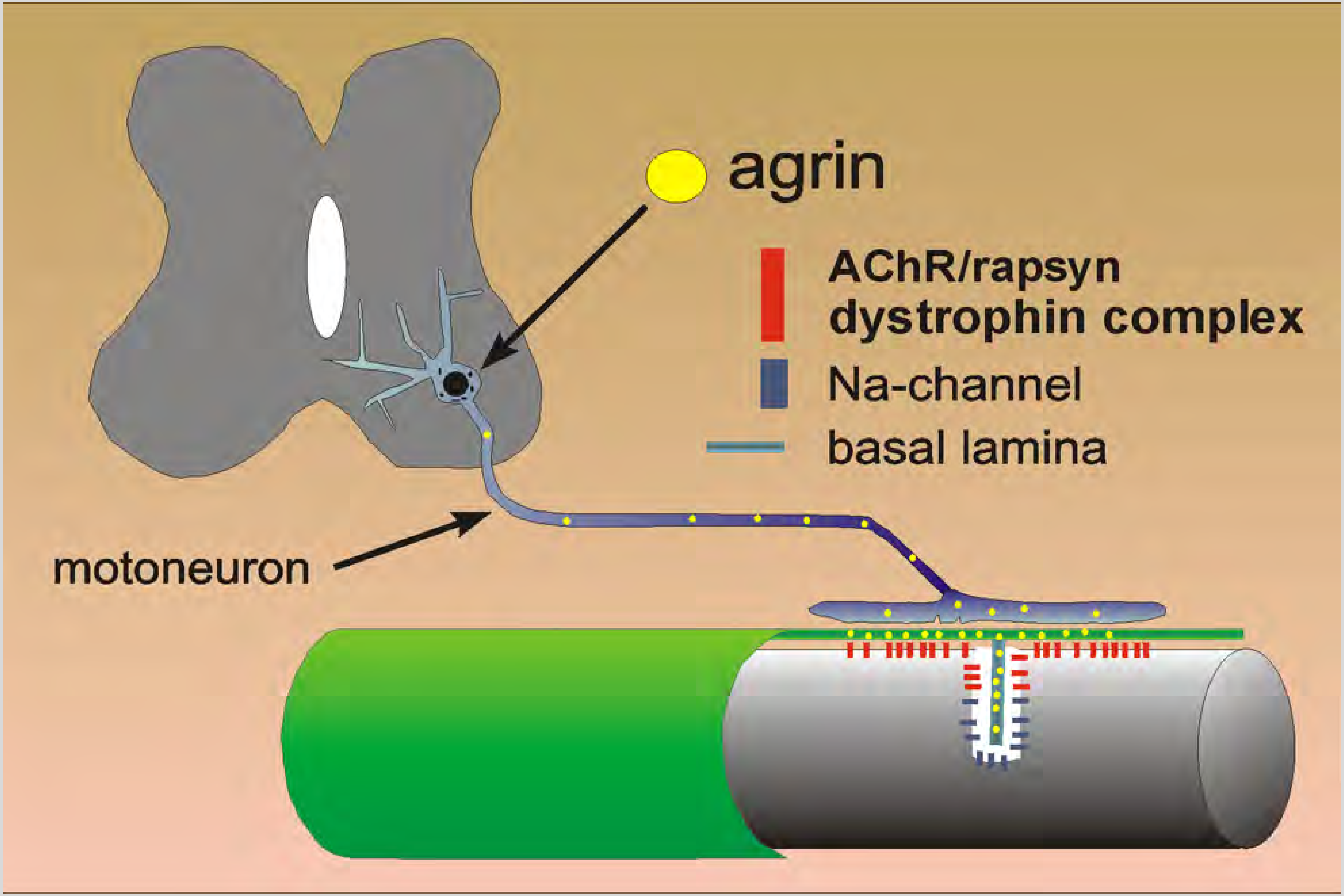
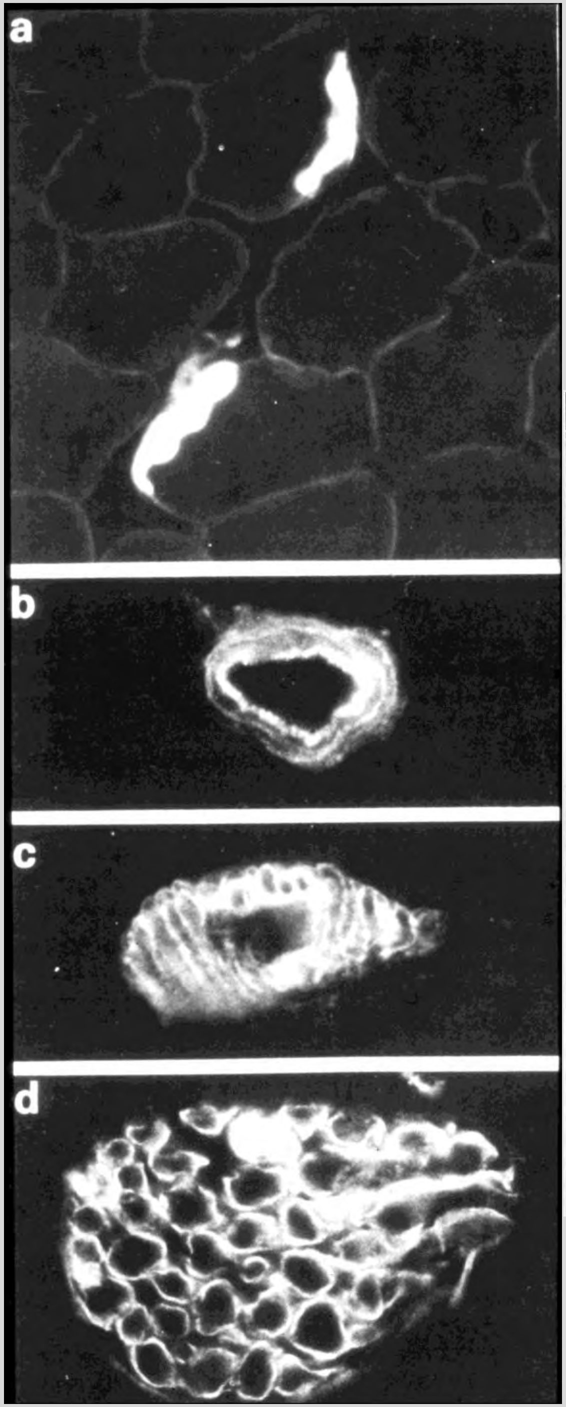
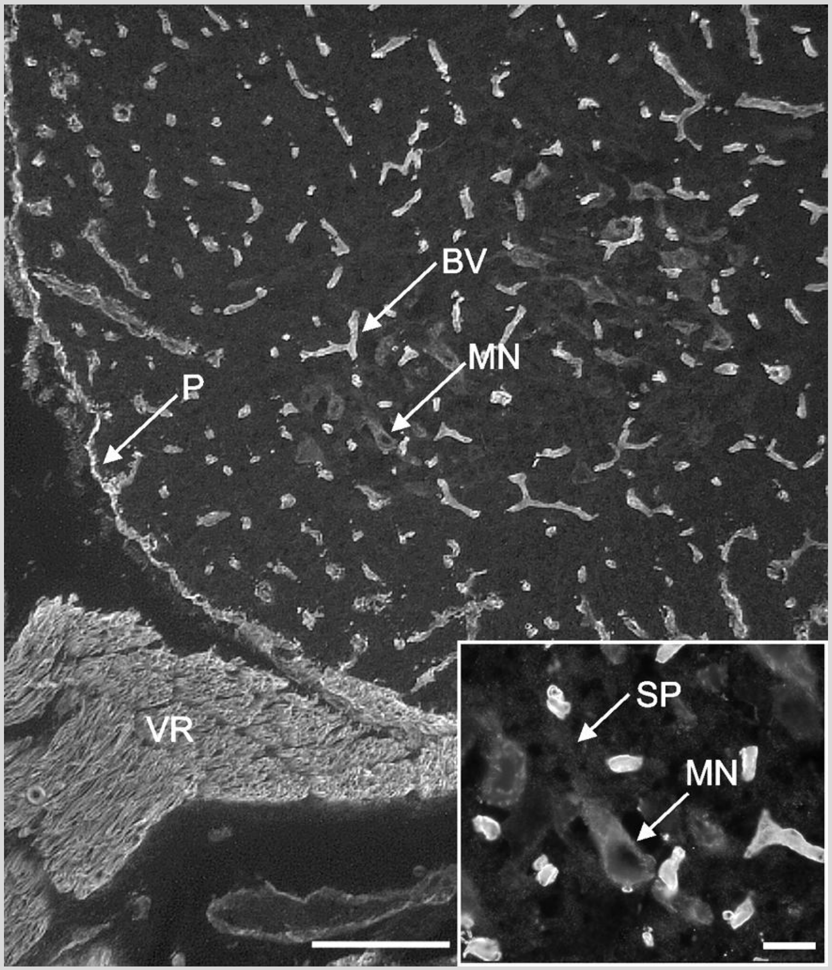
The Agrin Hypothesis (McMahan, 1990)



