



**UNIVERSITÀ
DEGLI STUDI
DI TRIESTE**



Dipartimento di
Scienze della vita



Corso di Laurea Magistrale in *Medicina e Chirurgia*

Corso di **FISIOLOGIA UMANA**

a.a. 2025-26

NEUROFISIOLOGIA

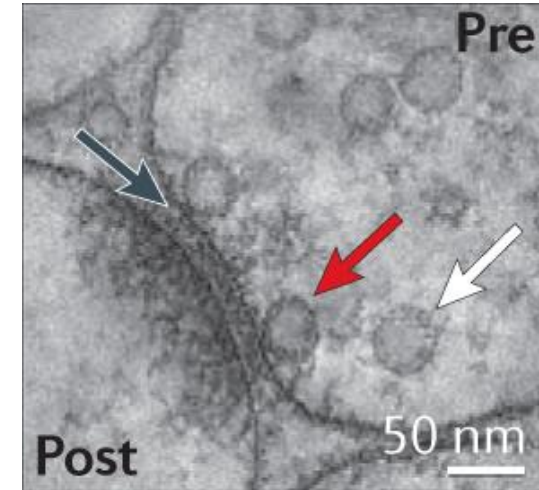
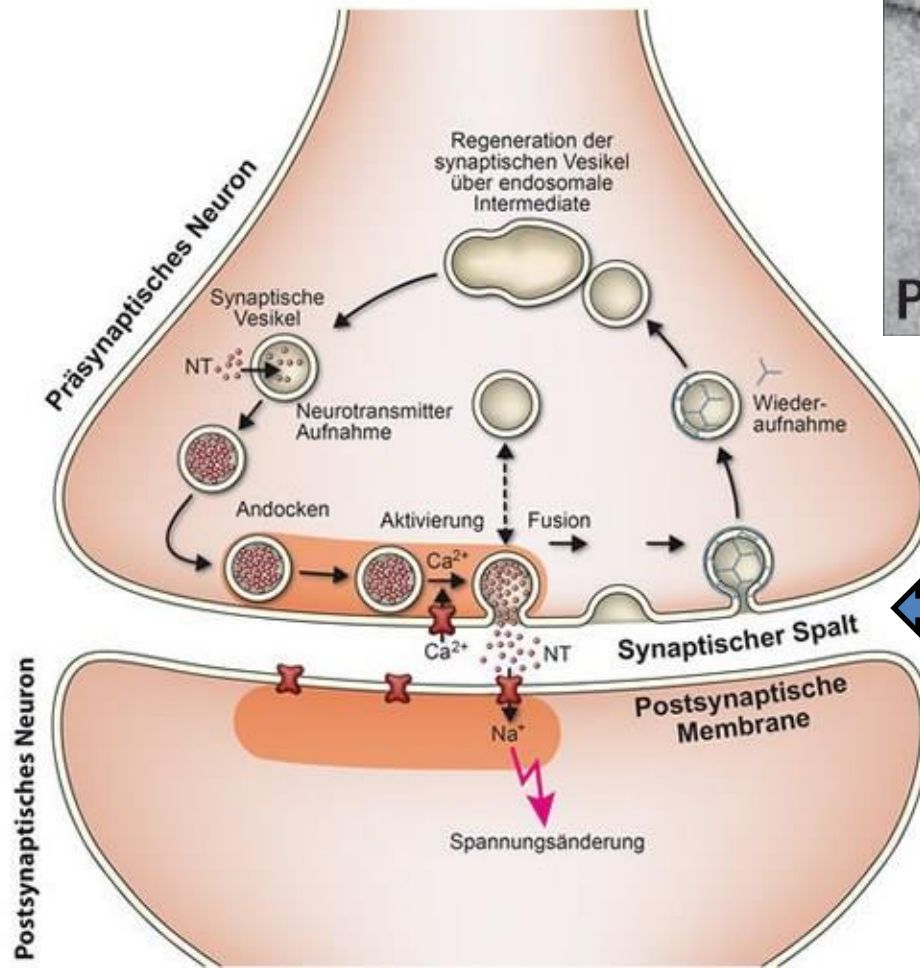
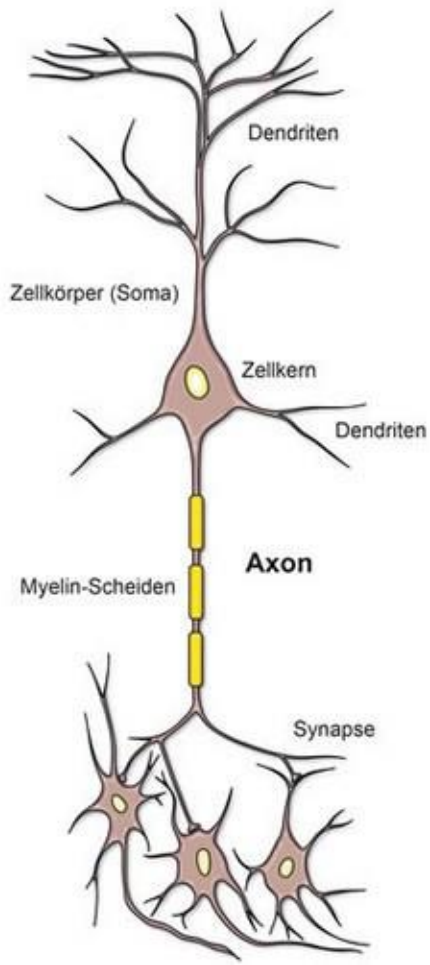
Sviluppo dei circuiti neuronali

