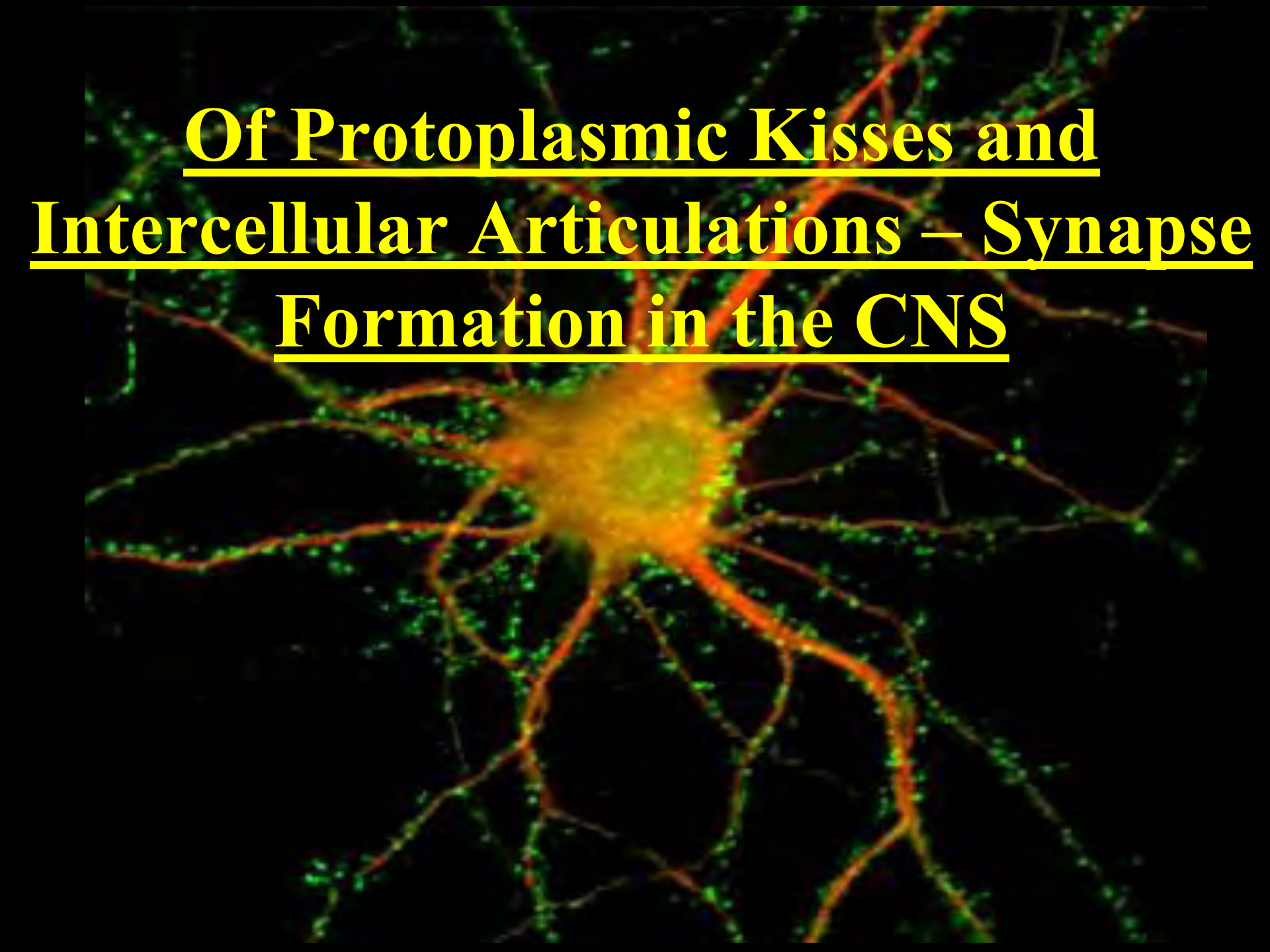
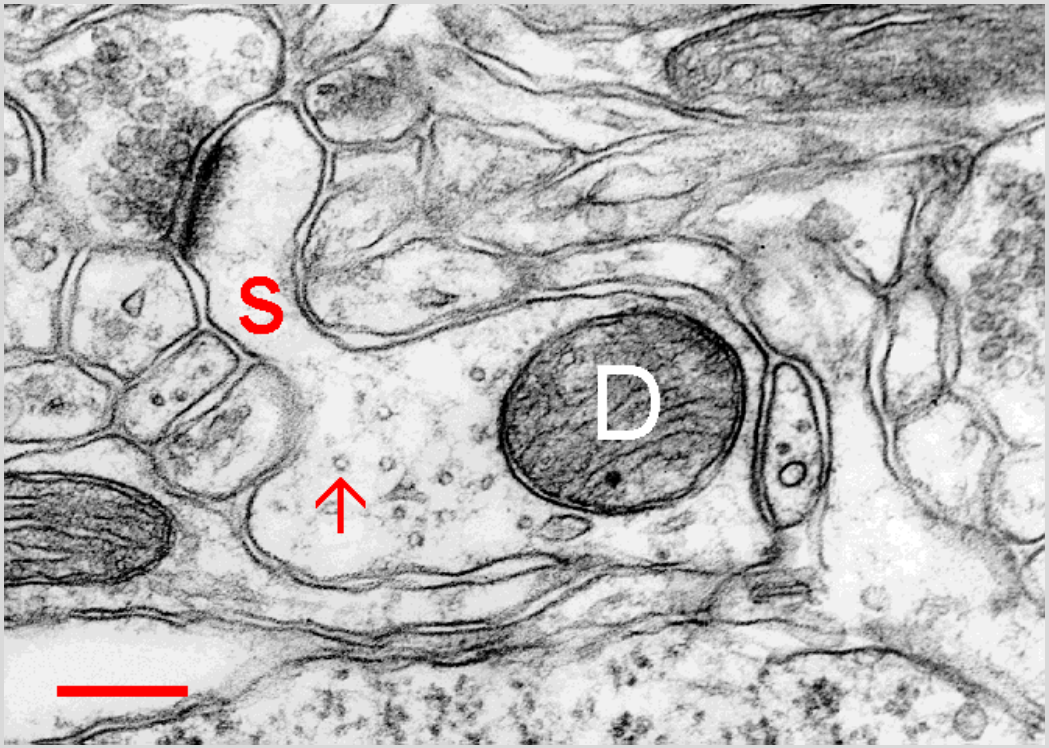
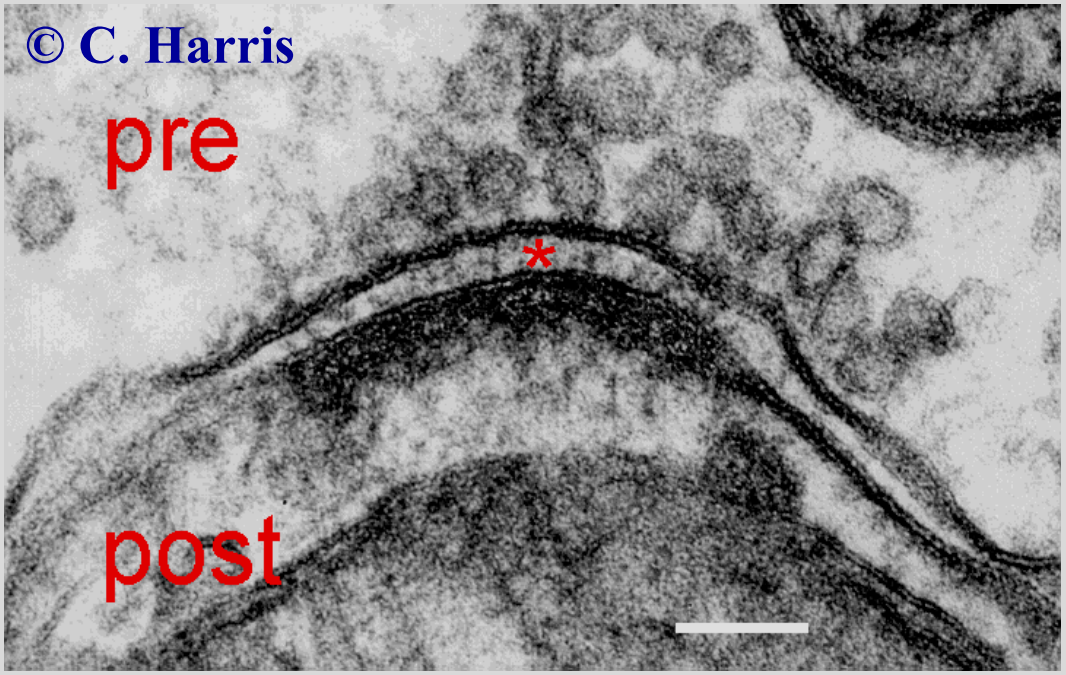

Of Protoplasmic Kisses and
Intercellular Articulations – Synapse
Formation in the CNS

„[Synapses,] ... those protoplasmic kisses, the intercellular articulations, ... seem to constitute the final ecstasy of an epic love story.“

- **Santiago Ramon y Cajal**





- Communication sites between 2 cells
- The synaptic cleft is only about 20 nm wide
- The synaptic cleft is **NOT** empty
- Distance is very precisely regulated
- Distance between cells at non-synaptic sites varies considerably more compared to synaptic distances

Why Study Synaptogenesis?