„Ectopic“ Expression of Agrin

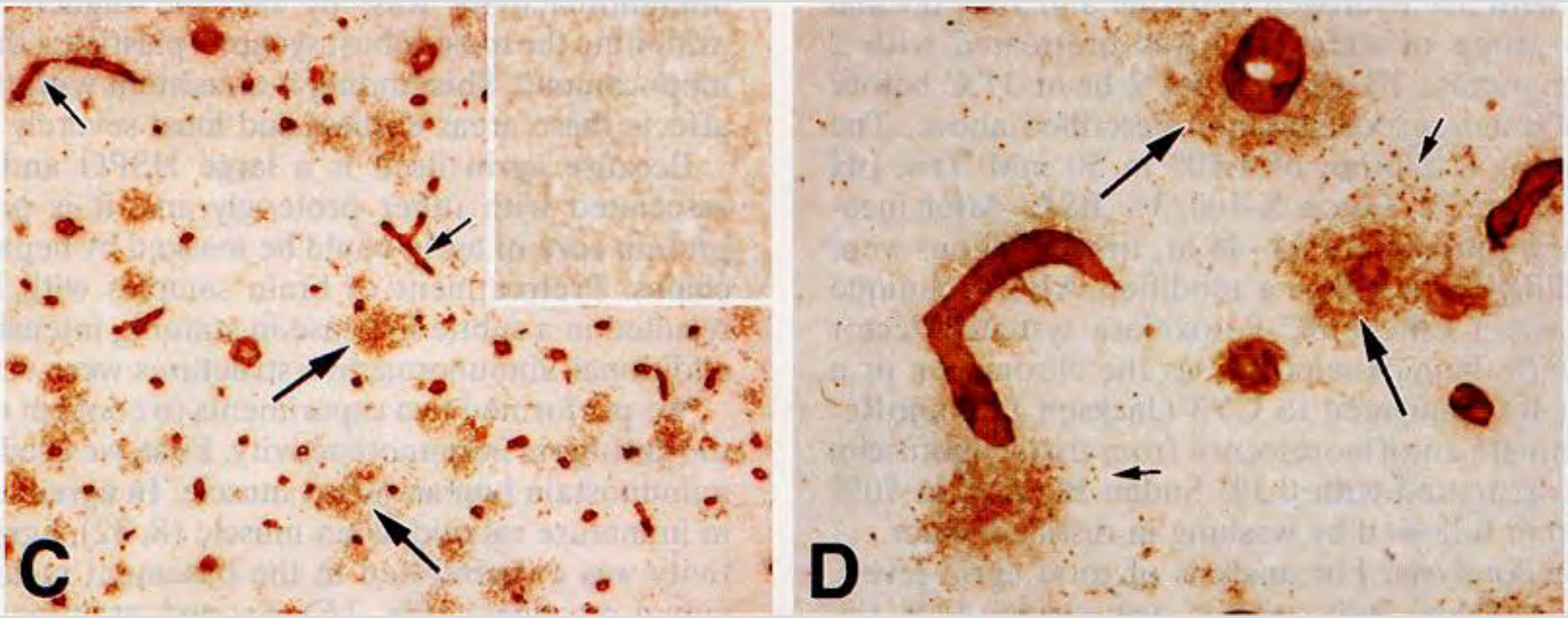


Reist et al., 1987



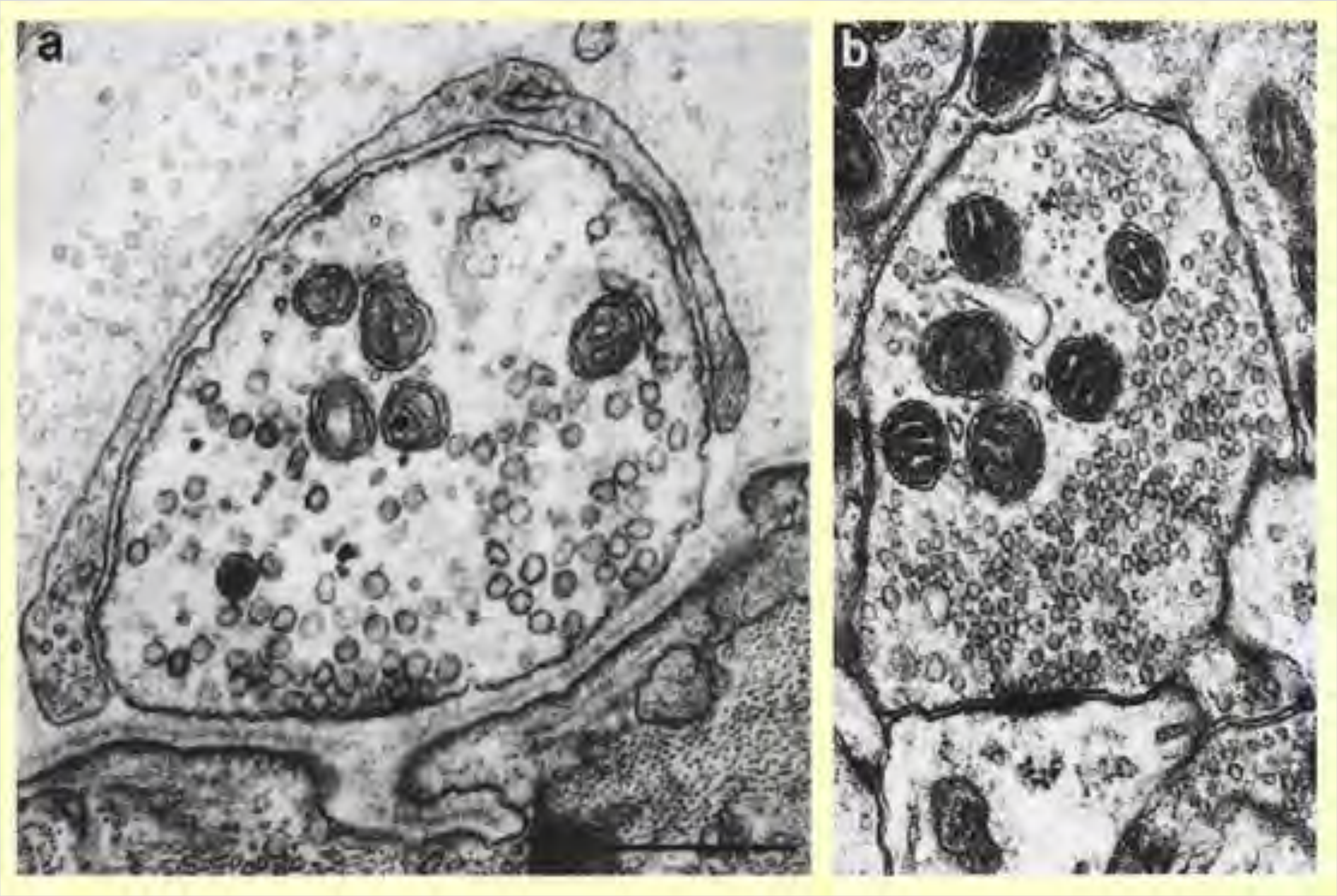
Kröger and Schröder, 2002

Agrin is a Component of β-Amyloid Plaques in Alzheimer's Brains

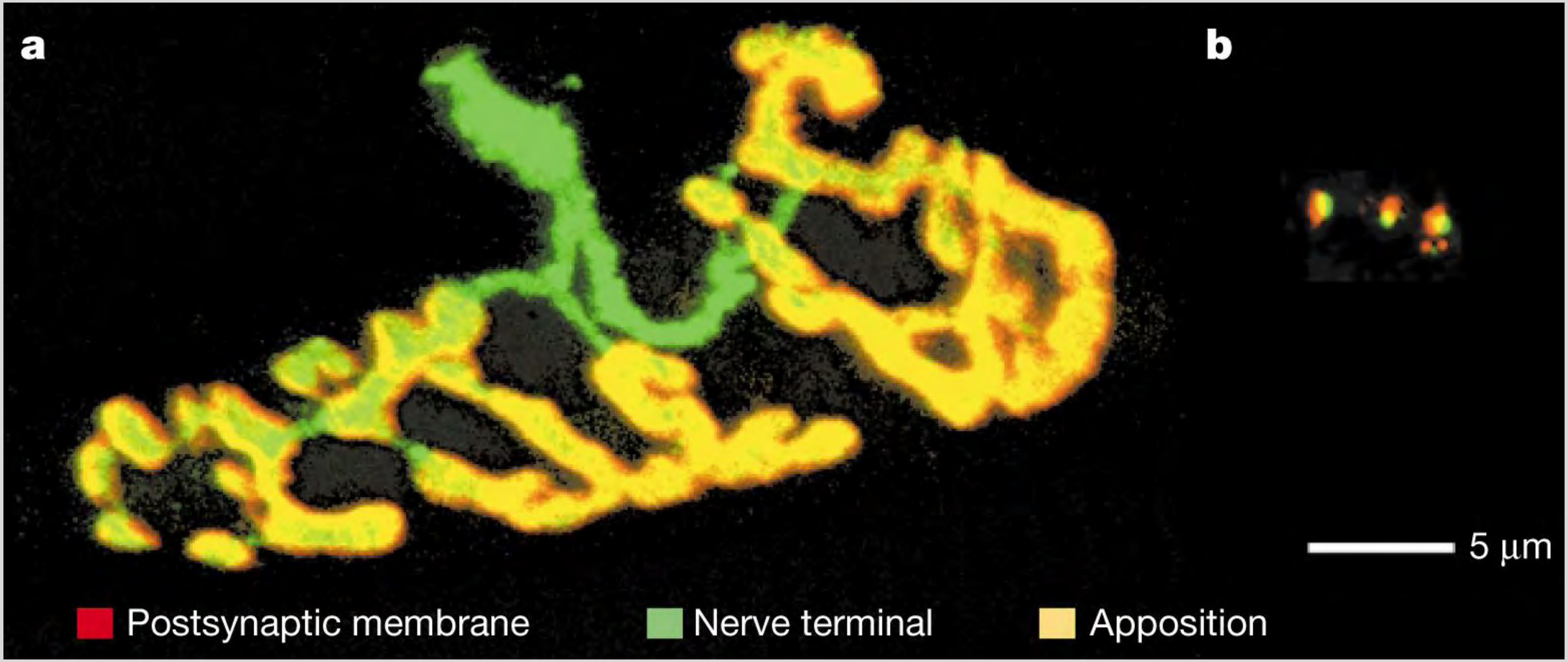


Donahue et al., (1999) PNAS **96:** 6468-6472