Lorenzo Cingolani
lcingolani@units.it

PROGRAMMA DETTAGLIATO

- **Target selection: il problema**
- **Base genetica e base funzionale del cablaggio cerebrale**
- **Il codice molecolare della specificità sinaptica. Tre esempi**
 - **Caderine**
 - **Protocaderine**
 - **Neuroligine- neurexine**
- **Malattie del neurosviluppo come patologie della connettività**

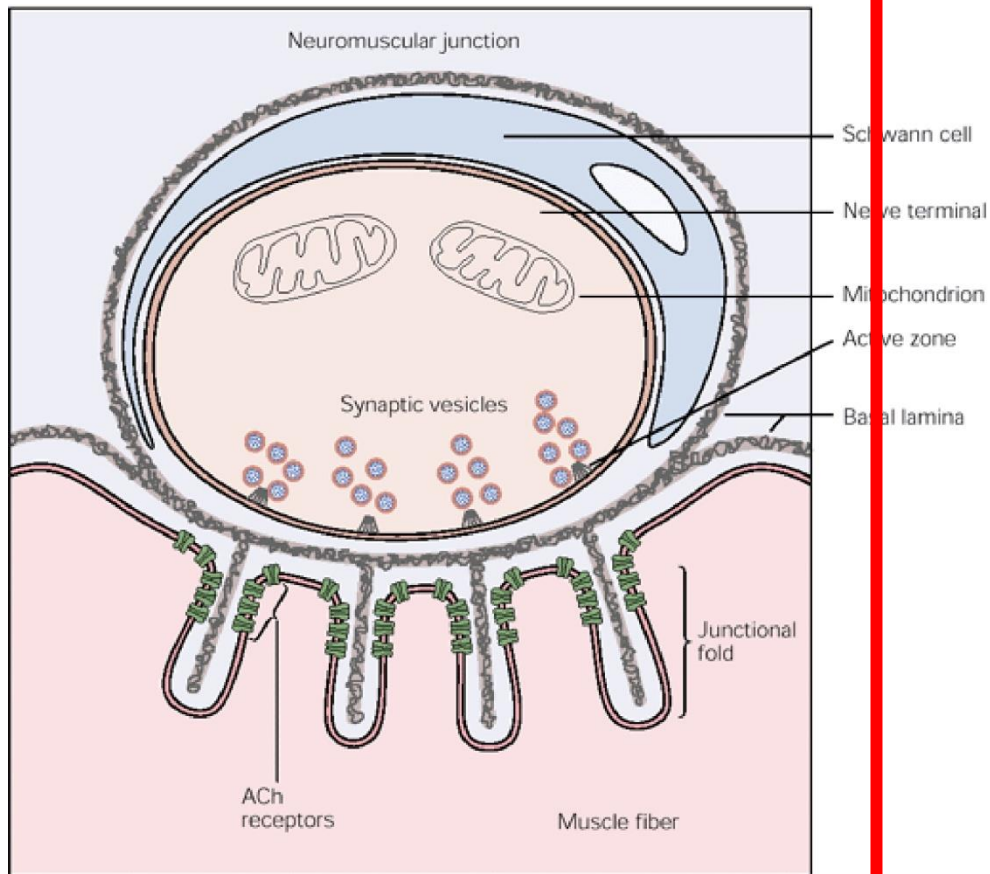
Synapses are specialized intercellular junctions