- Formation of synapses is important during development
- Active remodeling of synapses underlie memory and learning
- Many pharmaceuticals target synapses
- Several neuro-psychiatric diseases are **synaptopathies**, including Alzheimers disease, epilepsies, schizophrenia and autism are caused by altered functions of synapse-associated proteins

Table 1 | **Synaptic proteins with genetic defects that have been associated with developmental psychiatric disorders**

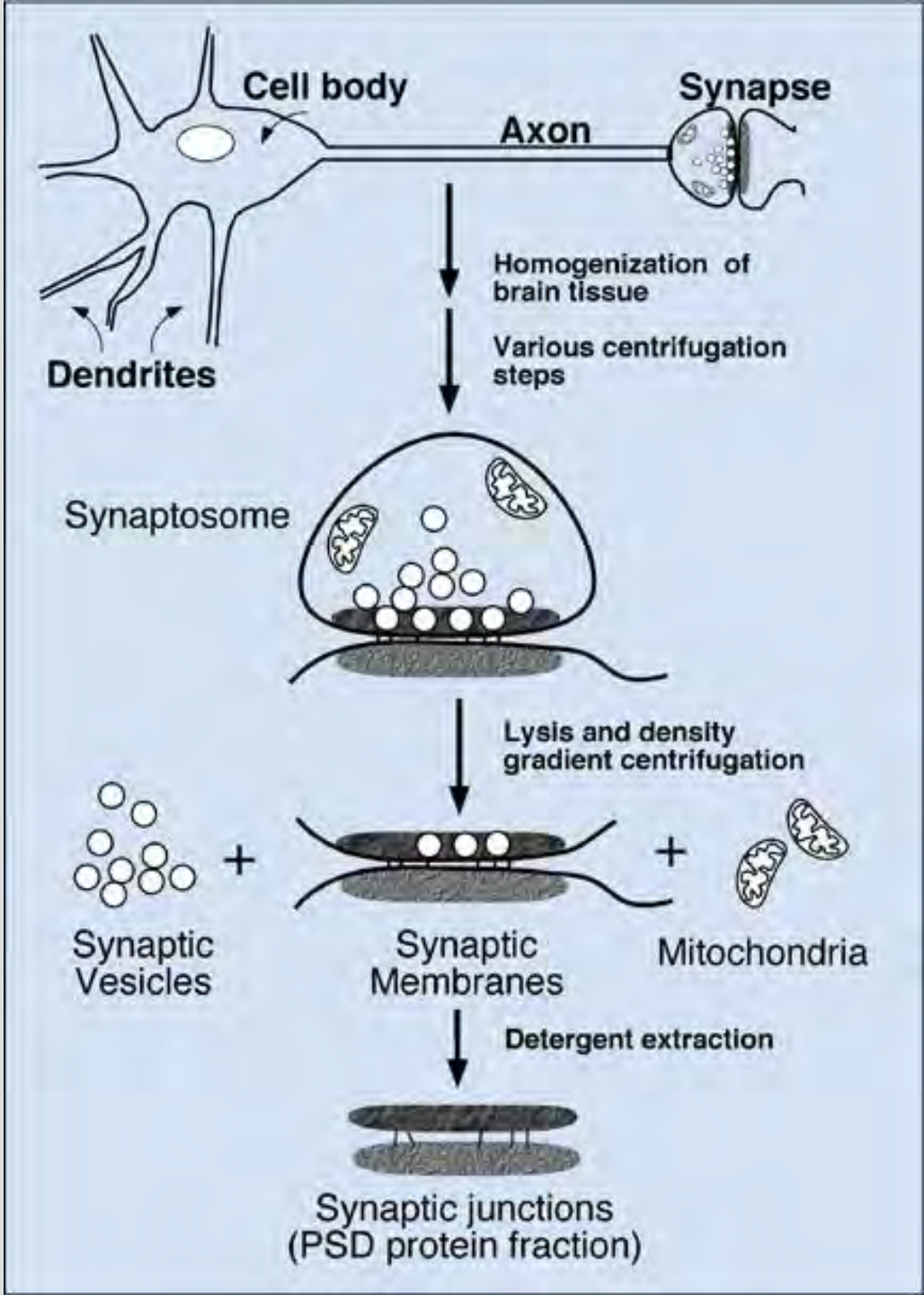
Protein	Function	Synaptic contribution	Disease
ARHGEF6	RAC GEF, regulation of actin cytoskeleton	Synapse formation and maturation	Intellectual disability
CYFIP1	Protein synthesis	Unknown	Fragile X syndrome
DISC1	Scaffold protein	Synapse formation and maturation	Schizophrenia
EPAC2	RAP GEF	Spine maturation	ASD
ERBB4	Receptor tyrosine kinase	Regulation of excitatory transmission	Schizophrenia
FMRP	Protein synthesis	Synapse stabilization	Fragile X syndrome
GABRB3, GABRA5, GABRG3	GABA receptor subunits	Excitation–inhibition balance	ASD
IL1RAPL1	Scaffold protein	Synapse formation	Intellectual disability
Kalirin	RAC GEF, regulation of actin cytoskeleton	Synapse formation and maturation	Schizophrenia, ASD
LIMK1	Protein kinase, actin skeleton	Spine maturation	Williams syndrome, intellectual disability
MINT2	Presynaptic adaptor protein	Neurosecretion	ASD, schizophrenia
Neuregulin 1	Trans-synaptic modulator of ERBB4	Regulation of excitatory transmission	Schizophrenia
Neurexin 1	Presynaptic adhesion molecule	Synapse stabilization	ASD
Neuroligin 3, neuroligin 4	Adhesion molecules	Synapse stabilization	ASD
Oligophrenin 1	RhoA GAP, regulation of receptor trafficking	Spine maturation	Intellectual disability
PAK3	Protein kinase, actin cytoskeleton	Synapse formation and stabilization	Intellectual disability
Protocadherins	Adhesion molecules	Unknown	ASD
PSD95	Scaffold protein	Synapse plasticity and stabilization	ASD, schizophrenia
PTEN	Tyrosine phosphatase, protein synthesis	Synapse stabilization	ASD, macrocephaly
RSK2	Protein kinase	Neurosecretion	Intellectual disability
SAP97	Scaffold protein	PSD protein trafficking	ASD, schizophrenia
SHANK2, SHANK3	Scaffold protein	Synapse stabilization	ASD
srGAP3	RAC1 GAP	Unknown	Intellectual disability
SSCAM (also known as MAGi2)	Scaffold protein	Receptor trafficking	Intellectual disability
SynGAP	RAS/RAP/RAC-GAP	Receptor trafficking and actin cytoskeleton	ASD, intellectual disability
TSC1, TSC2	Protein synthesis	Synapse stabilization	Intellectual disability
UBE3A	Protein degradation	Synapse formation	Angelman syndrome, intellectual disability

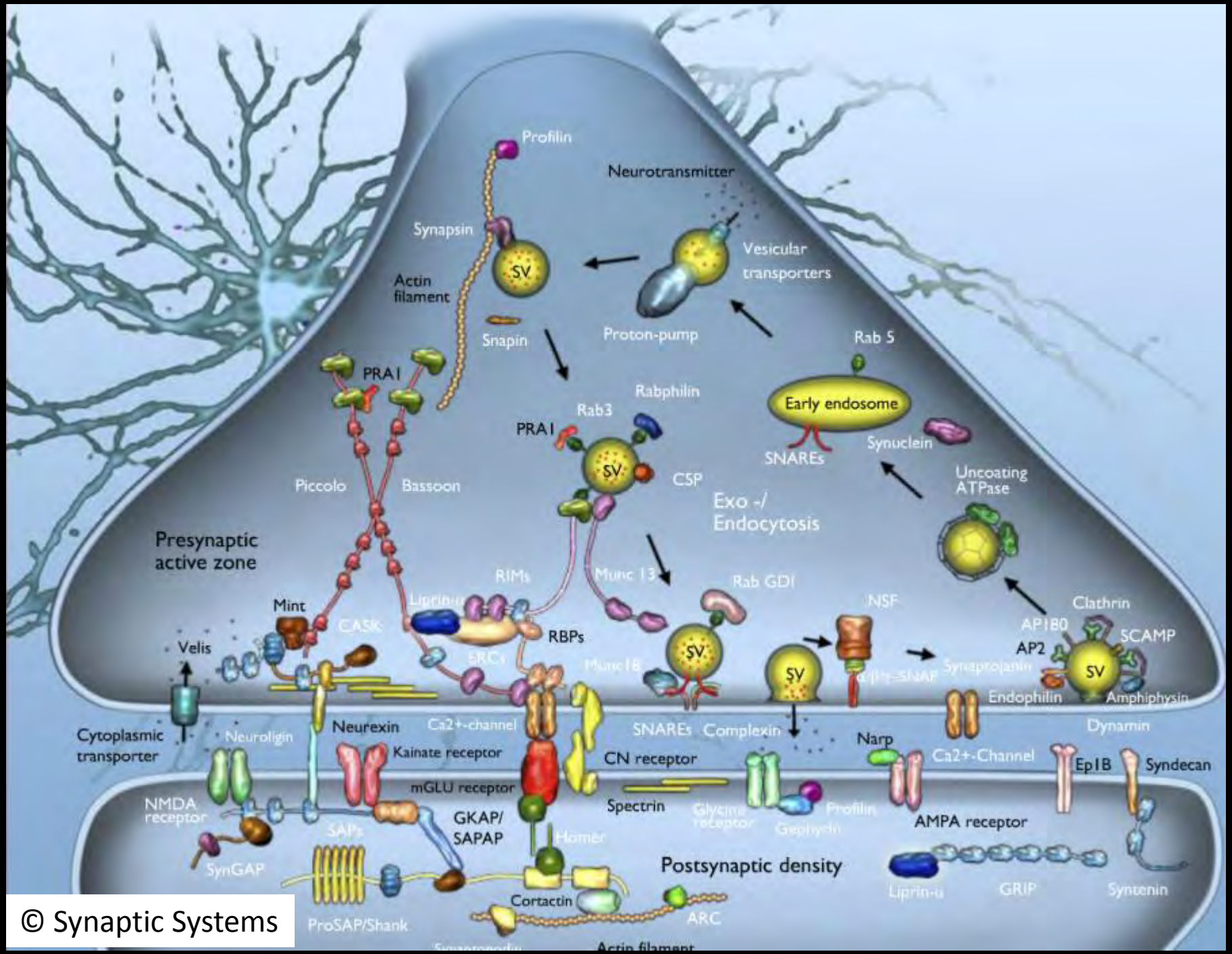
Synaptic proteins for which genetic defects (single point mutations, deletions, translocations or copy number variations (CNVs)) have been associated with autism spectrum disorders (ASDs), intellectual disability or schizophrenia. Supporting references can be found in recent reviews[72,103,114,115]. ARHGEF6, Rho guanine nucleotide exchange factor 6; CYFIP1, cytoplasmic FMR1-interacting protein 1; DISC1, disrupted in schizophrenia 1; EPAC2, Rap guanine nucleotide exchange factor 4; FMRP, fragile X mental retardation protein; IL1RAPL1, interleukin-1 receptor accessory protein-like 1; LIMK1, LIM domain kinase 1; MINT2, MUNC18-interacting protein 2; PAK3, p21-activated kinase 3; PSD, postsynaptic density; PTEN, phosphatase and tensin homologue; RSK2, ribosomal S6 kinase 2; SAP97, synapse-associated protein 97; SHANK, SH3 and multiple ankyrin repeat domains protein; srGAP3, SLIT-ROBO Rho GTPase-activating protein 3; SSCAM, membrane associated guanylate kinase, WW and PDZ domain containing 2; SynGAP, Ras GTPase-activating protein; TSC, tuberous sclerosis; UBE3A, ubiquitin protein ligase E3A.

