T. Sudhof, personal communication-some of this has been published, there are slight effects on synapse #, but subtle (animals don't live too long after birth)).

It is plausible that some molecules involved in synapse function can be made to "drive" synaptogenesis *in vitro*, but don't necessarily control this developmental process *in vivo*.

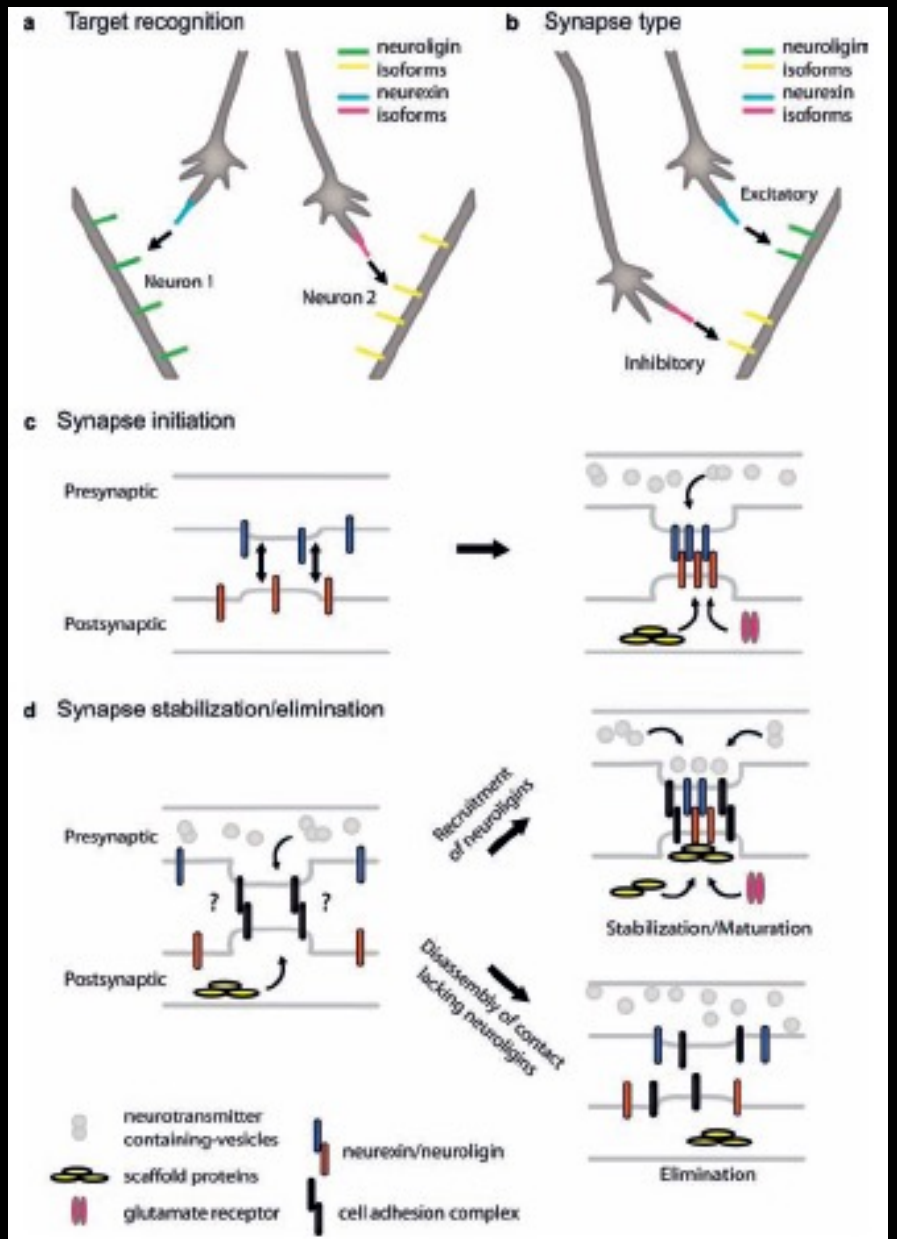
Neuroligin/Autism

- Other Caveats:
  - Human NLGN mutations are not *disease-specific:*
    - In a single family, some affected individuals have autism, others have mental retardation *without* autistic features.
    - Variable *expressivity* of phenotype resulting from a single mutation.
  - The autism "phenotype" is certainly not *gene-specific,* and may not even be *mechanism-specific* at a cell biological level.
    - NLGN mutations only account for a small minority of cases.
    - Many different genes involved in synaptogenesis or synapse function could have the same gross behavioral consequences.
    - There may be other cell biological ways to produce "autistic behavior" (e.g. defects in neuronal migration, axon pathfinding, etc).
  - Regardless: The genetic connection to Neuroligin suggests that synapse development and/or synapse function play a central role. This represents huge progress in understanding this disorder.