← Synaptic cleft

Synaptic cleft

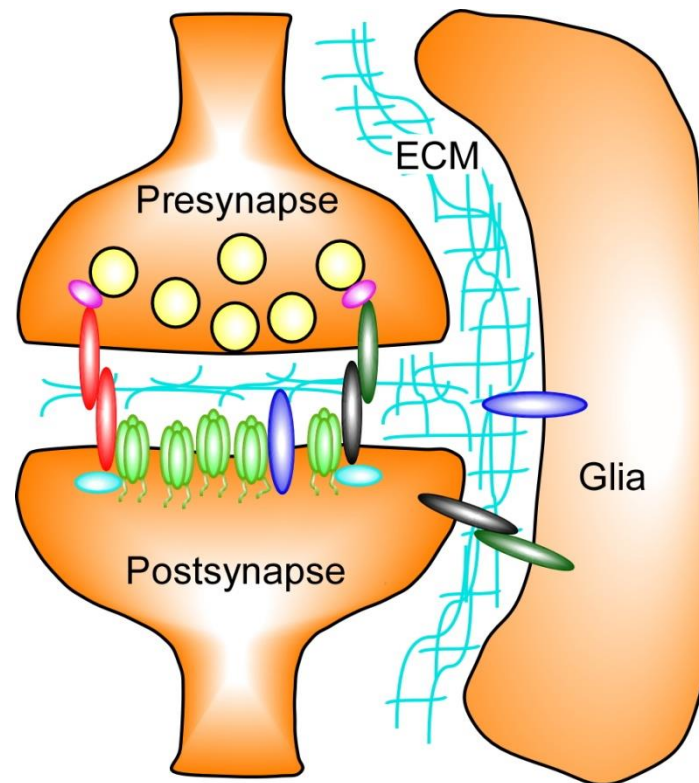
Neuromuscular junction



100 nm

Interposed Basal lamina

Central synapse

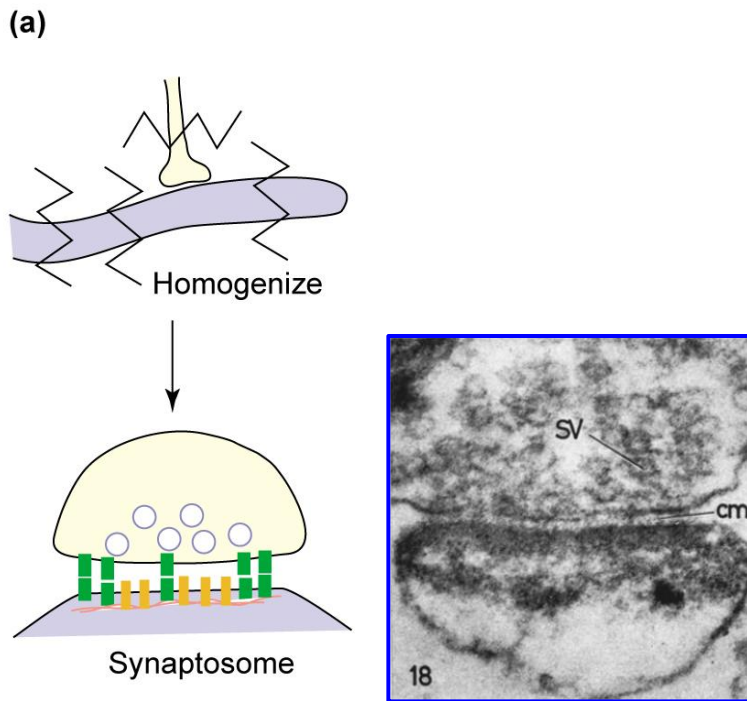


20 nm

Direct cell-cell interactions possible

Synaptic cell adhesion molecules

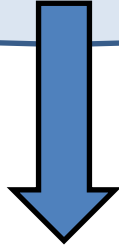
Stability



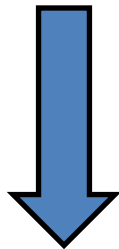