Caroni et al., (2012) Nature Neurosci. **13:** 478

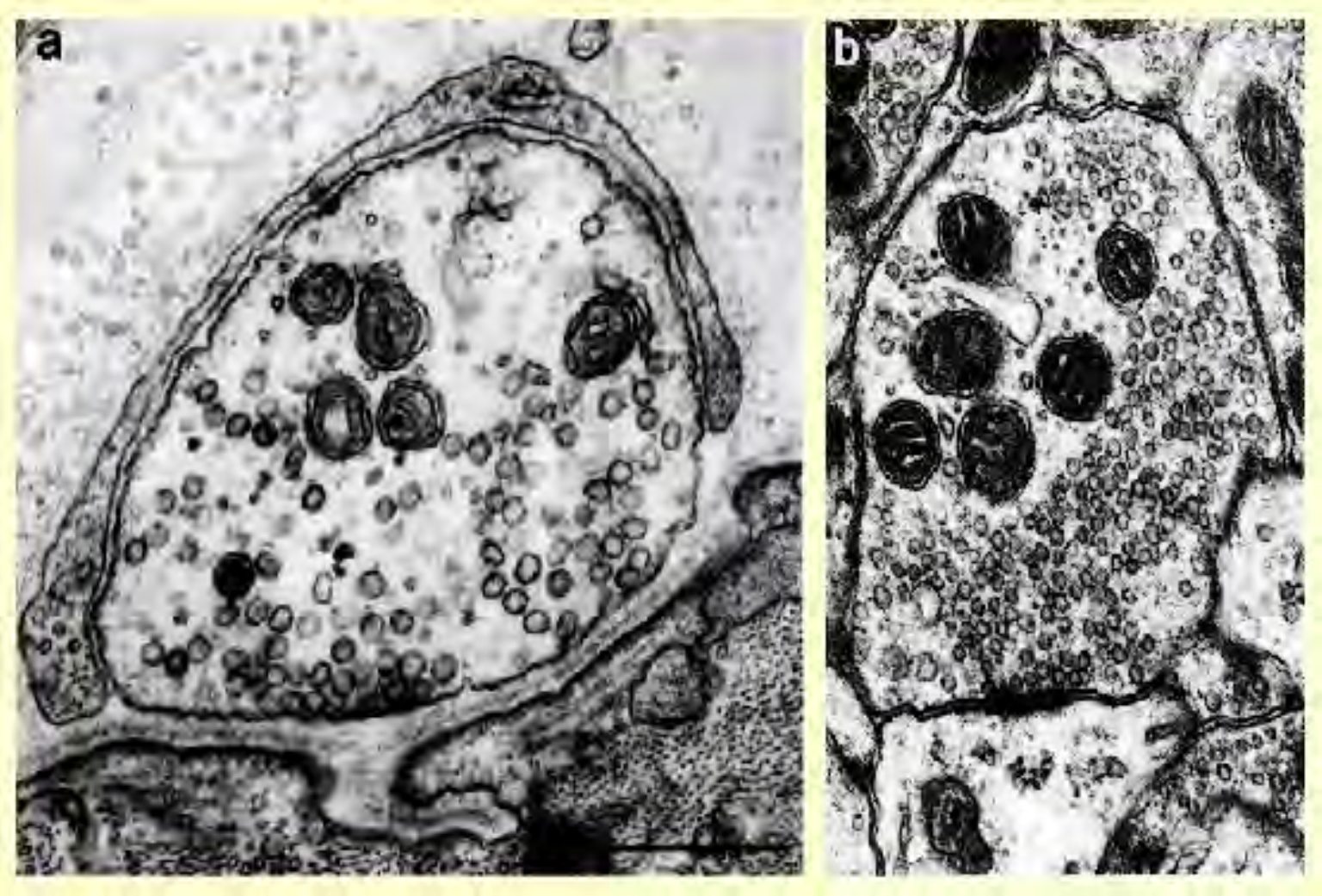
Synaptic connections are „sticky“ and can be biochemically isolated

- Synaptosomes as specimens to analyze the release of neurotransmitters
- Synaptic junctions (post-synaptic densities, PSDs) for the characterization of synaptic proteins
- Apparently pre- and post-synapse are **transsynaptically** stably connected
- synaptosomes more than 3,000
- Synaptic vesicle fractions contain over 400 protein species
- PSDs from 200 to over 1,000 (data from O’Rourke et al., Nature Rev. Neurosci. 2012)
- Synapses from forebrain and Cerebellum differ substantially (Cheng et al., 2006)





The Neuromuscular Junction and CNS Synapses are very Similar



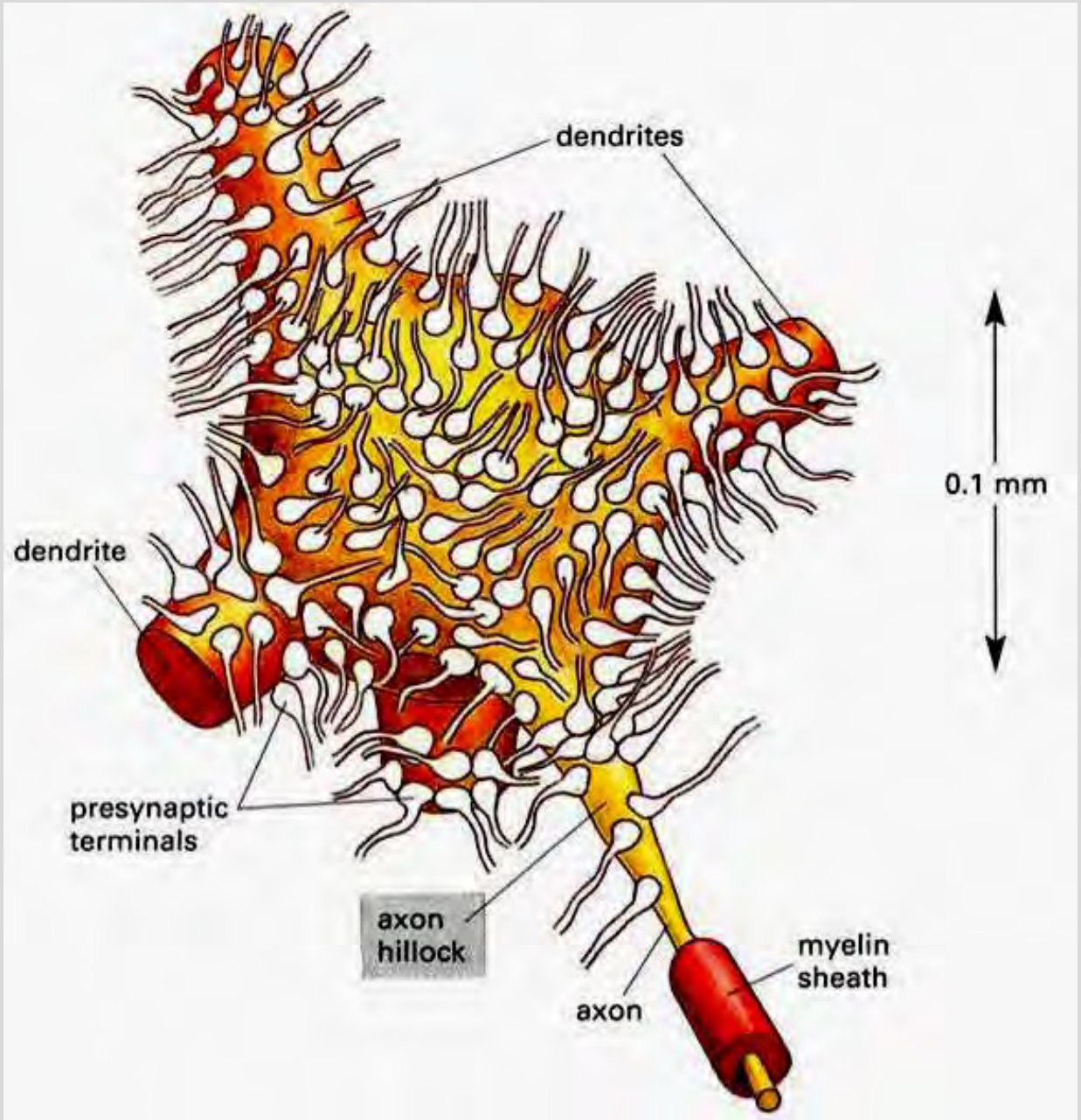
Differences Between the NMJ and Interneuronal Synapses in the CNS

- CNS synapses have no basal lamina in the synaptic cleft and no synaptic folds in the postsynaptic membrane
- synapses are much more flexible and have a higher turnover rate
- The formation of a mature CNS synapse takes hours rather than days/weeks
- CNS synapses are plastic and their morphology as well as the strength of synaptic transmission is severely dependent on their activity whereas the NMJ is rather static
- Major players for NMJ formation are known