Modified View of the role of Nrxn and Nrlgn

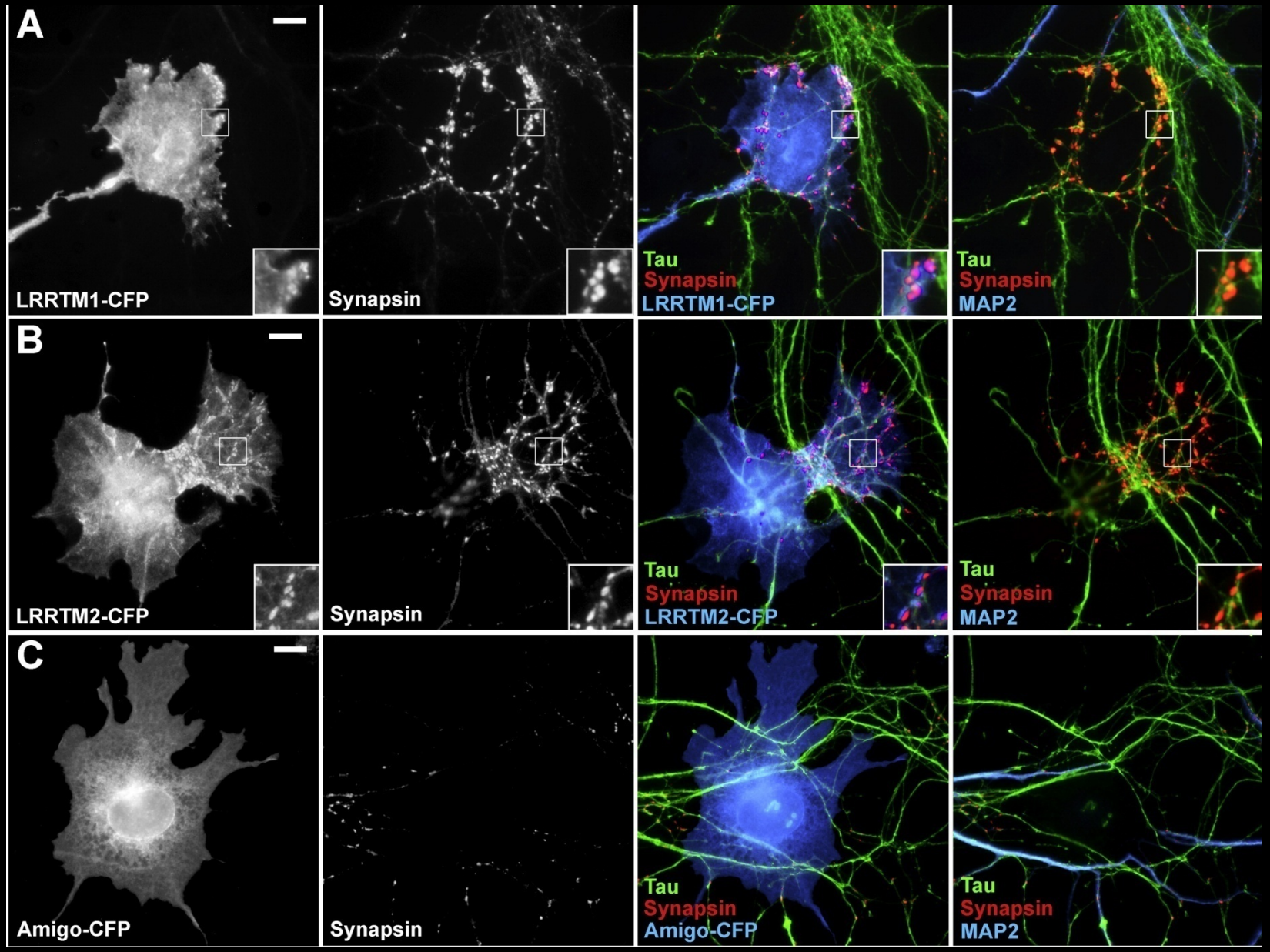
Promote identity of synapse – inhibitory vs. excitatory (nlg2 vs nlg1)

1) Help maintain synaptic structure
2) Functional role at synapse

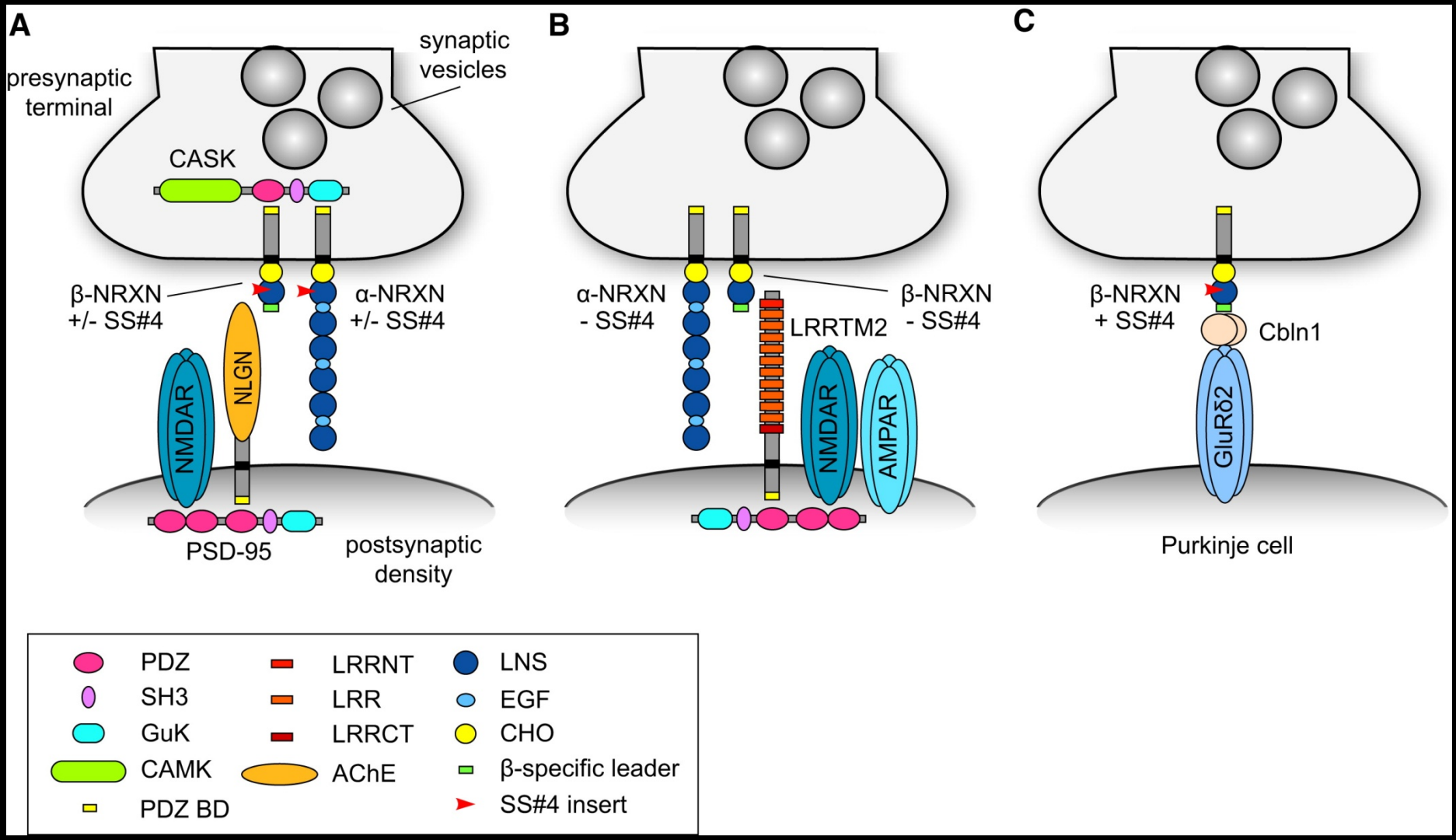


Huseini et al.