1) Non cell autonomous process

2) Direct physical interaction

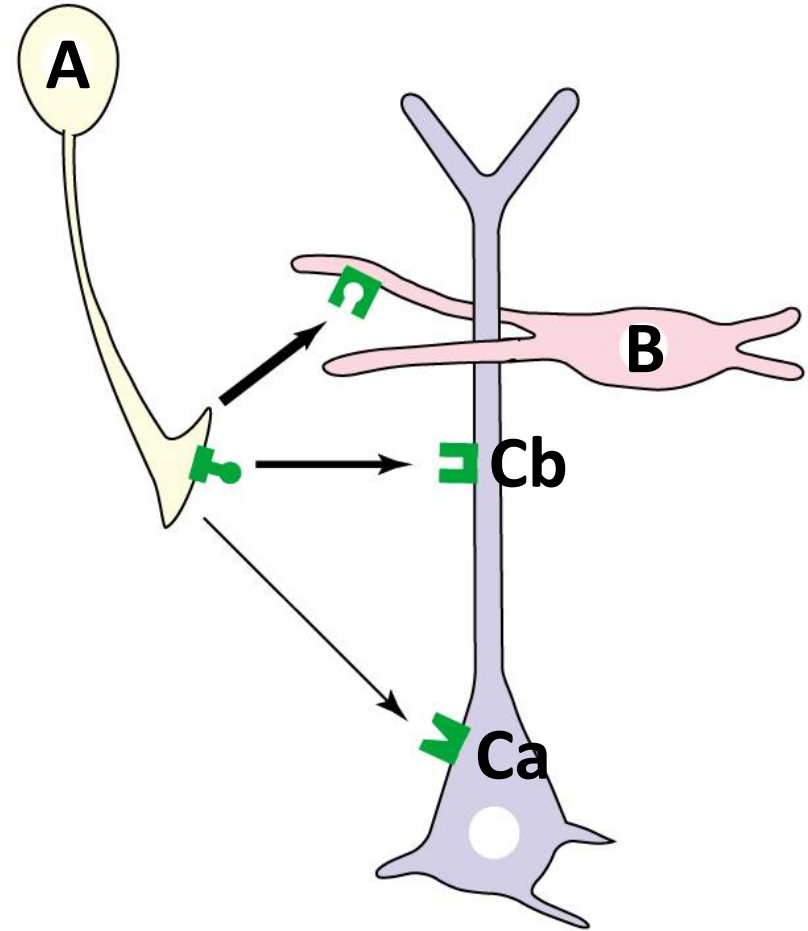
Target selection



Synaptic maturation



Synaptic function

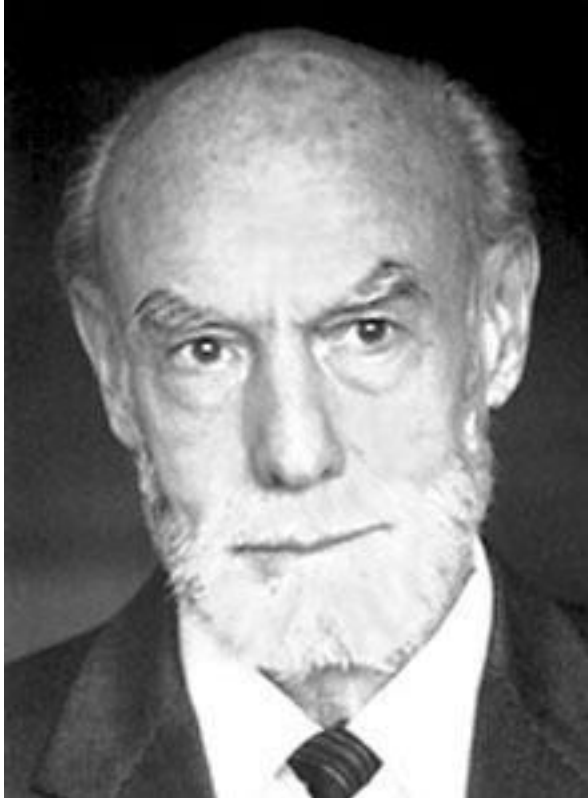


Every connection is genetically specified



Connections sort themselves out through functional validation

Roger Sperry



1913 – 1994