The Neuromuscular Junction and CNS Synapses are very Similar

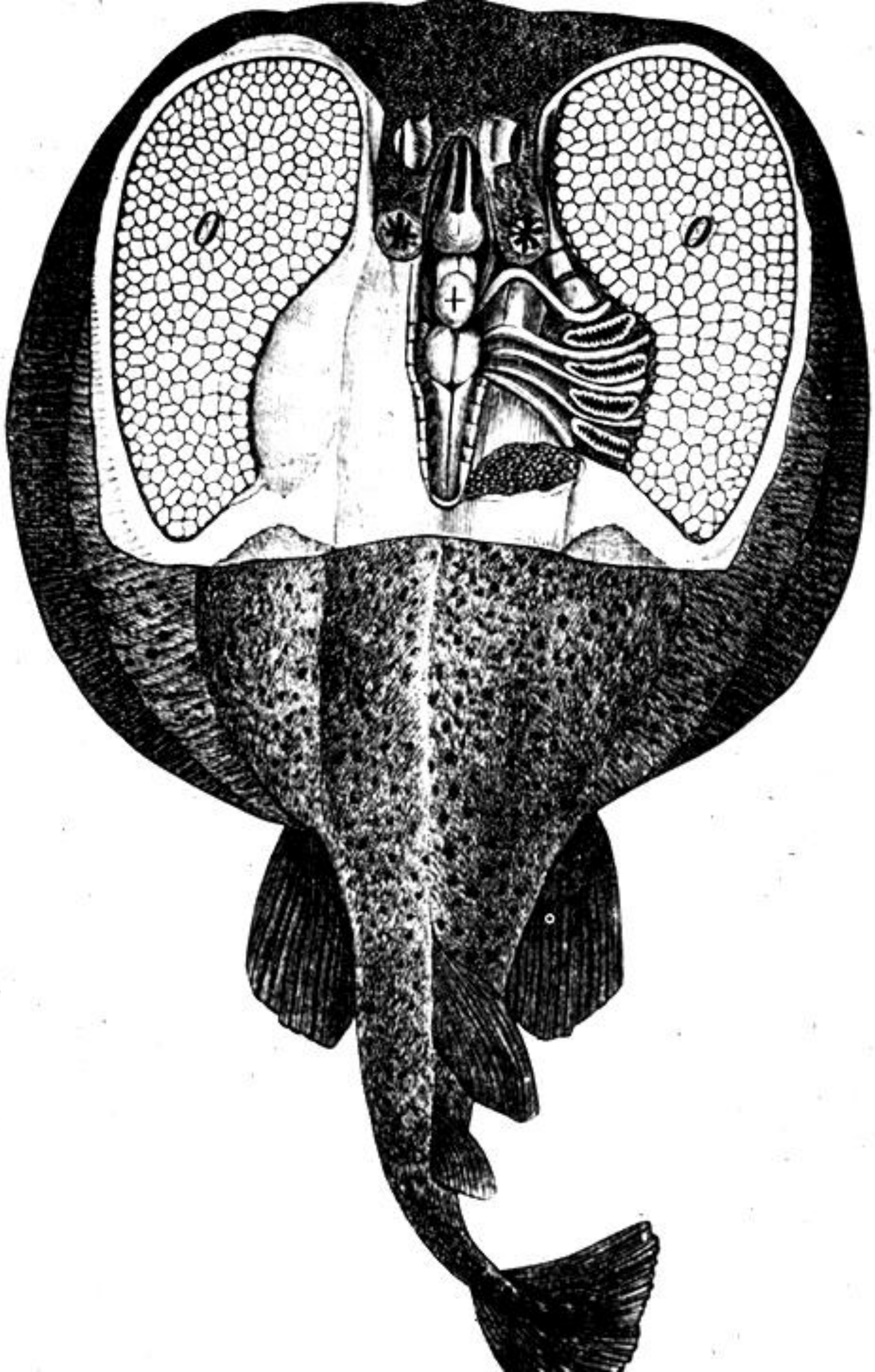


Advantages of the Neuromuscular Junction



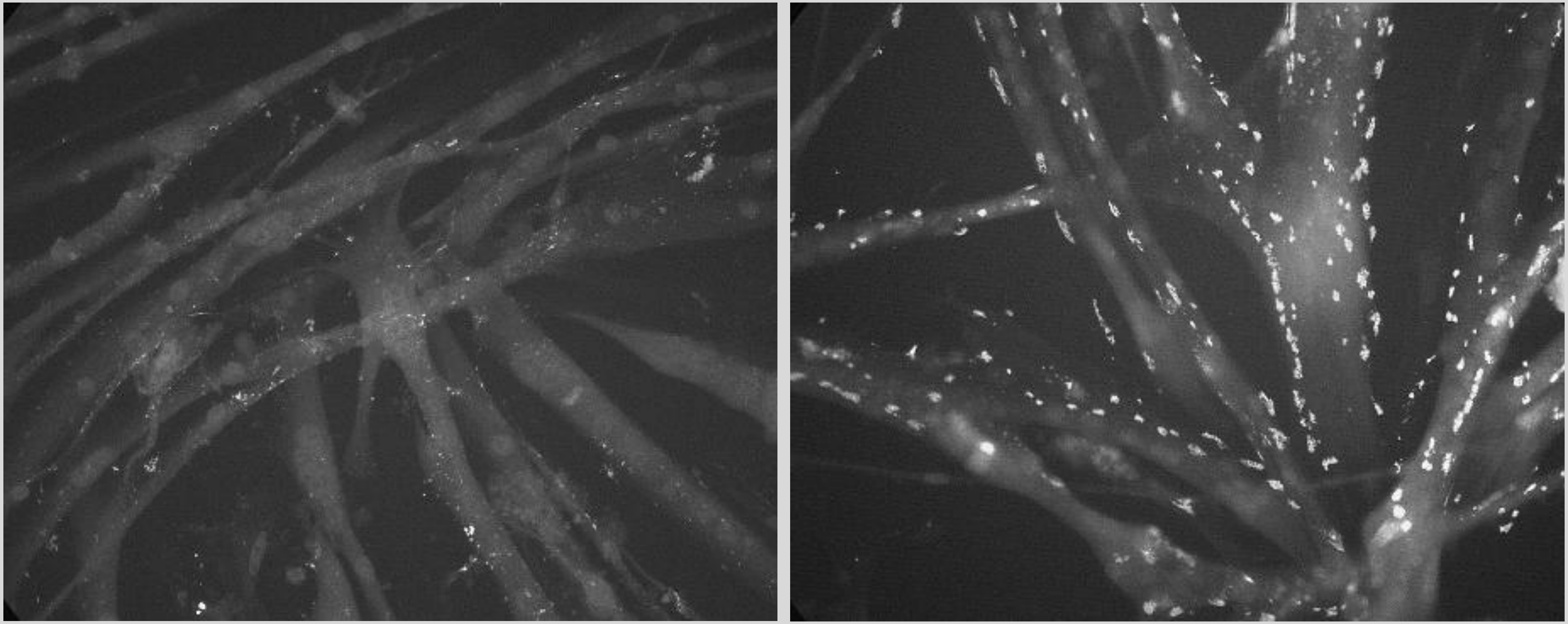
- Size
- Ability to regenerate
- Accessibility
- Tools for the analysis are available