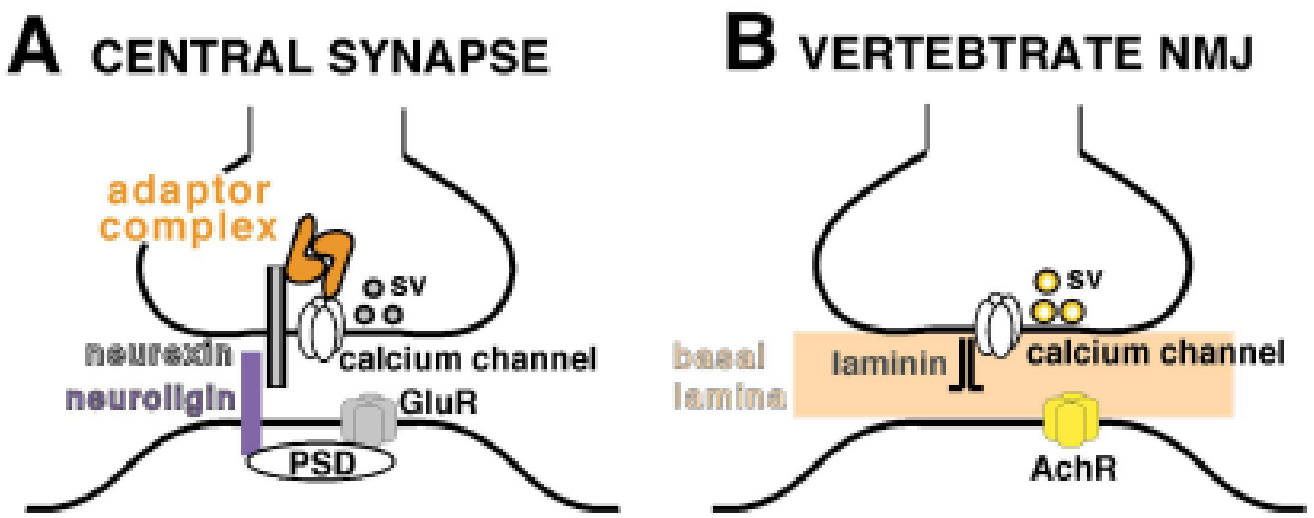
LRRTM proteins induce presynaptic puncta



The NRXN/NLGN Centric View of Synapse Formation

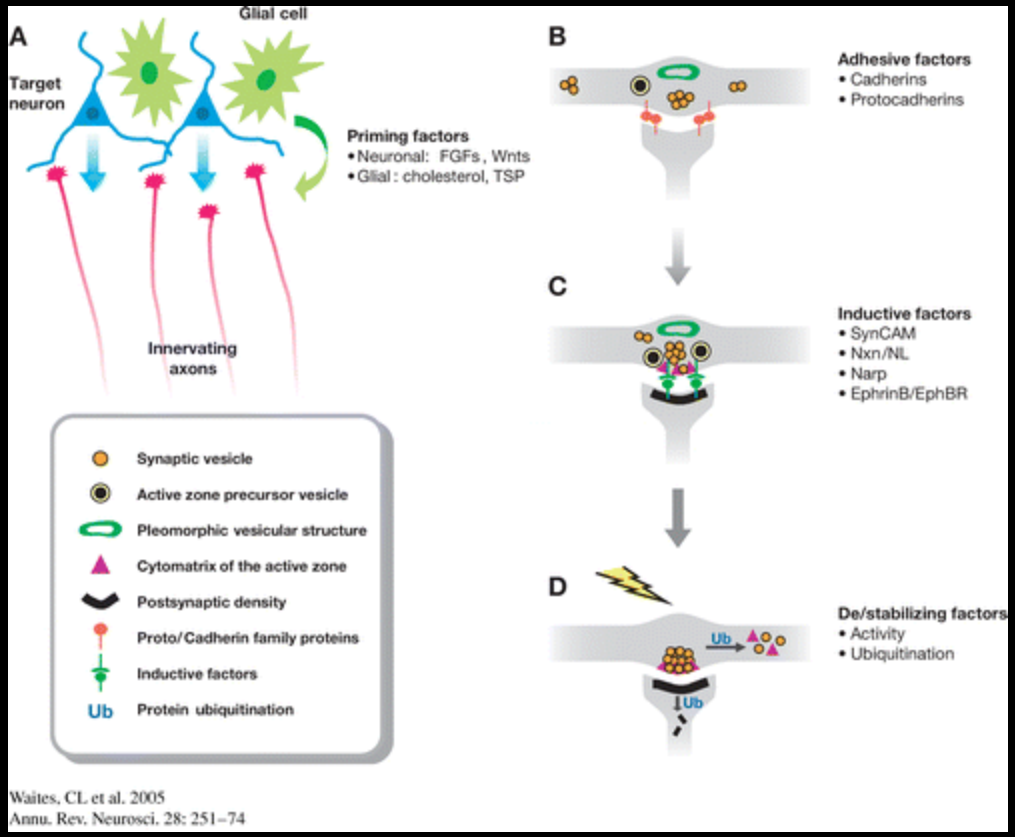


Trans-synaptic scaffolding

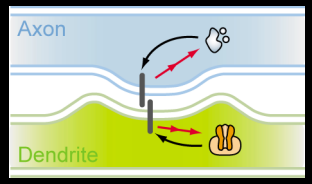


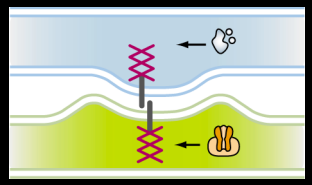
It is common that molecules that are sufficient for synapse formation are not necessary in vivo. Why?