American neuropsychologist and neurobiologist

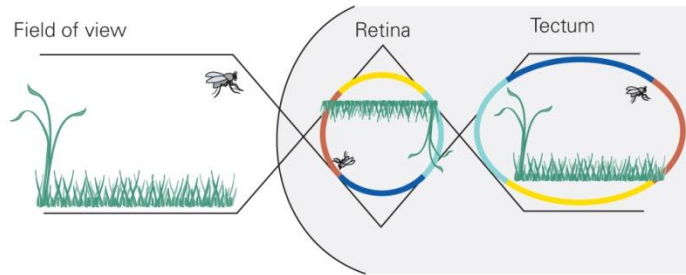
1981: Nobel Prize in Physiology or Medicine for his work on the split-brain

Roger Sperry's classical experiment

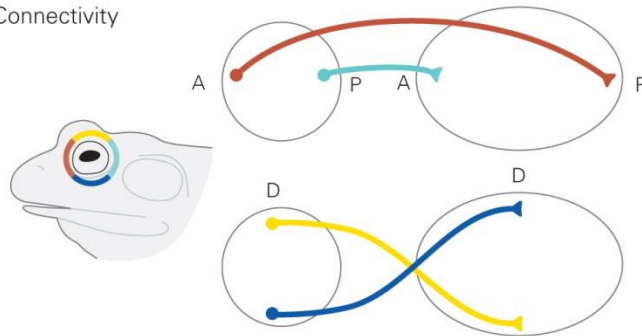
A Normal

Optics

Field of view

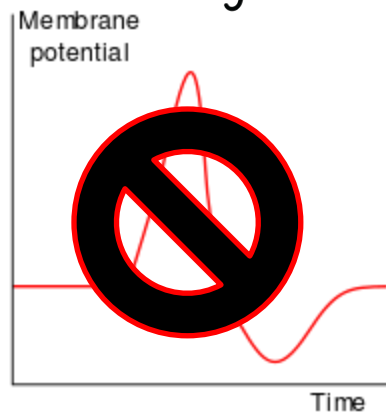
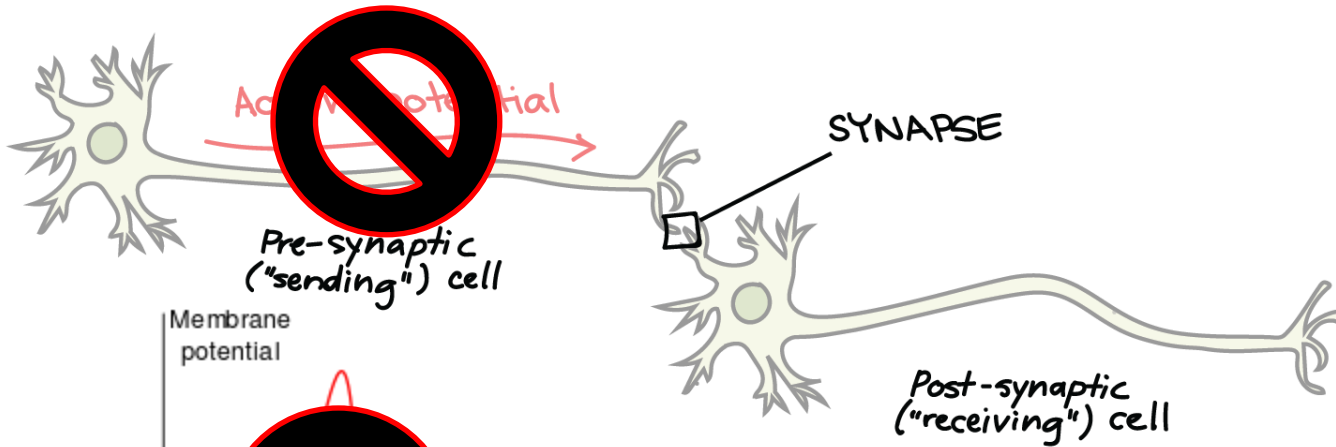