The Banded Krait: Venom Contains α-BTX



The Marine Ray *Torpedo californica*

In vitro Bioassay for Agrin



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

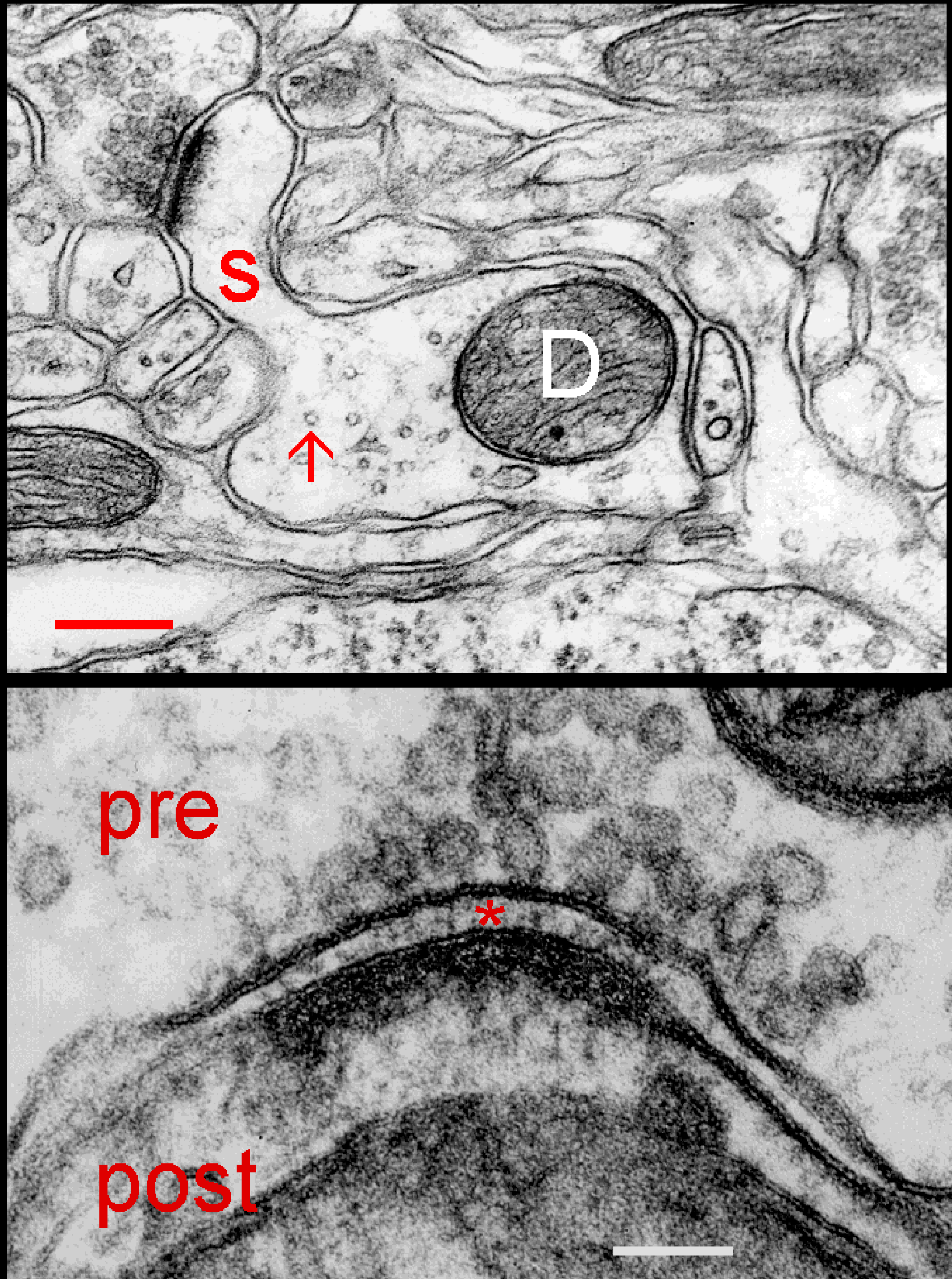
Cell adhesion molecules

Axon guidance molecules

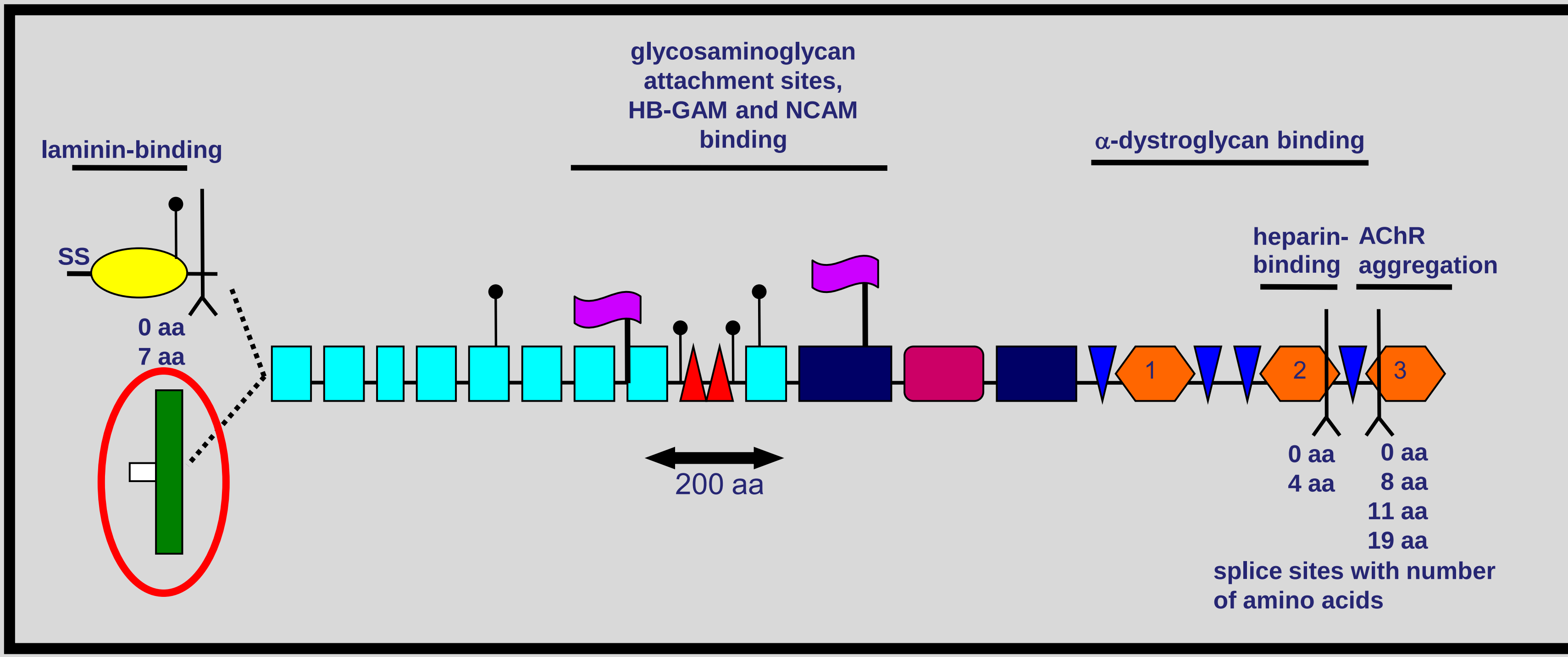
Glia-derived factors

Distribution of Agrin mRNA in the Adult Mouse Brain

Source: Allen Brain Atlas


S
D
pre
*
post

Domain Structure of Agrin



intracellular domain

transmembrane domain (TM)

NtA-domain

follistatin-like domain

conserved GAG chain attachment site

potential N-linked glycosilation site

laminin EGF-like domain

Ser/Thr-rich region

SEA (sea urchin sperm protein, enterokinase and agrin) domain

EGF-like domain

laminin globular domain

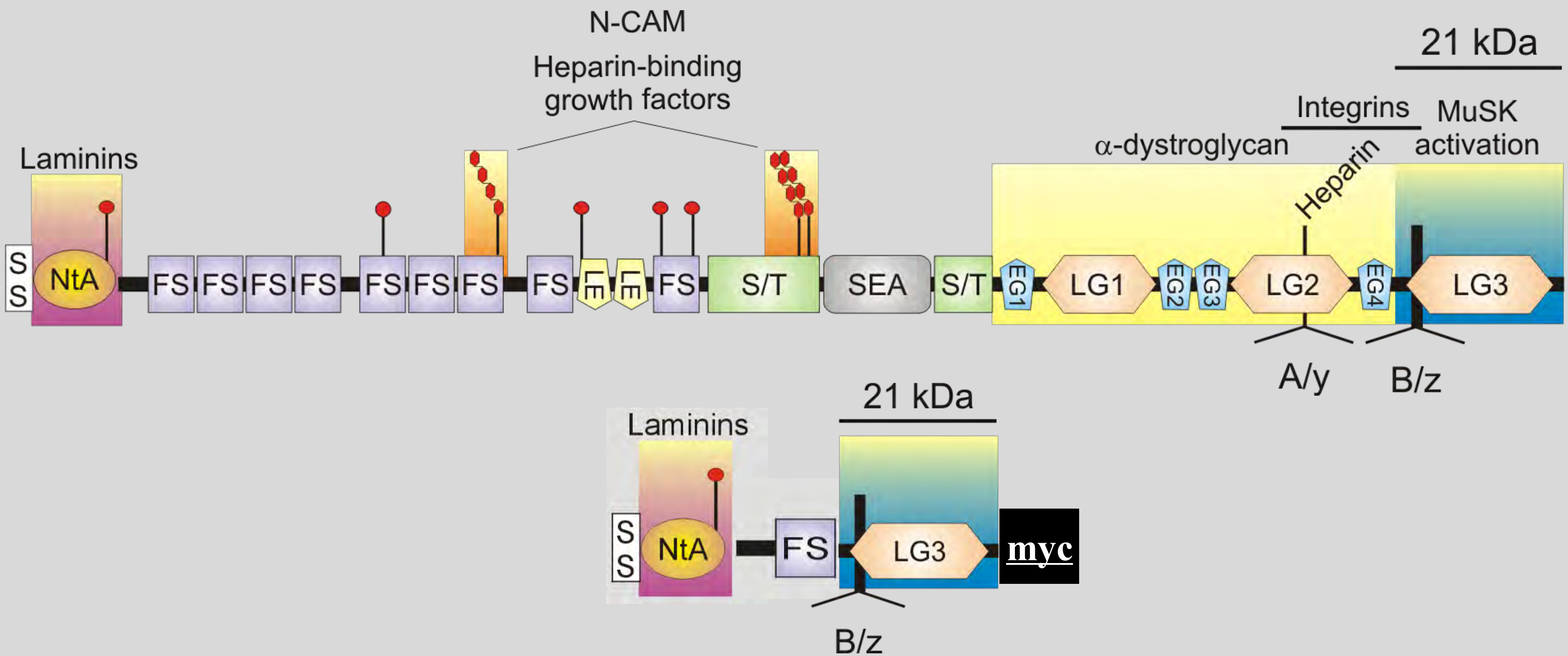
Evidence for a Role of Agrin during CNS Synaptogenesis

- All agrin isoforms are expressed in CNS tissue during development as well as in the adult
- Agrin expression is not limited to cholinergic neurons (O'Connor et al., 1994)
- Neurons and glial cells express agrin – but different isoforms; neurons: mostly TM-agrin, glial cells: mostly NtA-agrin
- Agrin is enriched in synaptosomal preparations of brain tissue (Böse et al., 2000)
- Agrin-mediated AChR aggregation activity can be extracted from brain tissue (Magill-Solc and McMahan, 1988)
- Agrin is present in the supernatant of cultured CNS cells (Mann and Kröger, 1996)
- Agrin mRNA is highest during phase of neurite growth and synaptogenesis – downregulated thereafter
- Individual CNS neurons express several agrin isoforms (Annies and Kröger, 2002)
- Long-lasting and rapid upregulation of agrin mRNA by kainate injection (induction of seizures; O'Connor et al., 1995) or by traumatic brain injury (Falo et al., 2008) suggesting a dynamic expression of agrin and maybe suggesting a need of agrin for recovery strategy during reorganization or new formation of synapses
- **Cultures** of hippocampal or cortical neurons from E18,5 agrin -/- mice form synapses that were indistinguishable from control cultures (Serpinskaya et al., 1999; Li et al., 1999) – why no effect??? Compensatory mechanisms?