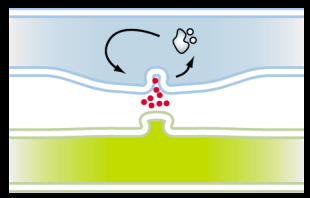
Current mechanisms of CNS synaptogenesis

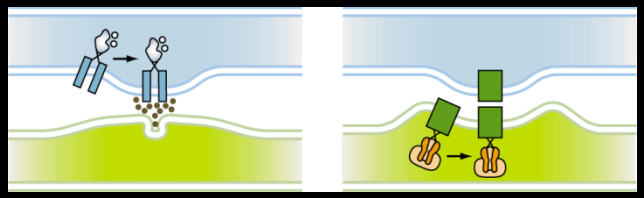


Waites, CL et al. 2005
Annu. Rev. Neurosci. 28: 251–74









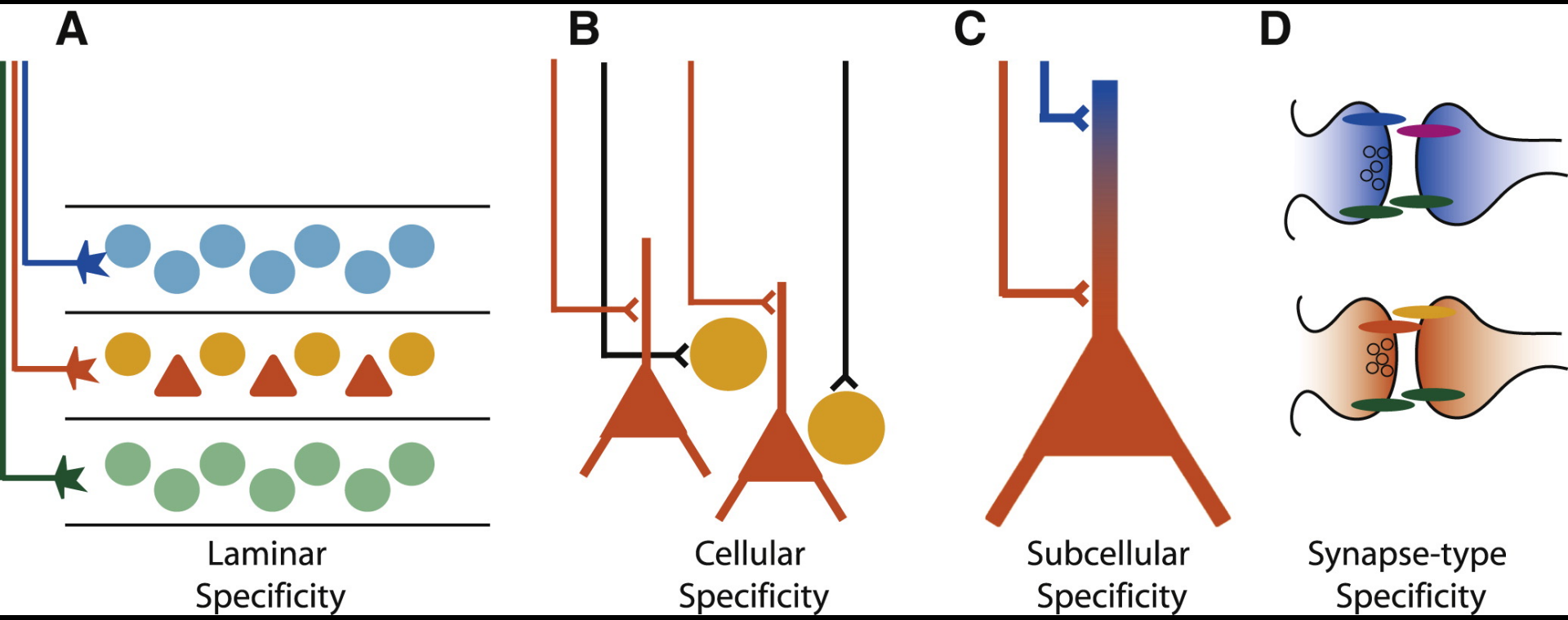
Synapse maturation and growth:

-following initial assembly, the synapse expands in size, alters its morphology and grows in strength
**for example, the mean # of SVs per synapse increases 4x during the first month of postnatal development

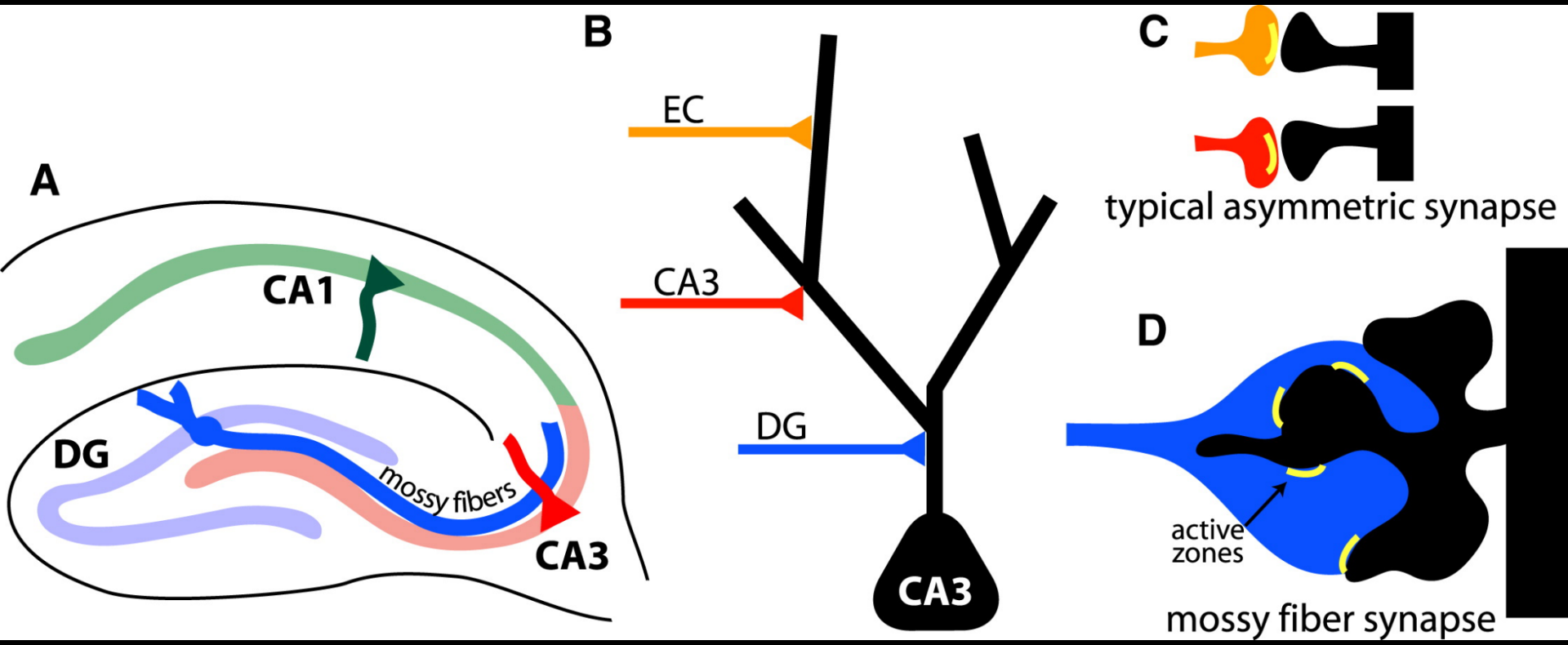
**AMPAfication of silent synapses
?? Changes in scaffolding molecules