Connectivity



First experiment suggesting the existence of **'biochemical tags'**

Target selection occurs accurately in the absence of neural activity



TTX



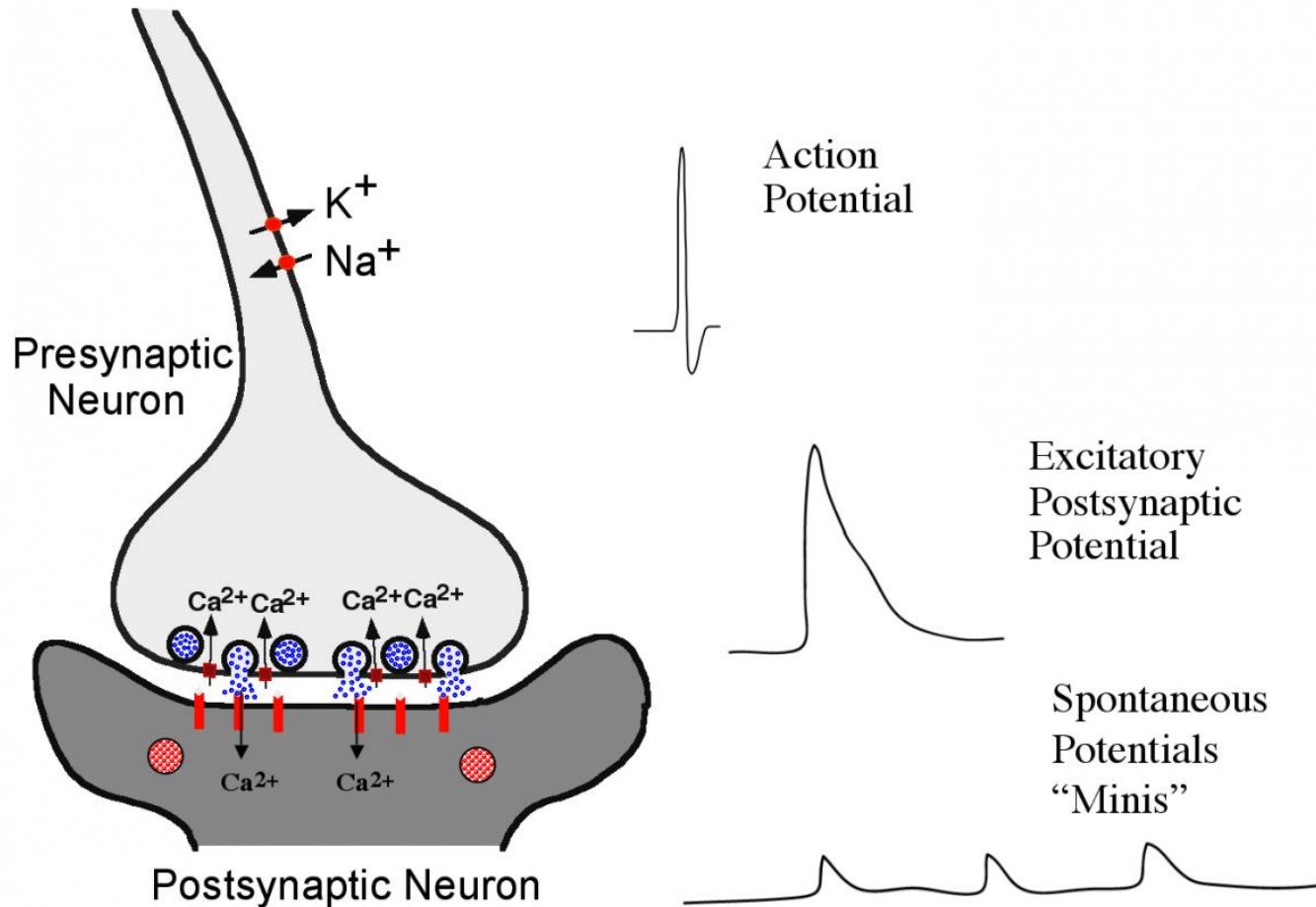
AXONAL PATHFINDING IN THE ABSENCE OF NORMAL PATHWAYS AND IMPULSE ACTIVITY¹

WILLIAM A. HARRIS

Department of Biology, University of California, San Diego, La Jolla, California 92093

Received September 2, 1983; Revised December 13, 1983; Accepted December 22, 1983

What about spontaneous neurotransmitter release?