The Advantage of ko Mice:

- „In many cases the animal dies, so it is concluded that the gene in question is important ..... In many more cases the effects of knockout are quite small (in which case the conclusion is often the same – the gene must be important because mechanisms must exist to compensate for its loss).“
- D. Colquhoun and B. Sakmann Neuron **20:** 381-387.

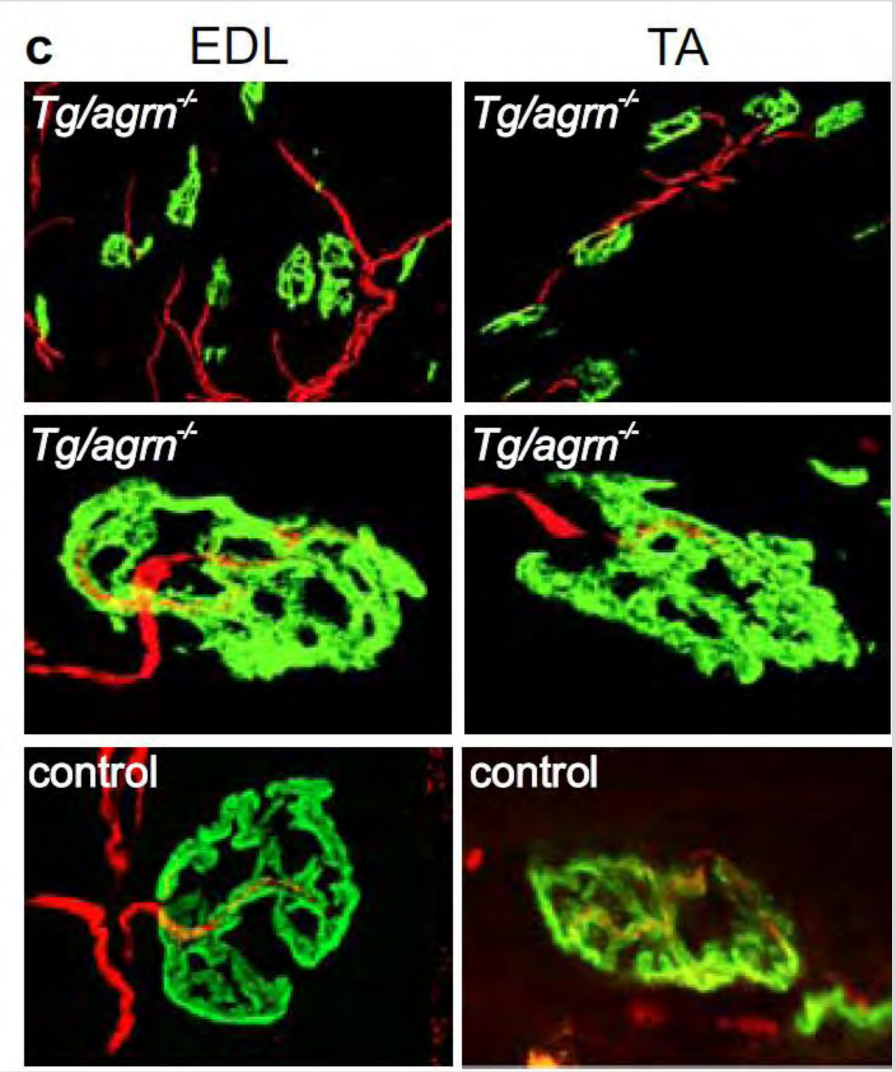
Agrin Rescue Mice



Mini-agrin transgenic mice:
Expression driven by a motoneuron-specific promoter!

Ksiazek et al., 2007

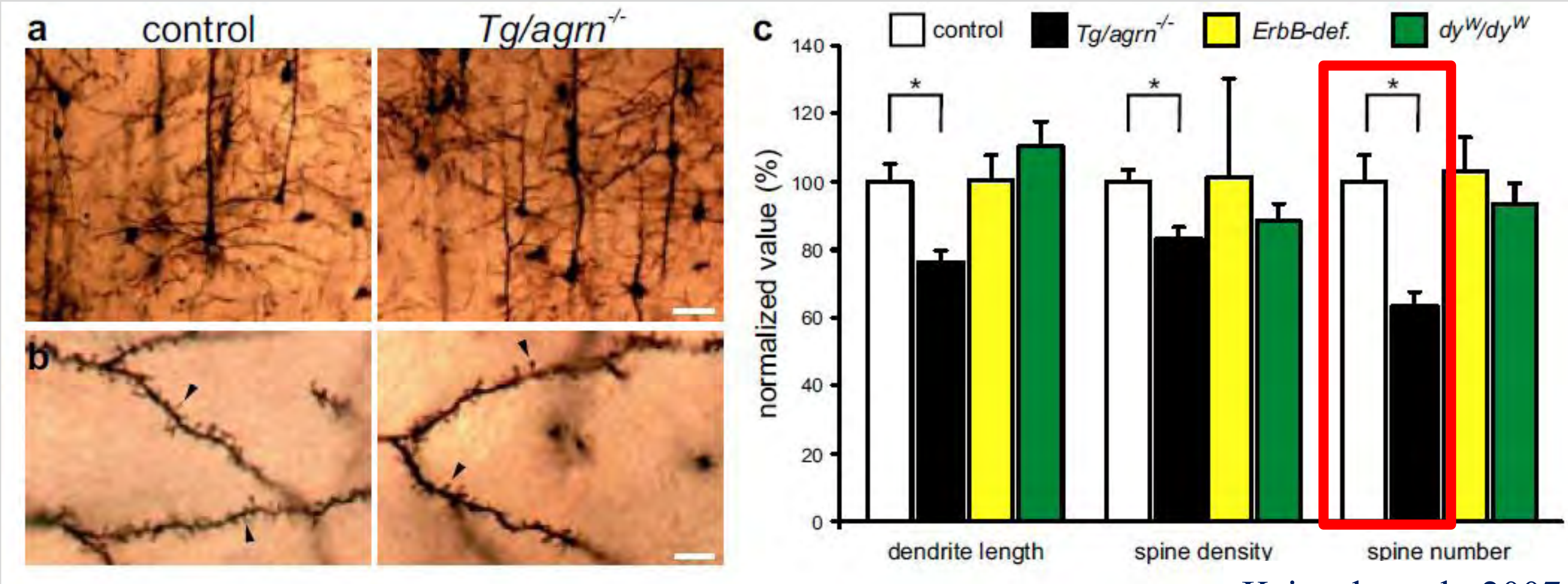
Neuromuscular Junctions in Rescue Mice have Normal Morphology



Ksiazek et al., 2007

- Red: motoneurons
- Green: α-bungarotoxin
- Some muscles (soleus or diaphragm): nerve terminals sprouted and muscle atrophied
- Mice die after ~50 days