-CaMKII promotes synapse maturation

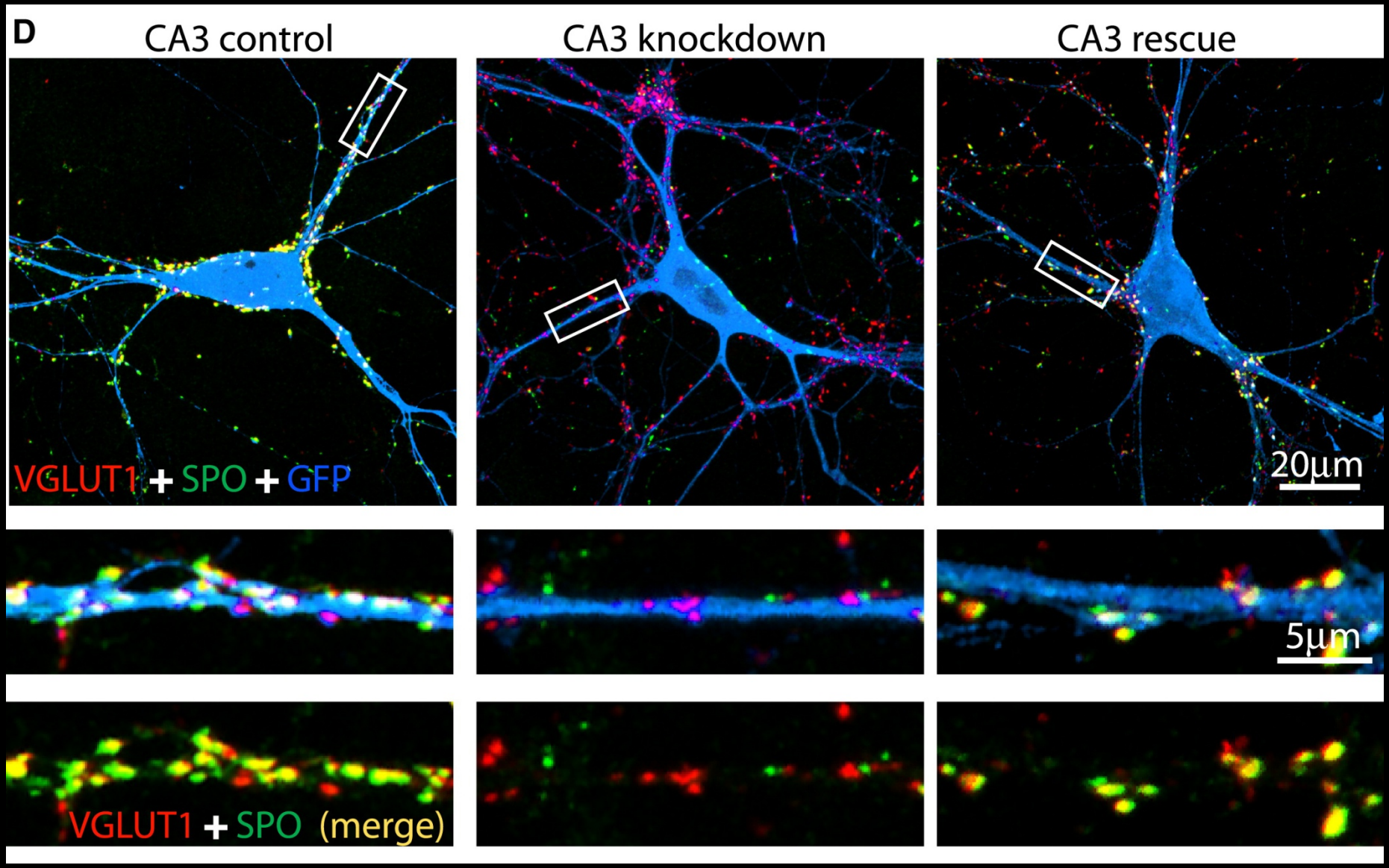
Synaptic Specificity