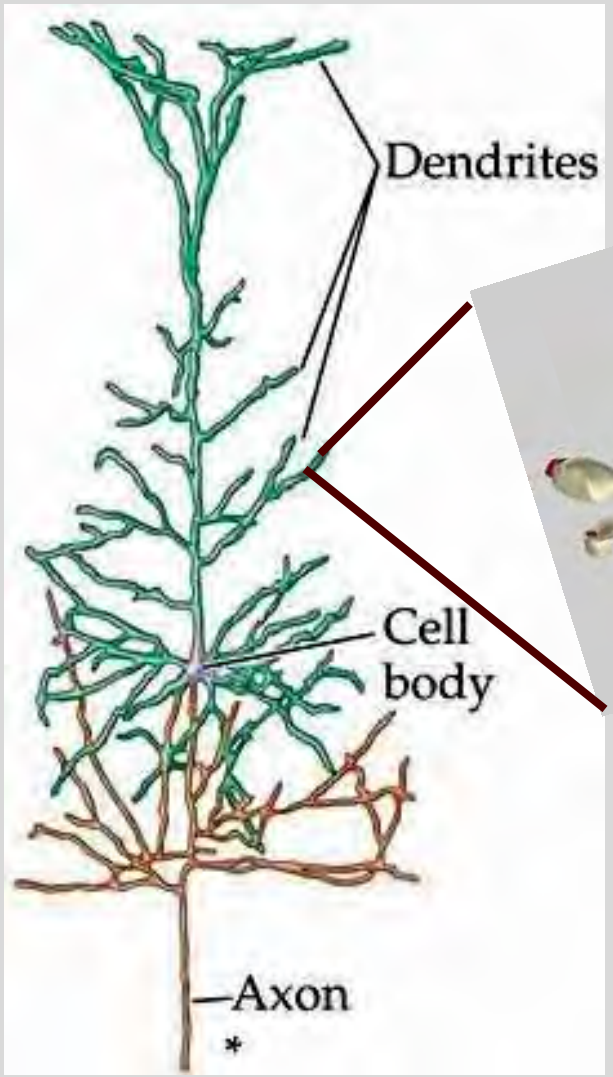
The Synaptic Complexity of the Brain

- Number of nerve cells per human brain: ~10^{11} (100 billion)
- The human brain contains on ~10^{14} synapses
- With one synapse per second, one would need about 3 million years to count all synapses in an average human brain
- If every person on the world (7 billion) would do 15.000 telephone calls simultaneously, we would have the same number of connections as a single average human brain (and a total breakdown of all telephone systems)
- If one synapse is enlarged to 1 cm diameter, an average human brain would be about 4 km long
- We still do not know what instructs a neuron to form a particular synapse at a specific position

Synaptic Complexity: the Absolute Numbers



Synaptic Complexity: Functional Complexity

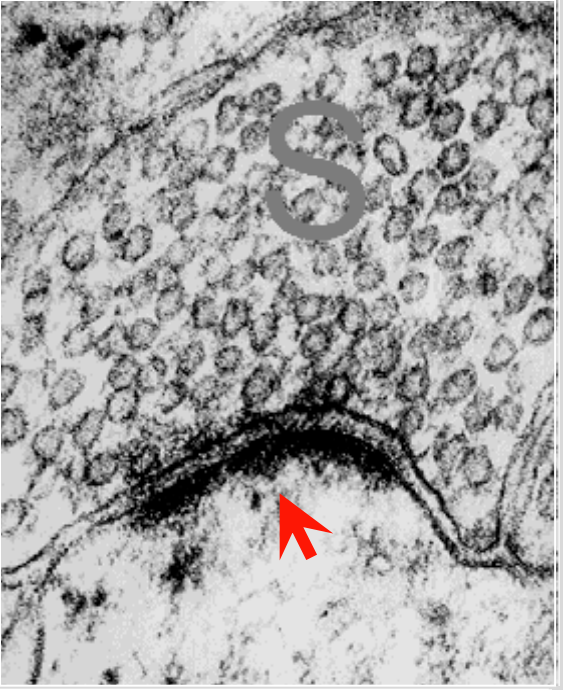


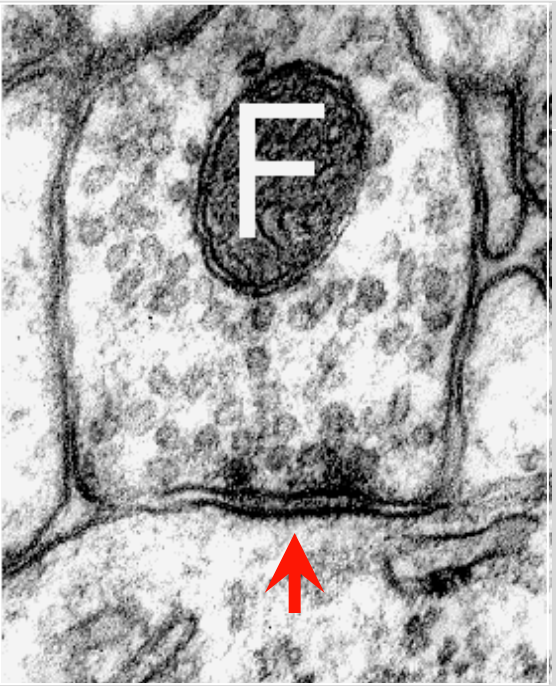
GABA-ergic synapse with $GABA_B$-receptor

GABA-ergic synapse with $GABA_A$-receptor

Kainate-type glutamatergic synapse

NMDA type glutamatergic synapse

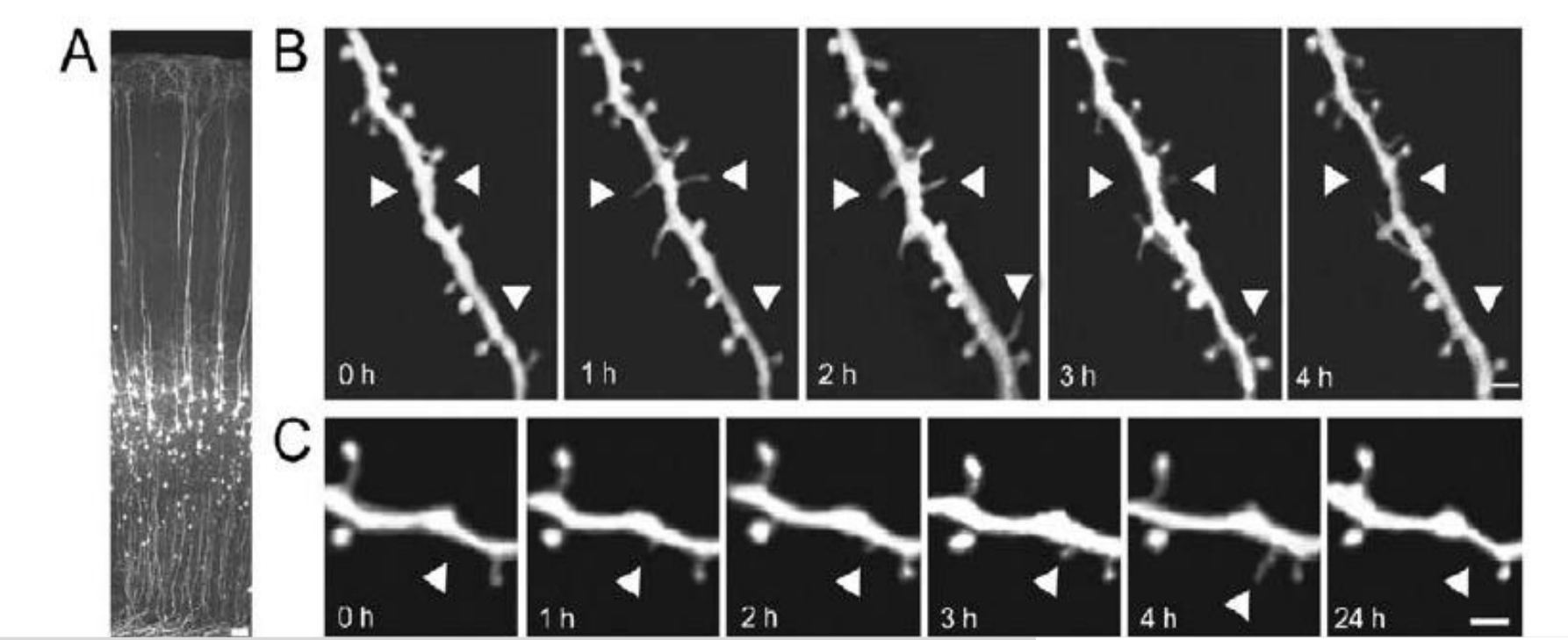




Synapses in the CNS

- Types according to GRAY (1959):
- **Type 1:** asymmetric, spines, synaptic cleft ~30 nm, prominent PSD, excitatory, round vesicles, neurotransmitter: glutamate
- **Type 2:** symmetric, on dendrite and soma, synaptic cleft ~20 nm, small electron-dense PSD, inhibitory, pleiomorphic vesicles, neurotransmitter: GABA or glycine

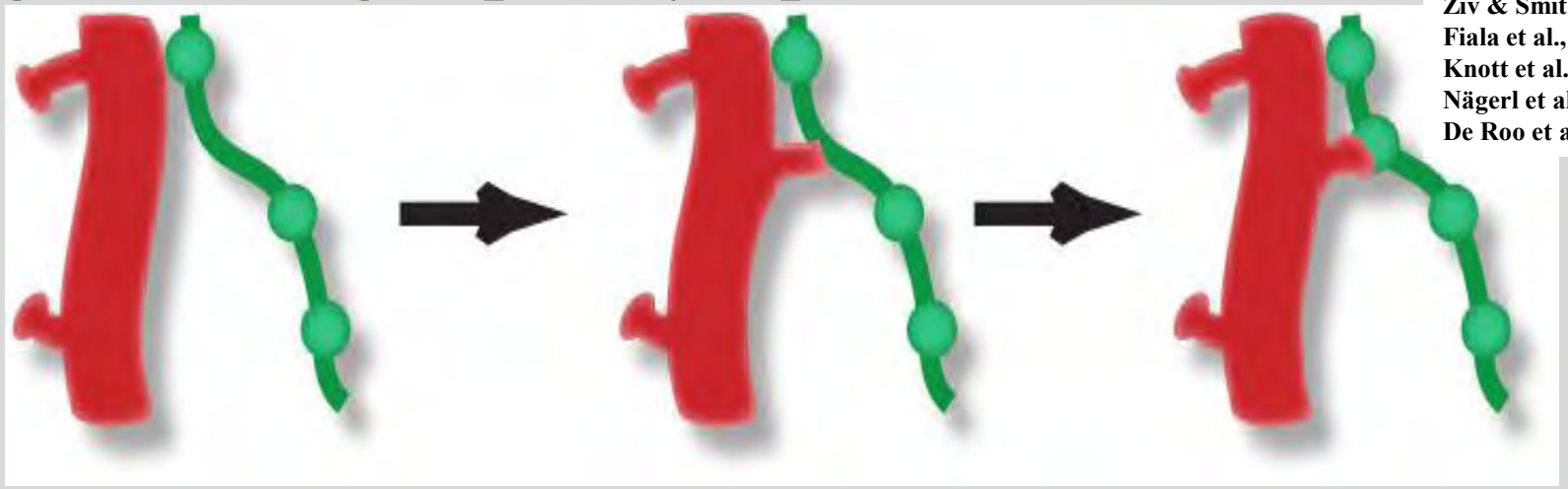
Formation of Glutamatergic Spine Synapses