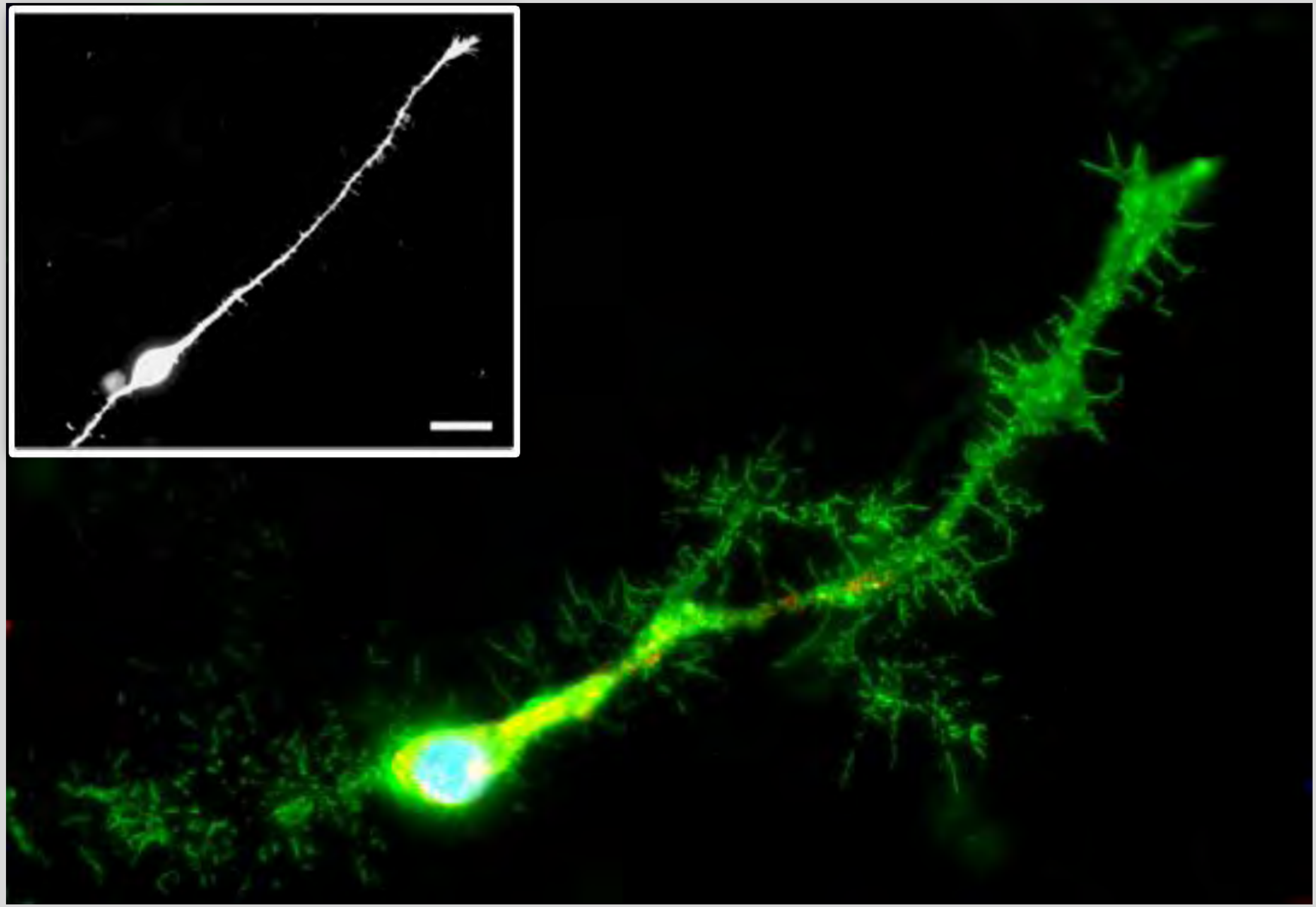
The Number of Synapses in the CNS of Agrin-Deficient Mice is 30% reduced



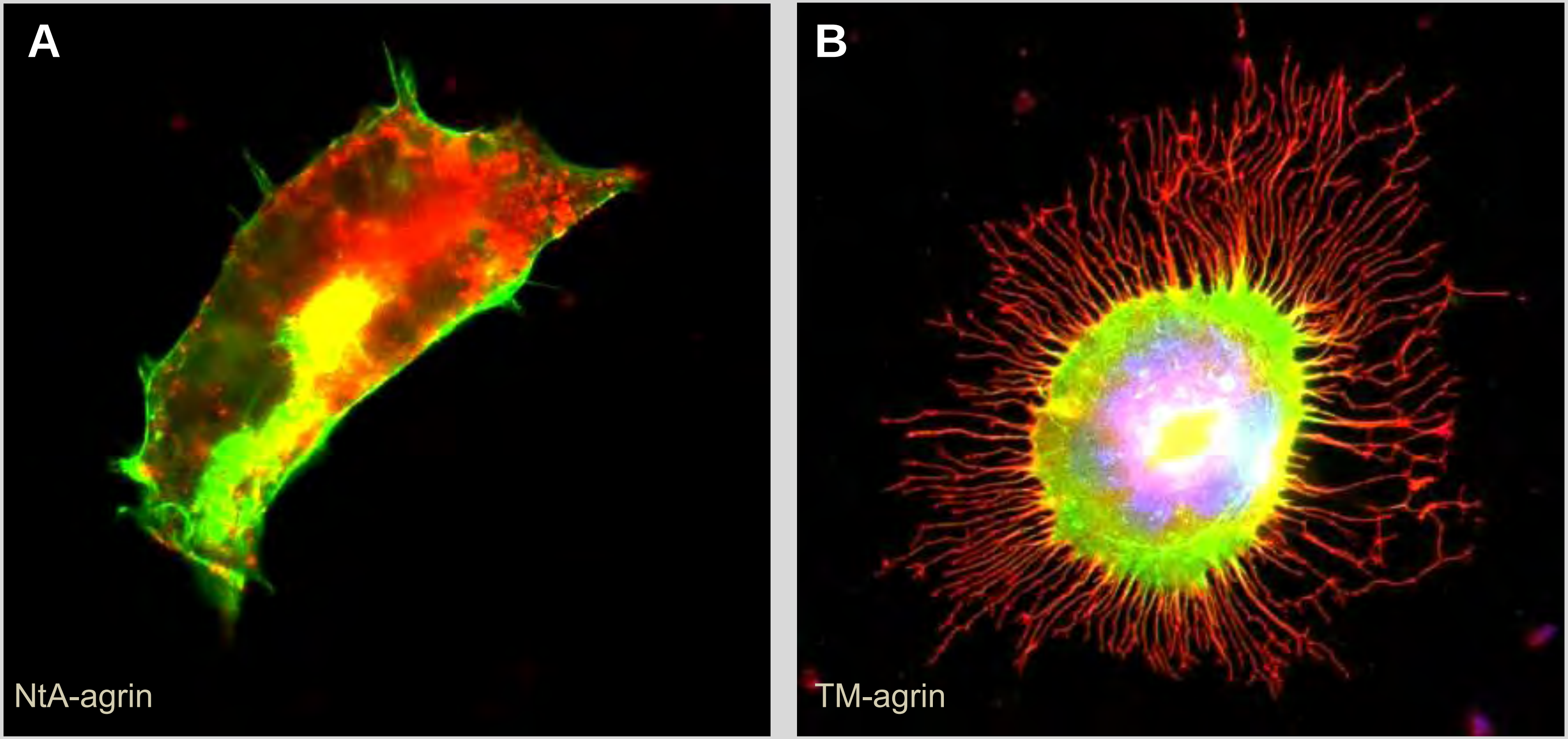
Ksiazek et al., 2007

- Golgi-Cox silver impregnation technique
- Length of dendrite, density of spines, and number of spines reduced in Tg/agrn -/- mice
- Since Tg/agrn-/- mice are smaller, ErbB-/- and dy^W/dy^W mice served as controls