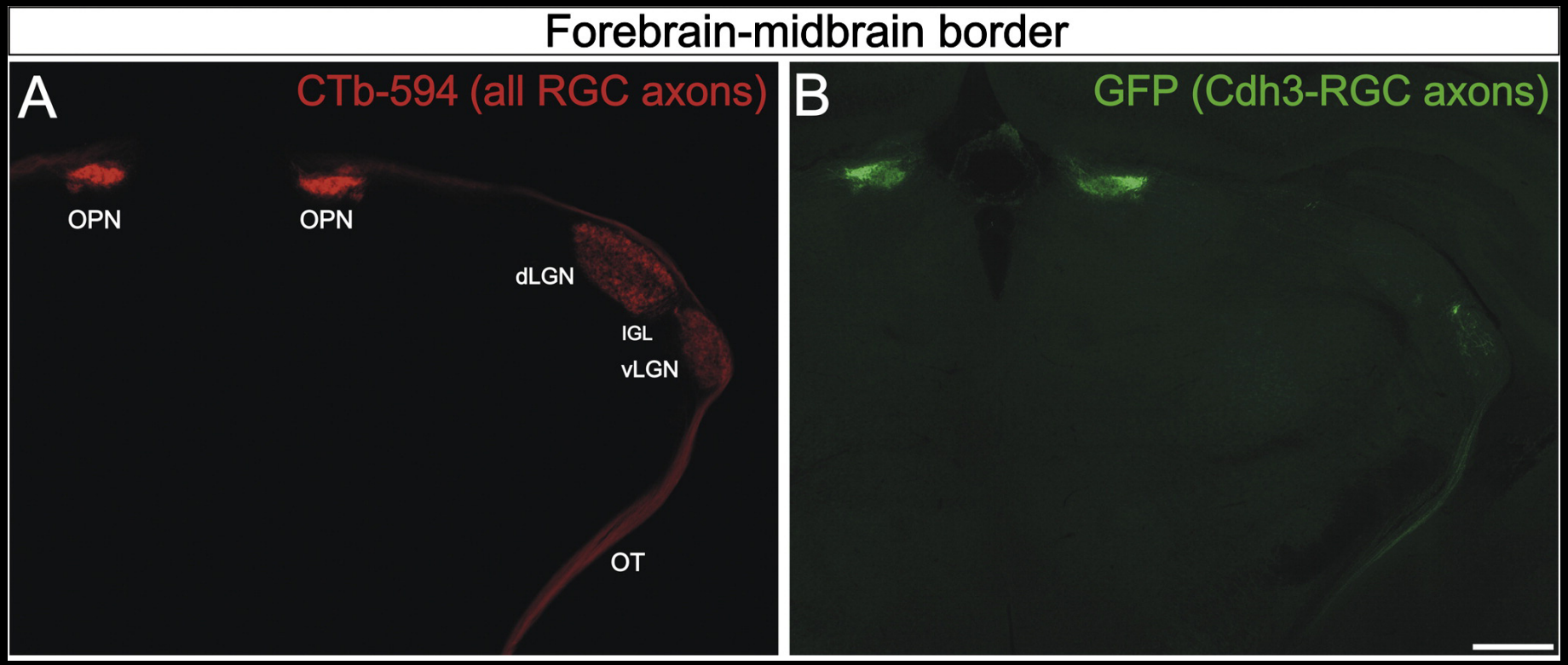
Types of Specificity in Hippocampus



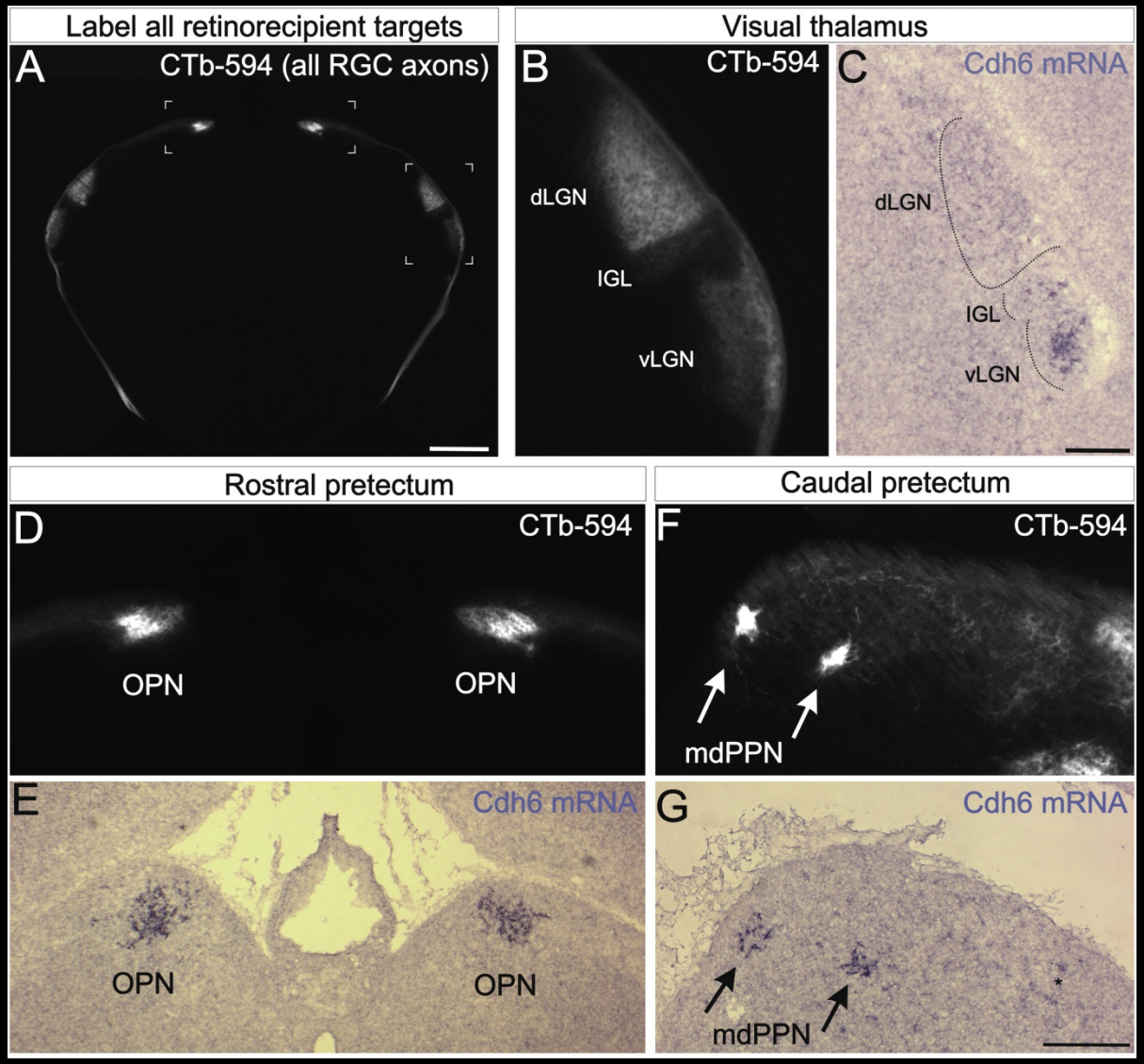
Synaptic Specificity – Cadherin 9