Ziv & Smith, Neuron 1996
Fiala et al., J. Neurosci. 1998
Knott et al., Nature Neurosci. 2006
Nägerl et al., JNS 2007
De Roo et al., Cerebr Cortex 2008

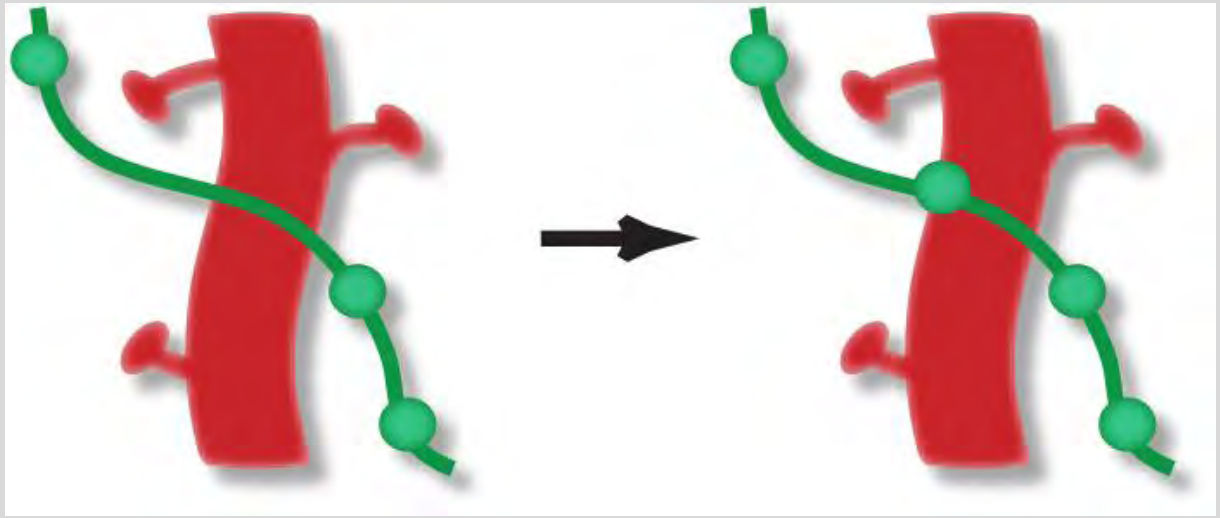
Formation of Synapses

glutamatergic spine synapse



Ziv & Smith, Neuron 1996
Fiala et al., J. Neurosci. 1998
Knott et al., Nature Neurosci. 2006
Nägerl et al., JNS 2007
De Roo et al., Cerebr Cortex 2008

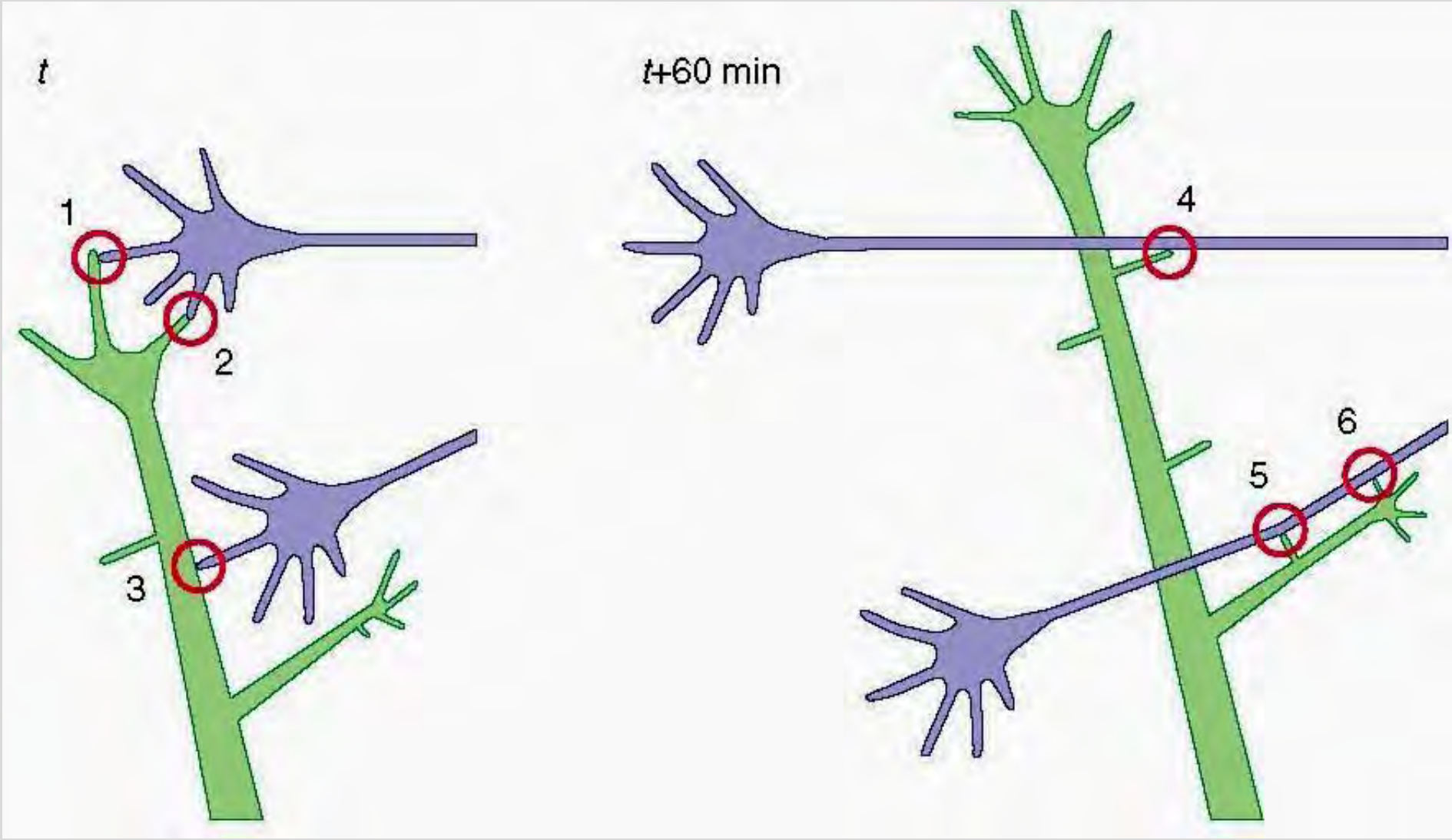
GABAergic shaft synapse



New inhibitory synapses are formed by new boutons at pre-existing axon-dendrite crossings

Wierenga et al., Nature Neurosci 2008

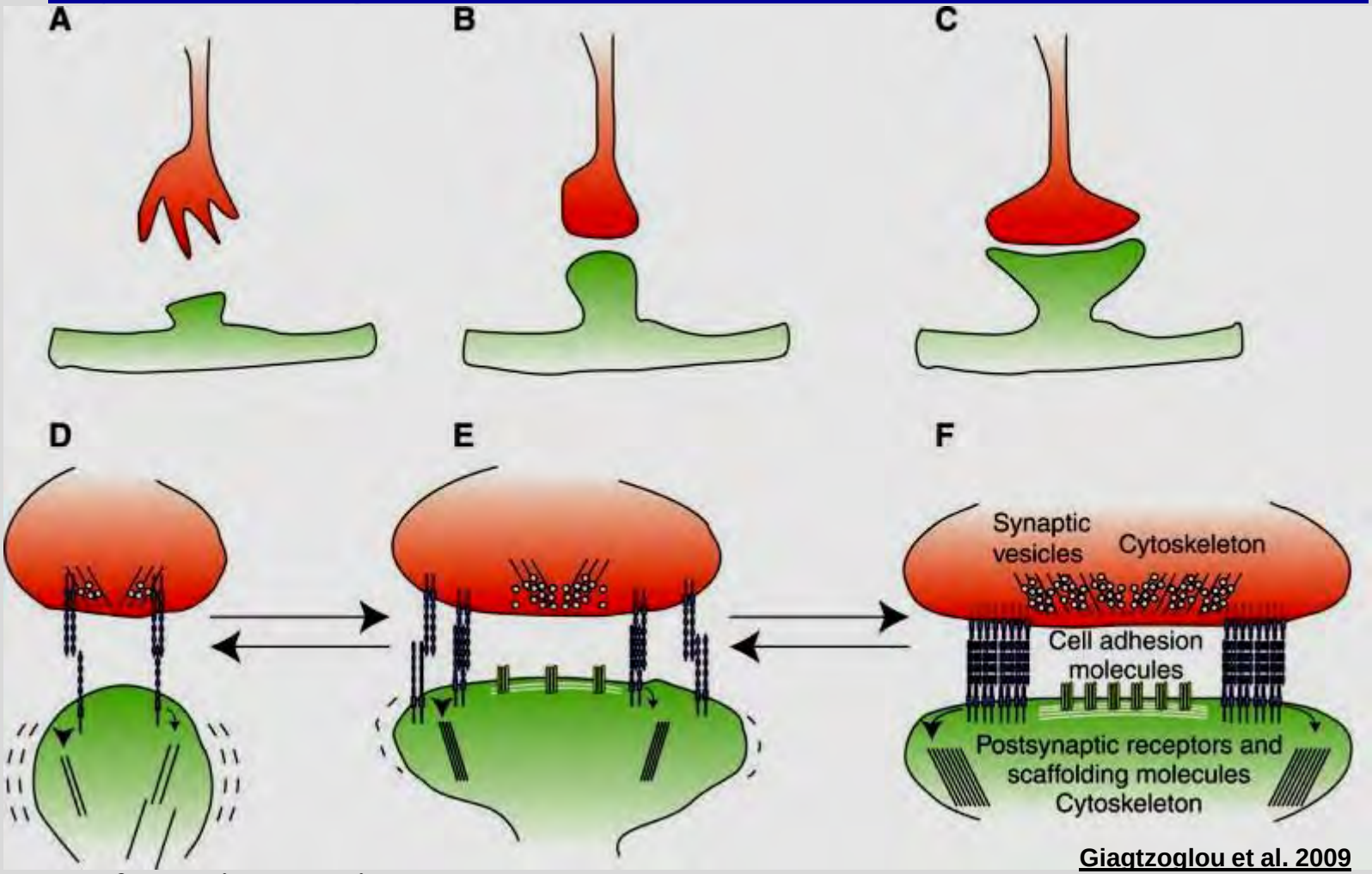
Dendritic Processes in Synapse Formation