Overexpression of TM-Agrin Induces Processes in CNS Neurons



Overexpression of TM-Agrin in non-Neuronal Cells Induces Processes



green: actin
red: agrin

red: agrin
green: actin

Process formation also on

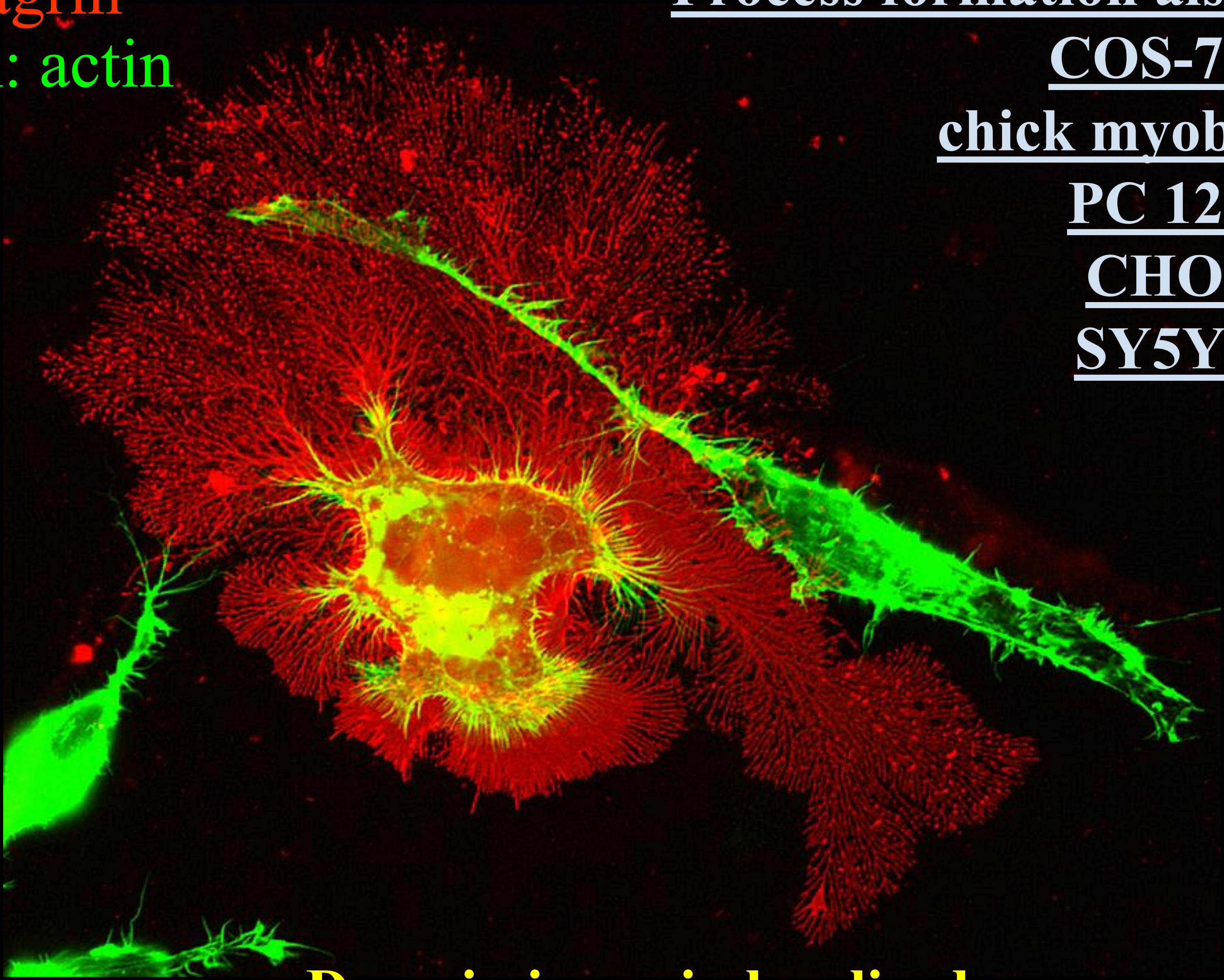
COS-7 cells

chick myoblasts

PC 12 cells

CHO cells

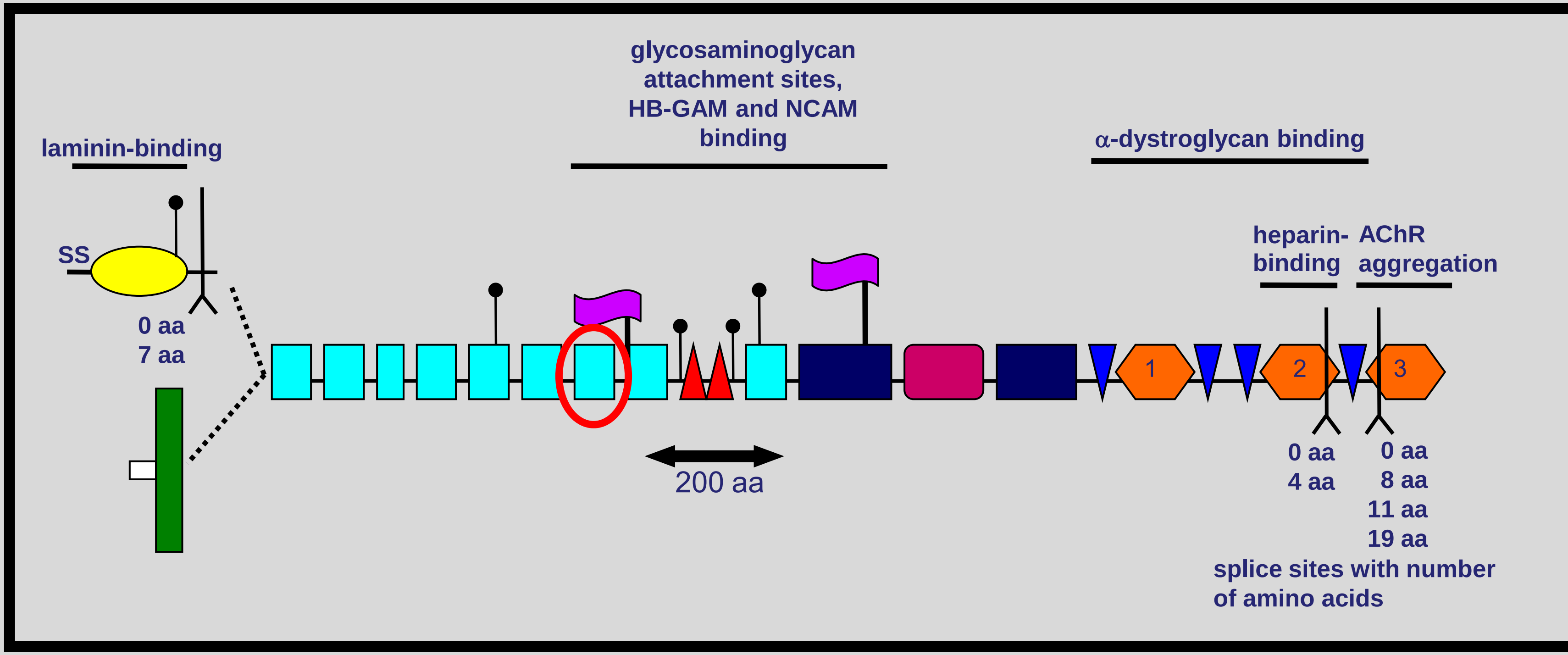
SY5Y cells



Domain in agrin localized

Signal cascade established

Domain Structure of Agrin



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SEA (sea urchin sperm protein, enterokinase and agrin) domain

potential N-linked glycosilation site

EGF-like domain

NtA-domain

laminin EGF-like domain

laminin globular domain

Is there evidence that the processes induced by TM-agrin are involved in synaptogenesis in the CNS?

Yes

Analysis during adult neurogenesis

Are Agrin-Induced Processes Involved in Synapse Formation in the Adult CNS?

The Journal of Neuroscience, March 14, 2012 • 32(11):3759–3764 • 3759

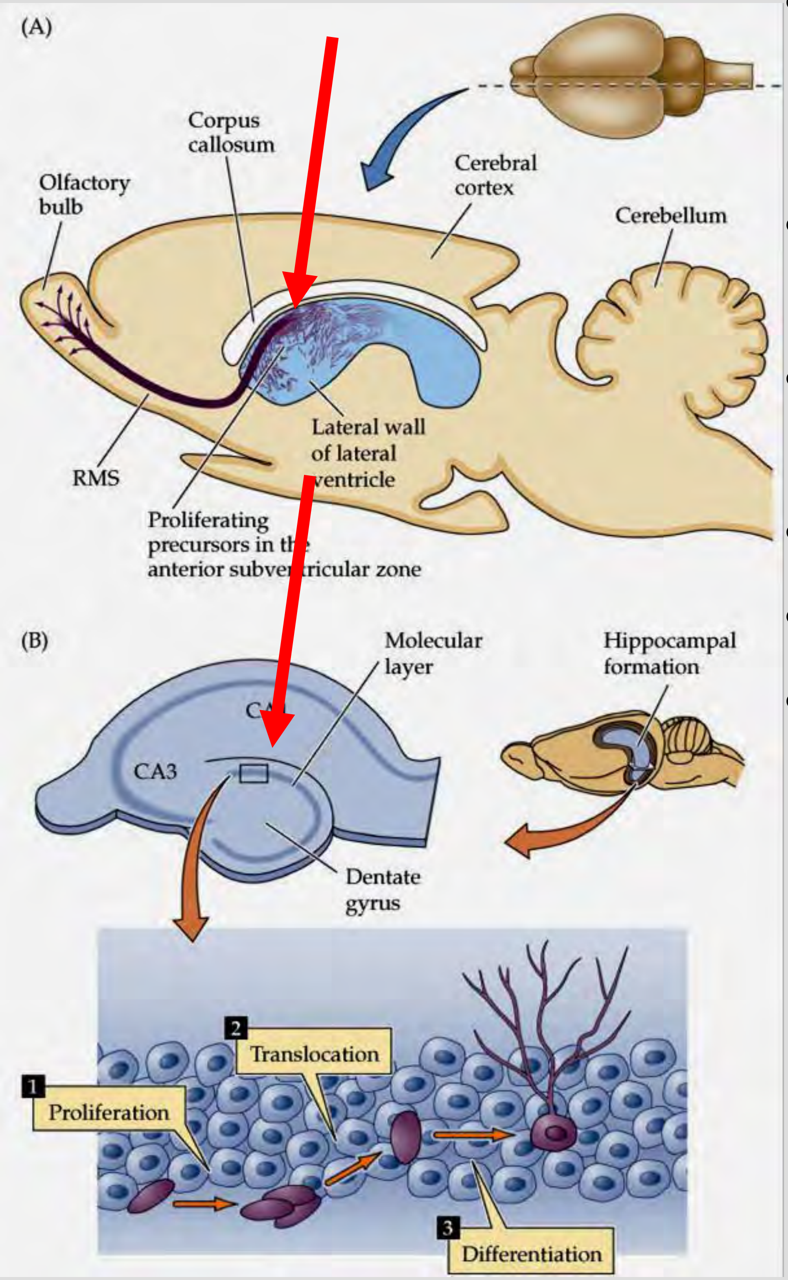
Brief Communications

Agrin-Signaling Is Necessary for the Integration of Newly Generated Neurons in the Adult Olfactory Bulb

Katja Burk,[1,2*] Angelique Desoeuvre,[1,2*] Camille Boutin,[1,2] Martin A. Smith,[3] Stephan Kröger,[4] Andreas Bosio,[5] Marie-Catherine Tiveron,[1,2†] and Harold Cremer[1,2†]