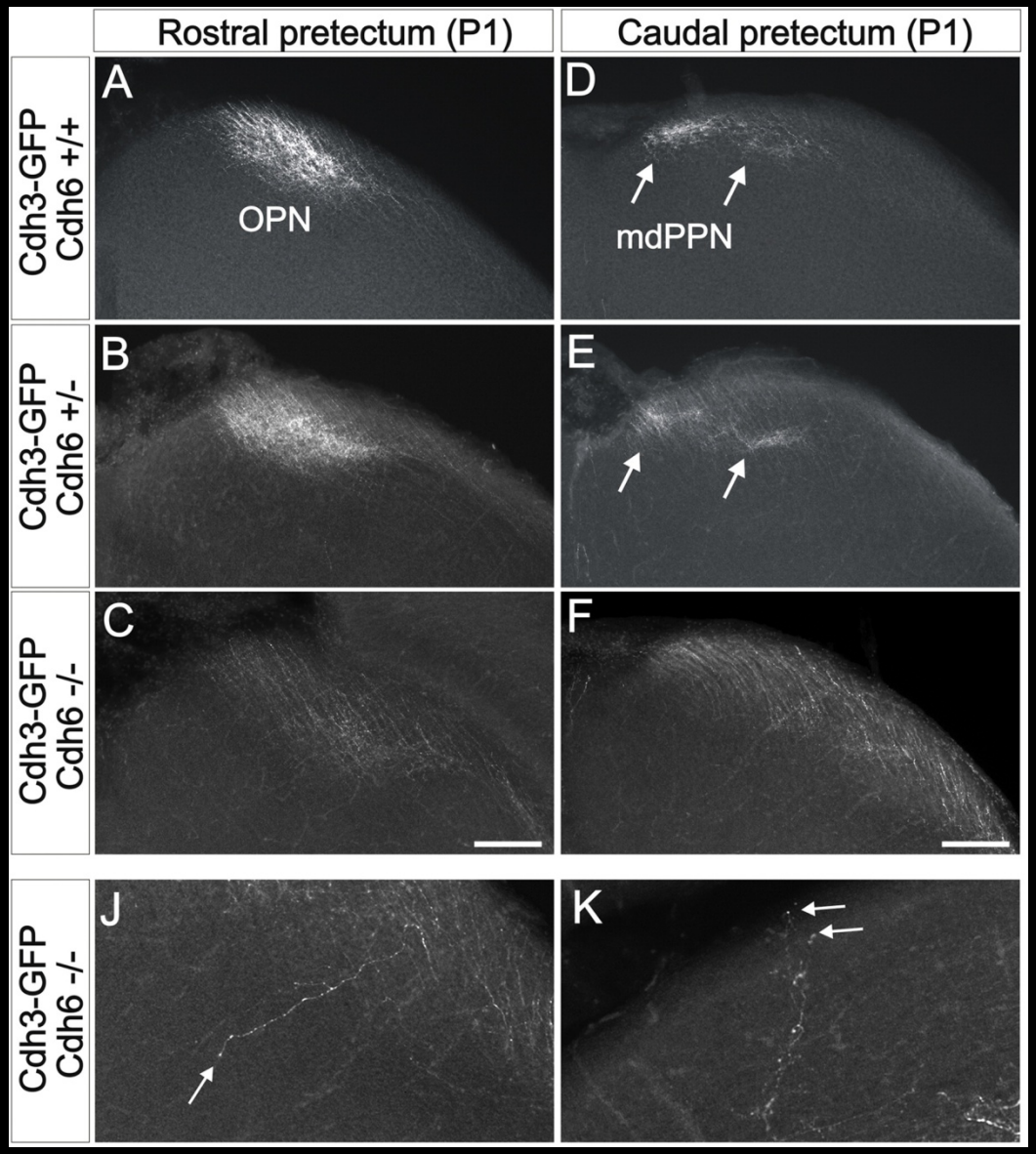
Synaptic Specificity – Cadherins and RGCs target specificity