90 % of all filopodia contacts are NOT stabilized
Only few transform into a synapse

Goda and Davis, 2003

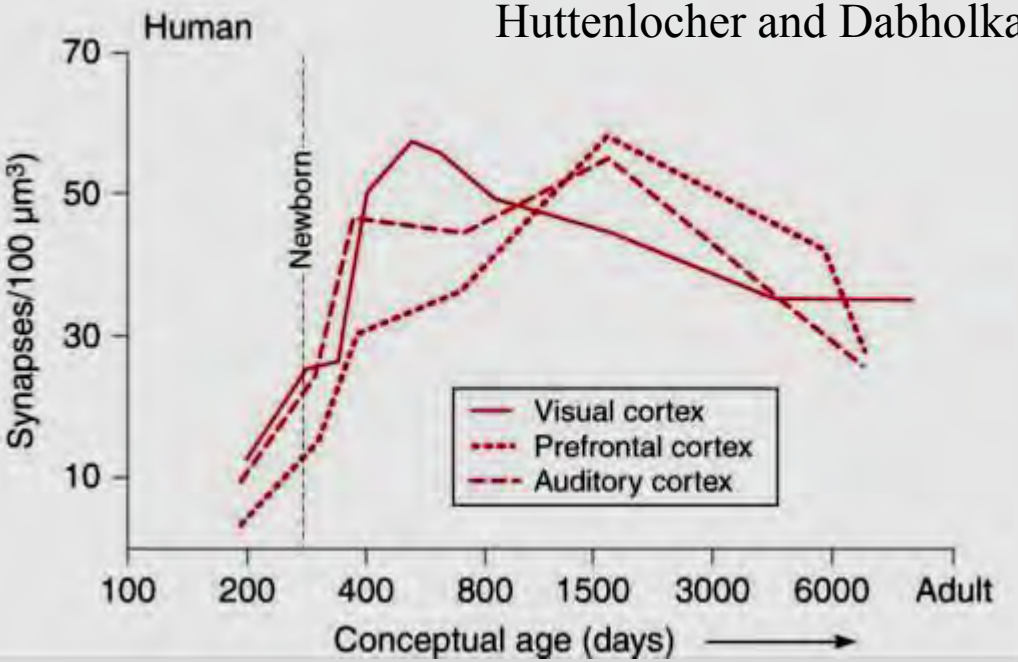
Synaptogenesis is a Multistep Process



Giagtzoglou et al. 2009

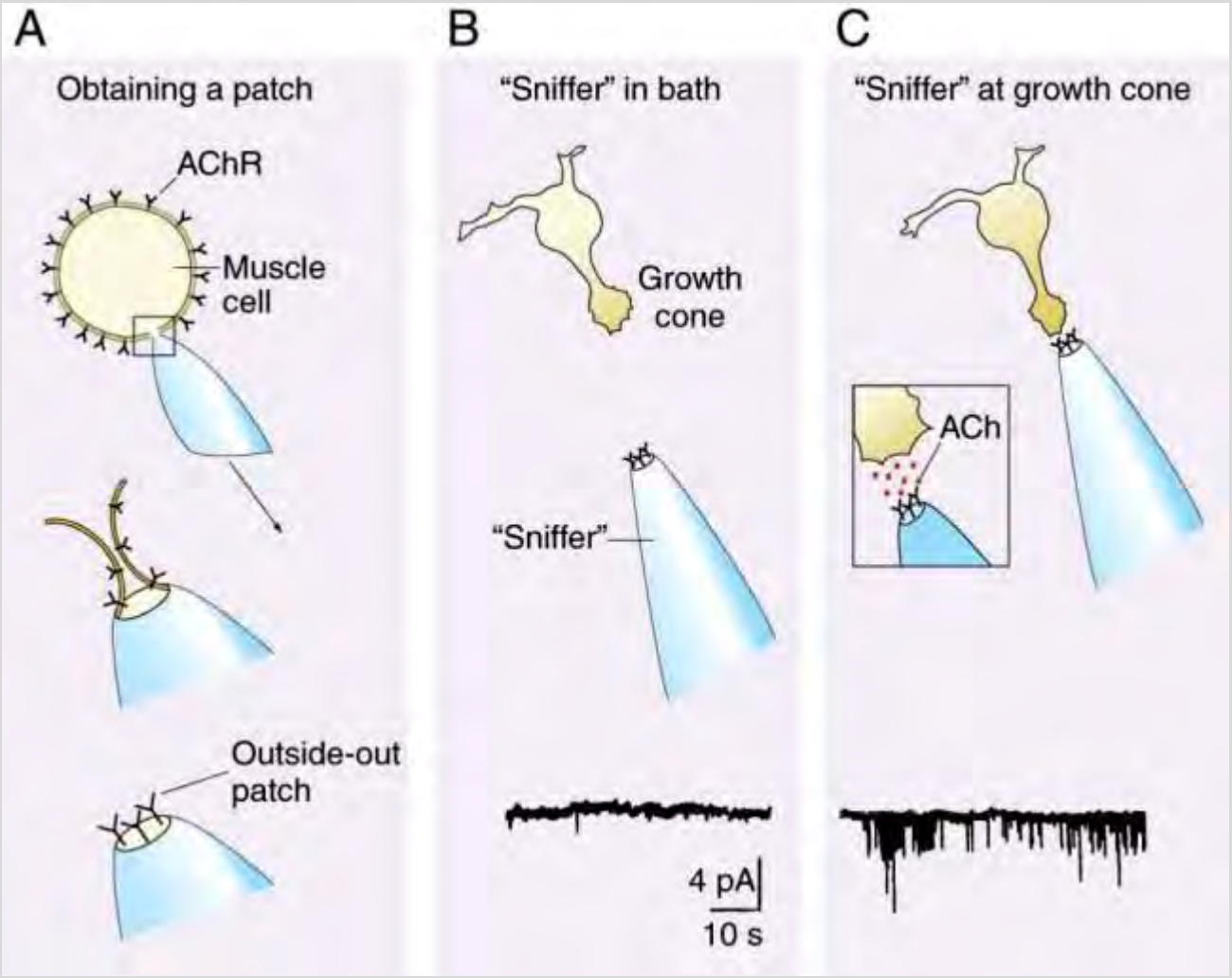
- Synapse formation requires:
- The halt of growth cone advance (mediated by increase of intracellular Ca^{2+}) and the transition of a highly motile growth cone to a stable presynaptic terminal capable of Ca-dependent neurotransmitter release
- The simultaneous specialization of the postsynaptic membrane

Synaptogenesis in the Developing CNS



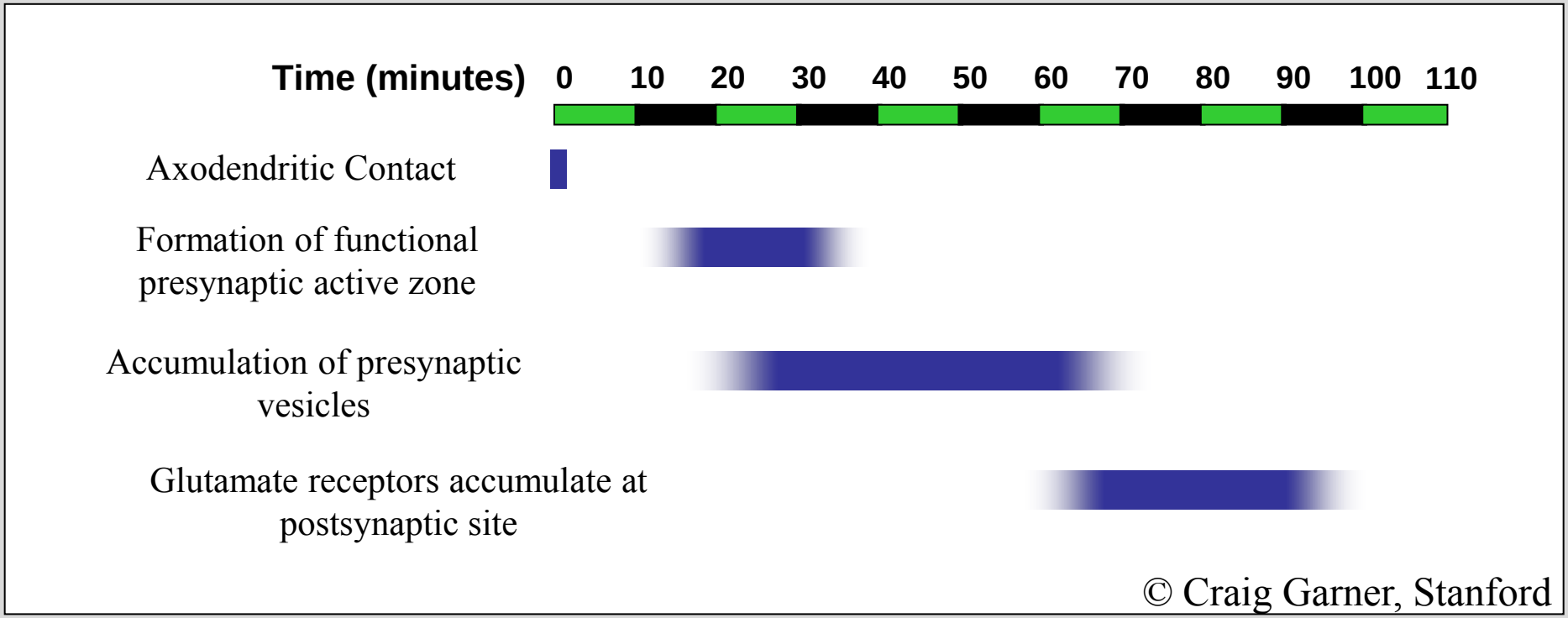
- Synaptogenesis starts shortly before birth and synapse density increases for months after birth
- Peak of synapse number in human cortex is 1-4 years after birth
- However: individual synapses form within minutes to hours
- Required: transmitter release already from growth cone and sensitivity of postsynaptic membrane to transmitter before arrival of growth cone
- Successful transmission is established within seconds of contact