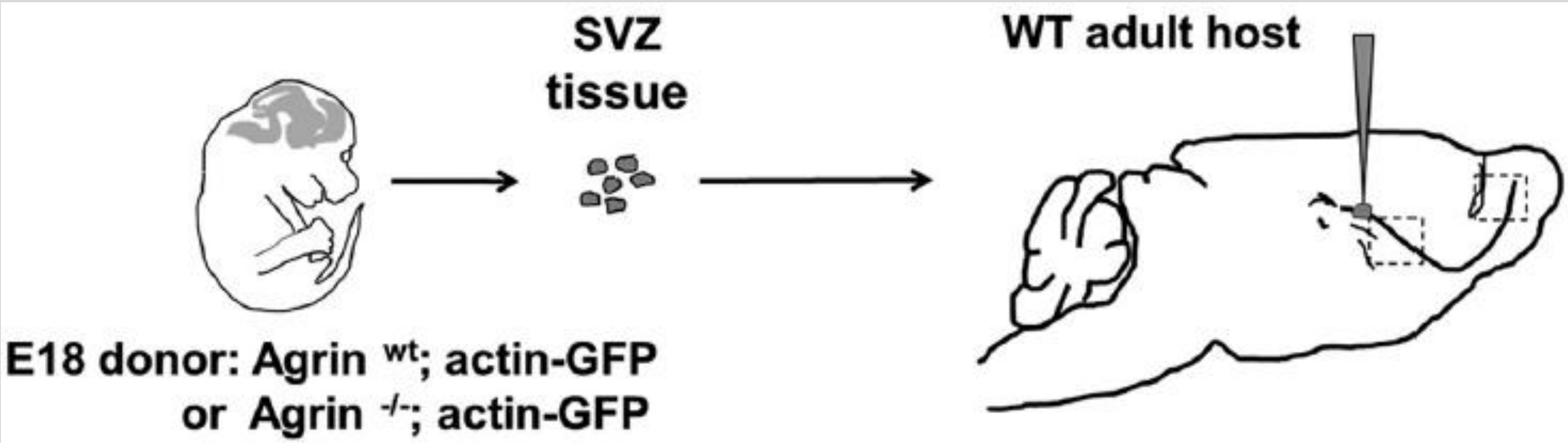
- Serial analysis of gene expression in neuroblasts of the RMS
- Compared to total brain cDNA
- Strong over-representation of agrin transcripts (Pennartz et al., 2004)

Neurogenesis in the adult mammalian brain



- No adult neurogenesis in the cortex, but the adult CNS generates new neurons!
- **HOWEVER ONLY IN TWO REGIONS:**
- 1. lateral wall of ventricle (olfactory system)
- 2. dentate gyrus of hippocampus
- Mostly interneurons are generated
- In the subventricular zone (SVZ) of the lateral ventricle new neuroblasts are generated that migrate along the rostral migratory stream (chain migration) to the olfactory bulb where they differentiate into neurons (mostly GABA-ergic interneurons – granule cells and periglomerular neurons – but also few glutamatergic neurons)

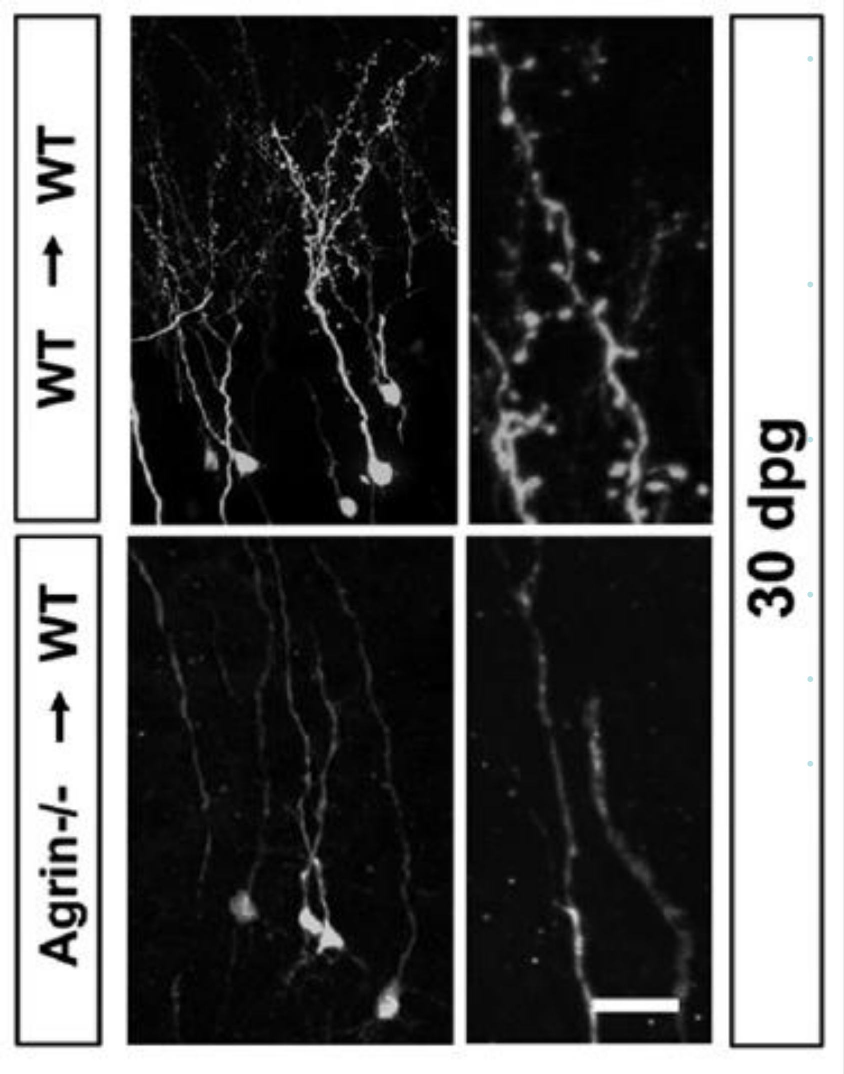
Transplantation Approach to Analyze the Role of Agrin During Adult Neurogenesis



Burk et al., 2012

- Mice deficient for all agrin isoforms crossed with actin-GFP mice
- SVZ tissue from E 18.5 was transplanted into adult host WT mouse SVZ
- 10 days later green cells were detected in RMS – no difference in migratory behavior of cells with different genotypes

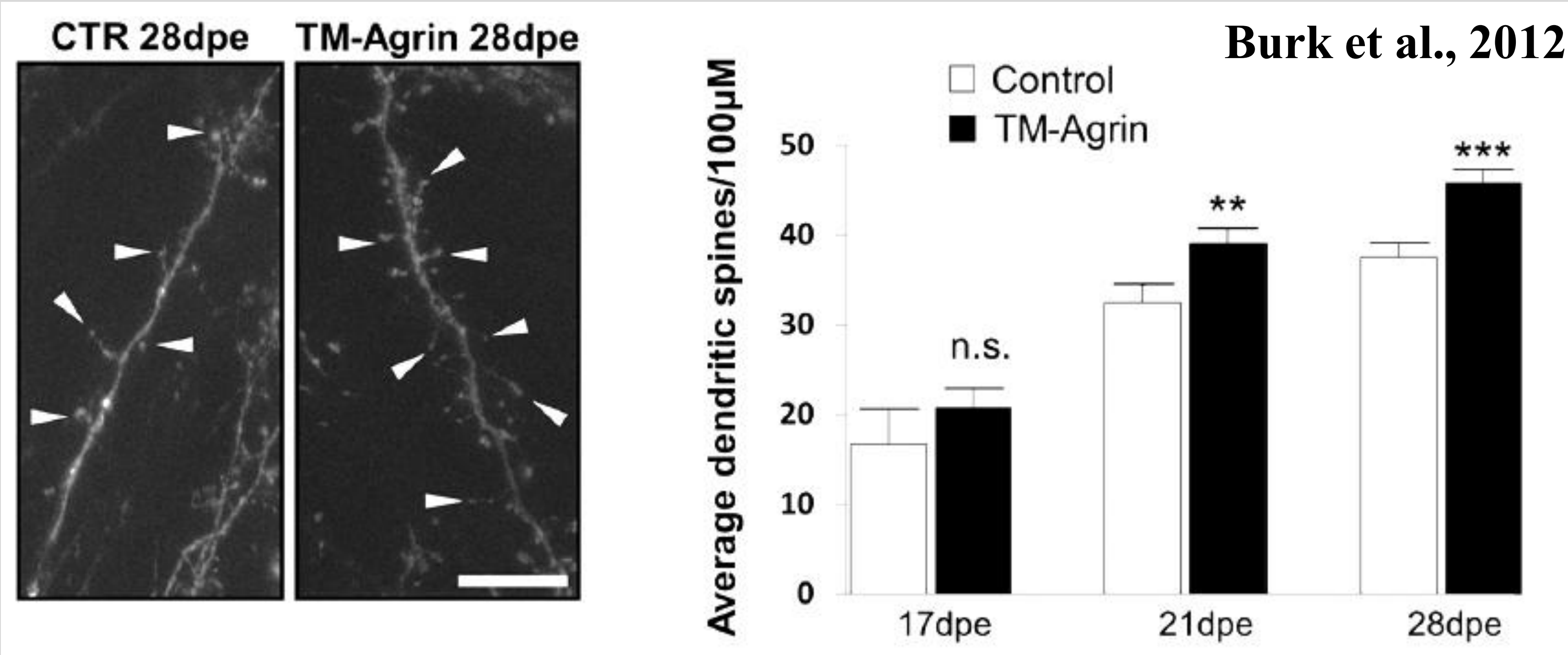
Agrin Deficiency Compromises Survival and Integration of Neurons in Olfactory Bulb



Burk et al., 2012

- 10 days postgrafting – no difference between the agrin -/- cells and the wt cells
- 30 days postgrafting: most agrin -/- cells had degenerated
- The few remaining have very "simple" morphology
- Few spines
- After 60 days hardly any cell survived
- Agrin deficiency compromises the survival and/or maintenance and/or integration of neuroblasts in olfactory bulb

TM-Agrin Overexpression Induces Formation of Spine-Like Structures in Transfected Neurons



- Knockdown specifically of TM-agrin mimicked the effect!
- Effect of knockdown was eliminated by coexpression specifically of TM-agrin
- More synaptic spines (synapses) are generated after TM-agrin overexpression *in vivo* – functional consequences??

agrin: the good, the bad, the ugly

- **Good:** undisputed that agrin is the "master organizer of synaptogenesis" at the developing and regenerating NMJ
- **Bad:** less important but necessary during CNS synaptogenesis
- **Ugly:** many additional functions – not well characterized