Synaptic Specificity –Cad 6 expressed in targets



Synaptic Specificity – Cad 6 KO has targeting defects

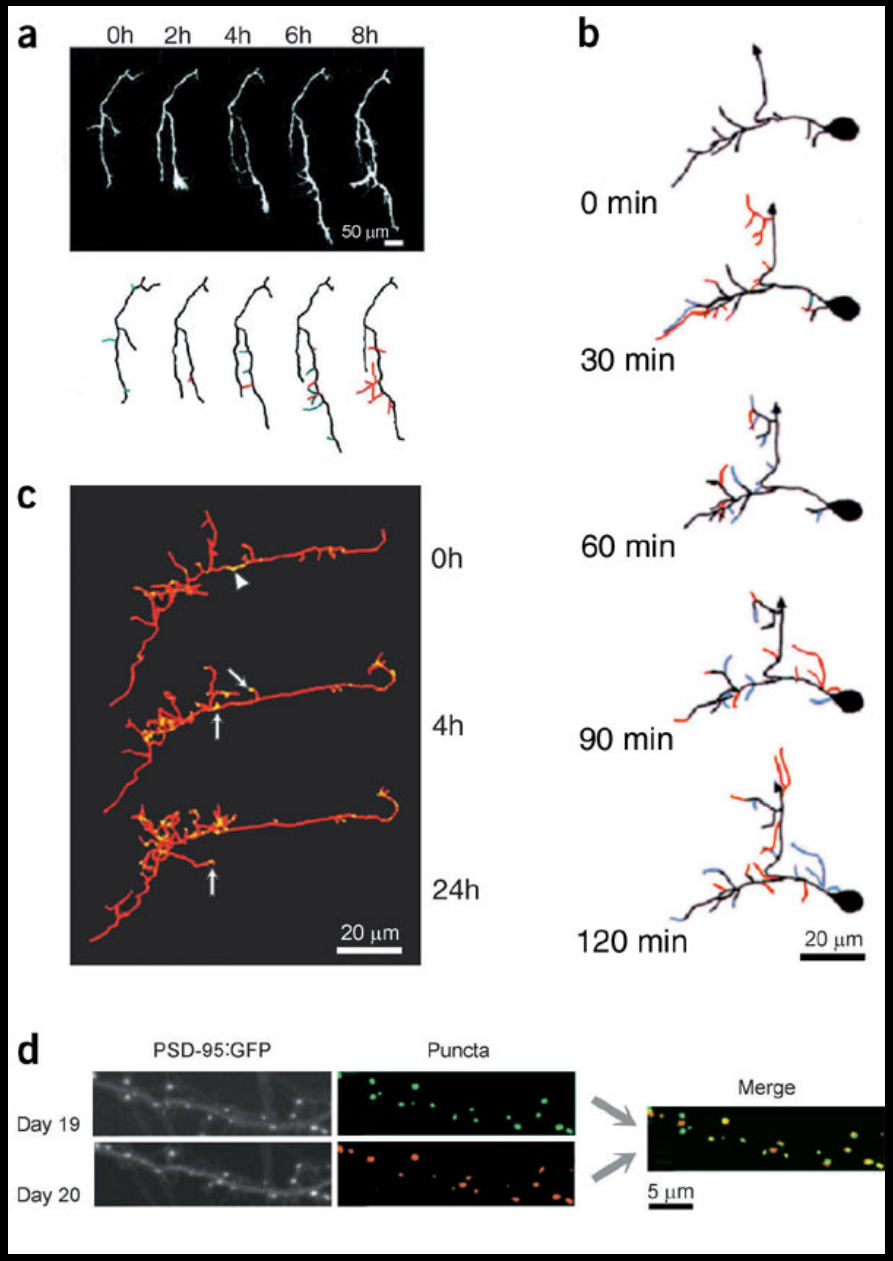


CNS Synapse Elimination?

- **In Mammalian CNS development too many synapses are initially formed in many brain regions**

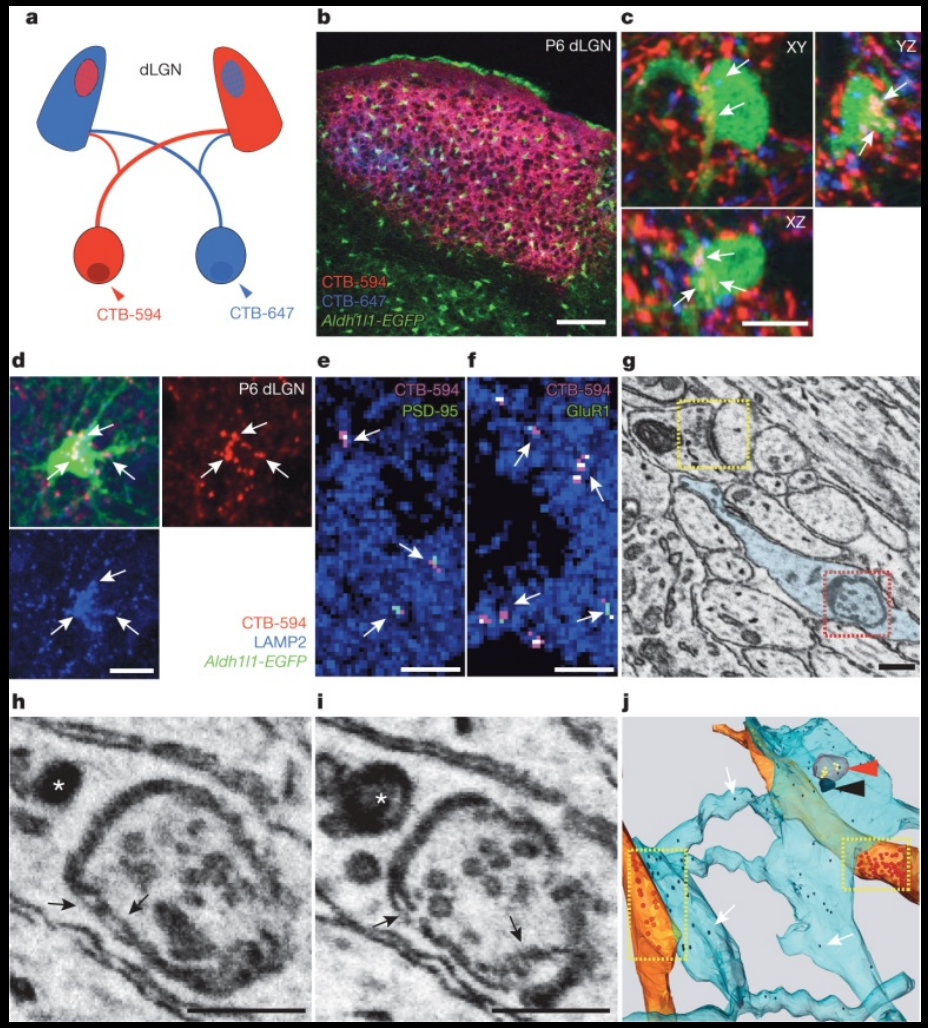
- **Correct synaptic partners are also not always initially found in mammalian CNS**

- **Synapse elimination is a prevalent but poorly understood process for generating the correct number and targets**



Concurrent formation and elimination of synapses?

Glia also play a role in CNS synapse elimination?



W-S Chung *et al. Nature* **000**, 1-7 (2013) doi:10.1038/nature12776

nature

Fundamental unanswered questions:

(1) What are the specific signaling molecules and their signaling pathways that control synapse formation?
(2) How is pre- and postsynaptic alignment and growth coordinated?
(3) How are excitatory vs. inhibitory synapses regulated (neurexin-neuroligin)?
(4) How do neurons control the density of synapses made on their dendrites (BDNF – MEF2; syncam)?
(5) How is synapse formation coordinated with dendrite extension?
(6) How are synapses specified to particular neurons and subcellular positions (Cads, L1 adhesion and inhibitory synapses)?
(7) How are synapses strengthened and stabilized or weakened and eliminated?