The Growth Cone releases Neurotrans-mitter



- Measure neurotransmitter release by "sniffing" with the appropriate neurotransmitterreceptor-containing membrane patch
- Not only ACh but also other transmitter

Temporal Order of Synapse Assembly



- *in vitro* at glutamatergic synapses the presynaptic differentiation precedes postsynaptic differentiation
- Differentiation of presynapse by insertion of preassembled active zone material from vesicles
- Assembly of PSD is sequential multistep process
- Glutamate receptors are present but not aggregated before synaptogenesis starts
- Activity at synapse is important but not essential for synapse formation

What are the molecules that mediate the formation of synaptic specializations in the developing CNS?

Incomplete List of Molecules IMPORTANT for CNS Synaptogenesis

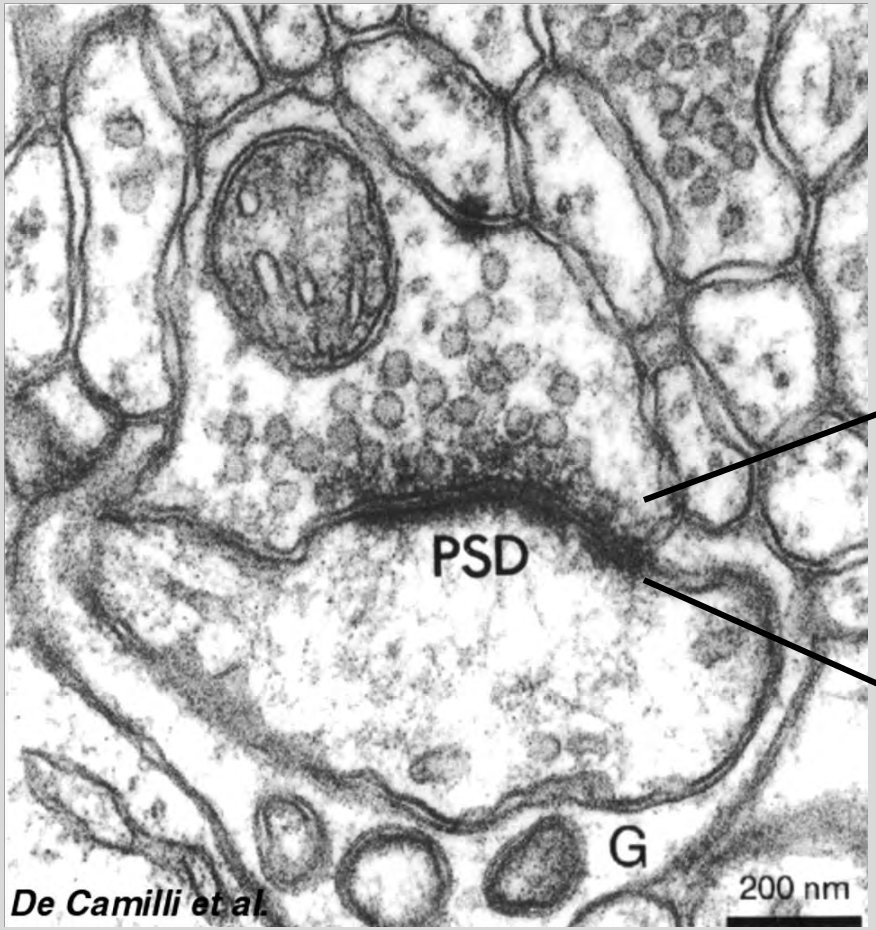
Sidekick-1, Sidekick-2,	Yamagata & Sanes 2008
Neurexin / Neurologin	Chih et al. 2005, 2006, Dahlhaus et al. 2
Dscam, and DscamL	Yamagata et al. 2002
LRRTM1 / LRRTM2	Linhoff et al. 2009; de Wit et al. 2009
SynCAMs	Biederer et al. 2002
NGL1 -2,-3 with LRRTM2	Kim et al. 2006; Linhoff et al. 2009; Woo et al. 2009
agrin	Ksiazek et al. 2007
Pentraxins like NARP /NP1	O'Brien et al., 1999, 2002; Sia et al. 2007; Passafaro et al. 2003
EphB2	Grunwald et al. 2001; Henderson et al. 2001
PTPRs (NGL-3 and LAR)	Woo et al. 2009
FGF22 and FGF7	Terauchi et al. 2010
thrombospondin	Christopherson et al., 2005; Xu et al., 2010
Gabapentin	Eroglu et al. 2009
cholesterol	Mauch et al. 2001
Cerebelin1	Uemura et al. 2010; Matsuda et al. 2010
Semaphorin ; Semaphorin 3E–Plexin-D1	Tran et al., 2009; Ding et al. 2012
β-adducin	Bednarek and Caroni, 2011
Cadherin-9	Williams et al. 2011
BDNF / TrkB	Martinez et al., 1998; Alsina et al., 2001
FLRT	O'Sullivan et al. 2012
GDNF	Ledda et al., 2007
Wnt7a	Hall et al., 2000; Ahmad-Annuar et al., 2006

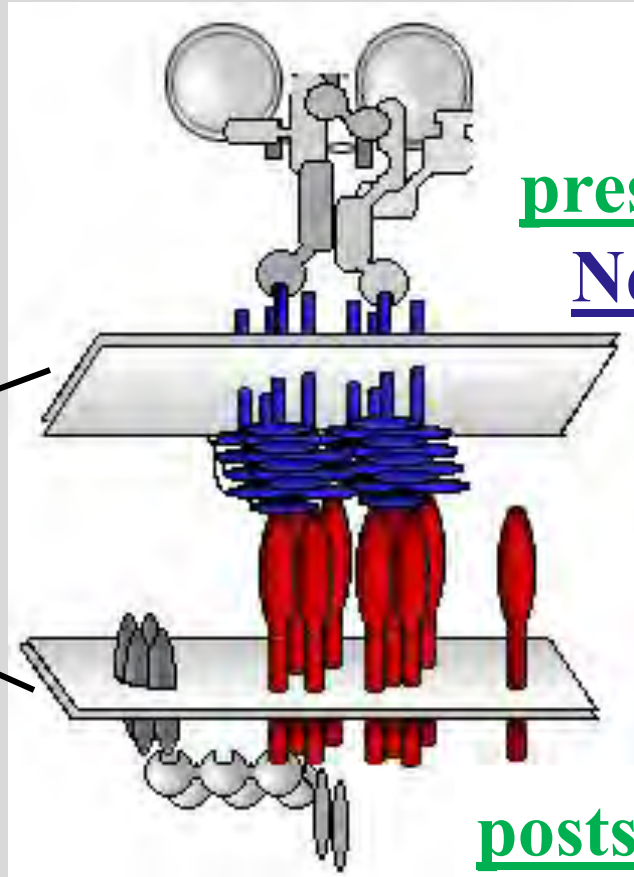
Cell adhesion molecules

Axon guidance molecules

Glia-derived factors

Model of the Synaptic Neuroligin-Neurexin Adhesion Complex



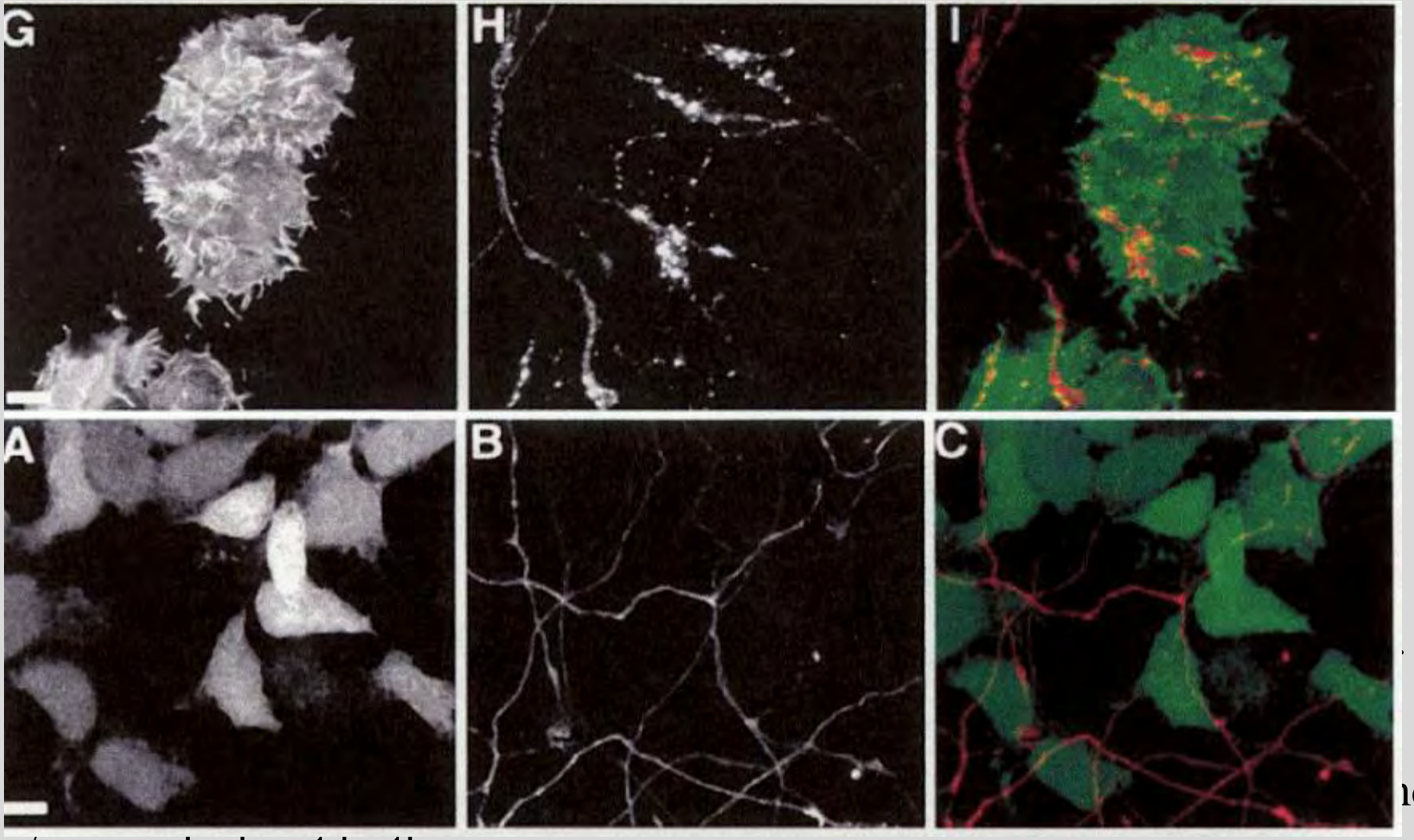


presynaptic: Neurexin

postsynaptic: Neuroligin

- NL1 is only present at excitatory synapses (Song et al. 1999)
- NL2 and NL4 at inhibitory synapses (Varoqueaux et al. 2004; Hoon et al. 2011)
- NL3 is present at both excitatory and inhibitory synapses (Budreck and Scheiffele 2007).

Non-Neuronal Cells Expressing Neuroligin Induce Accumulation of Synapsin in Contacting Axons



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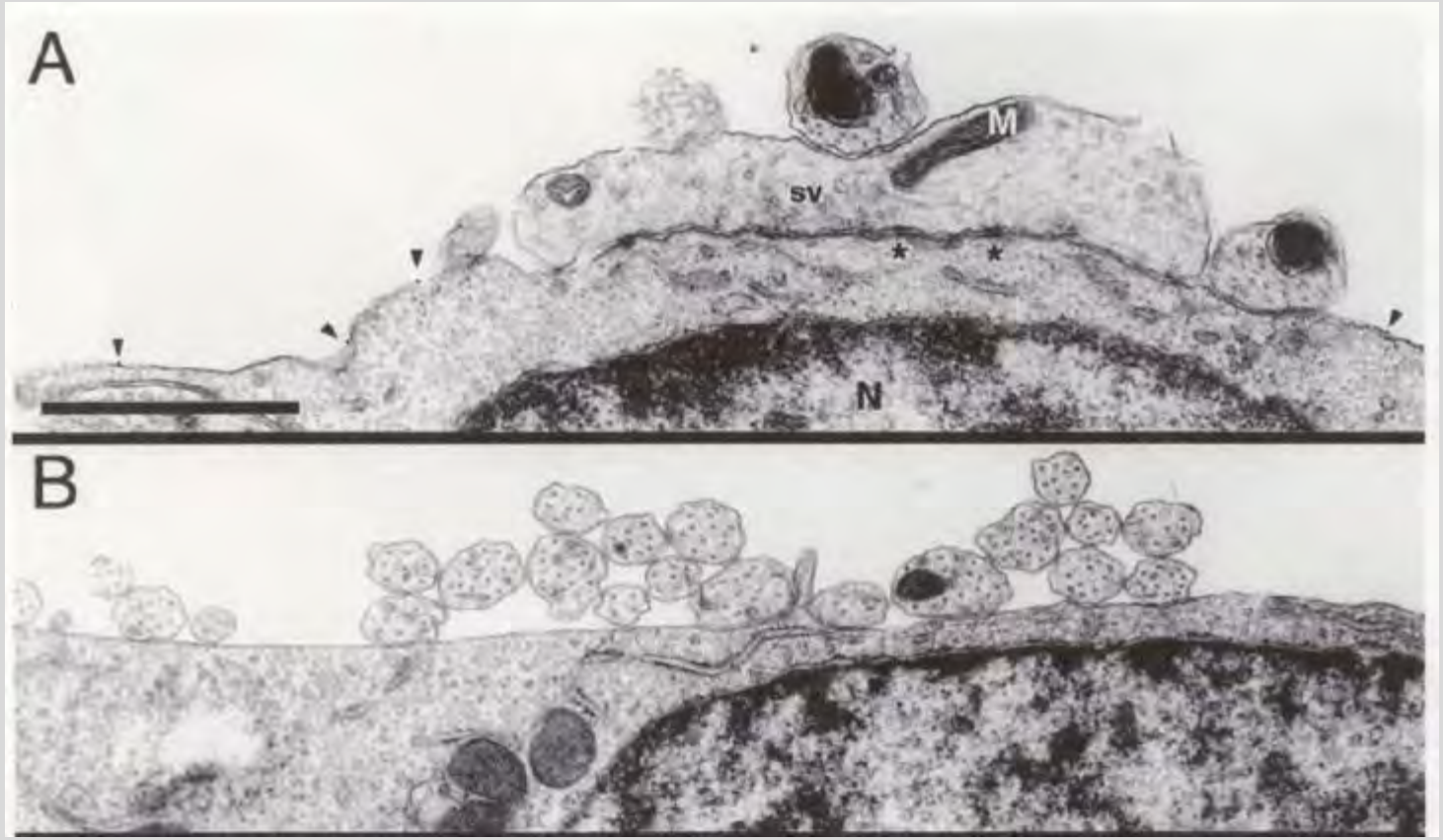
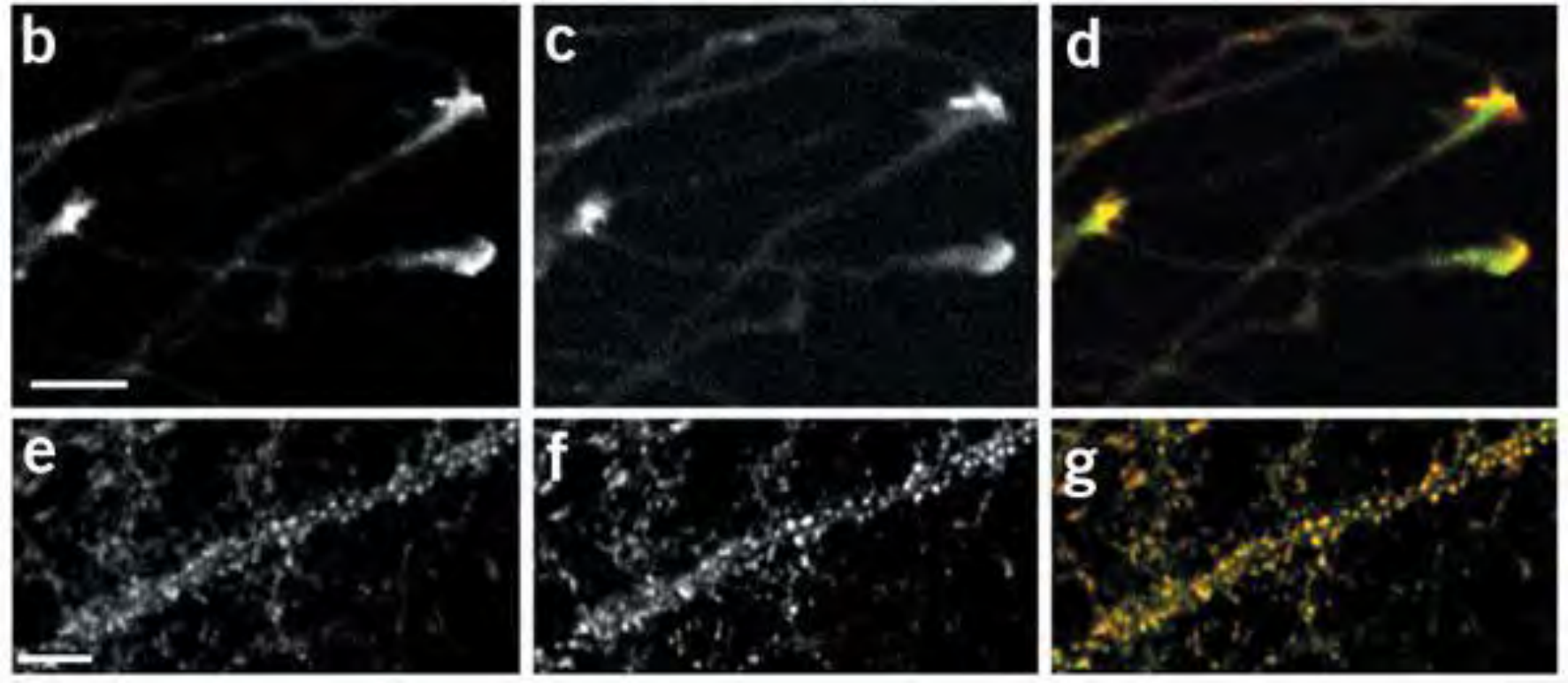
Non-Neuronal Cells Expressing Neuroligin Induce Accumulation of Synapsin in Contacting Axons



- EM verification of presynaptic specializations
- NL1 is only present at excitatory synapses (Song et al. 1999), NL2 and NL4 at inhibitory synapses (Varoqueaux et al. 2004;
- Synaptic vesicles undergo cycles

Endogenous Neurexins are Concentrated at Synapses



- Before synaptogenesis neurexins are concentrated in growth cones (pontine explants)
- After synaptogenesis clusters of neurexin colocalize with pre- and postsynaptic specializations