Synaptic Assembly of the Brain in the Absence of Neurotransmitter Secretion

Matthijs Verhage,^{1,2,*} Ascanio S. Maia,¹ Jaap J. Plomp,³
Arjen B. Brussaard,⁴ Joost H. Heeroma,¹ Hendrika Vermeer,¹
Ruud F. Toonen,¹ Robert E. Hammer,⁵ Timo K. van den Berg,⁶
Markus Missler,^{2,†} Hans J. Geuze,⁷ Thomas C. Südhof^{2*}

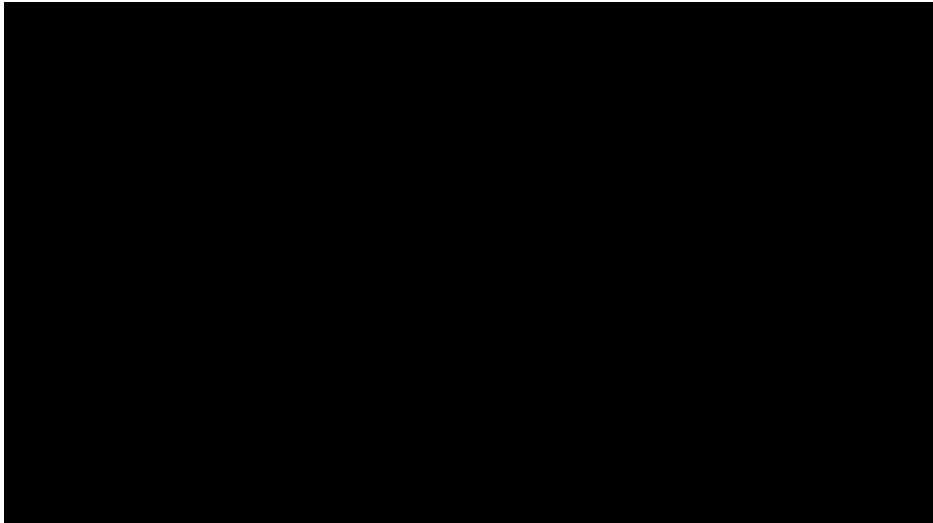
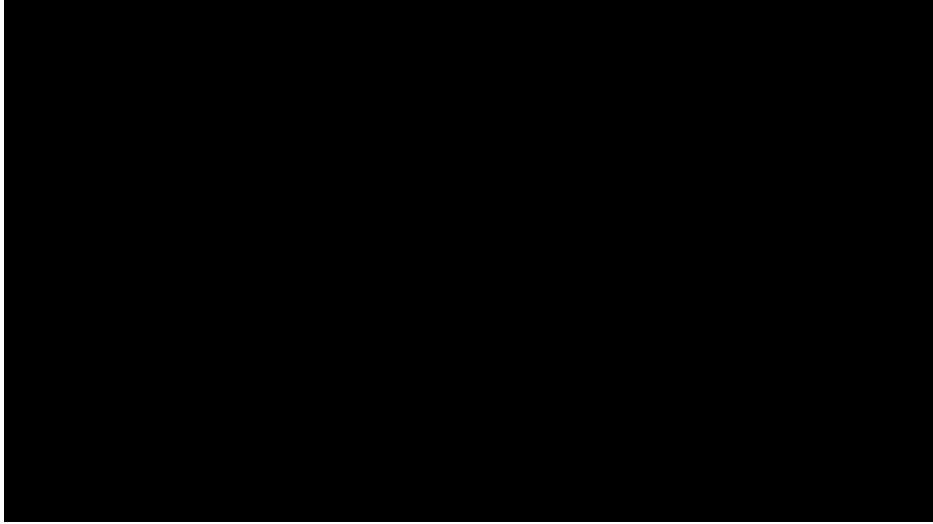
Brain function requires precisely orchestrated connectivity between neurons. Establishment of these connections is believed to require signals secreted from outgrowing axons, followed by synapse formation between selected neurons. Deletion of a single protein, Munc18-1, in mice leads to a complete loss of neurotransmitter secretion from synaptic vesicles throughout development. However, this does not prevent normal brain assembly, including formation of layered structures, fiber pathways, and morphologically defined synapses. After assembly is completed, neurons undergo apoptosis, leading to widespread neurodegeneration. Thus, synaptic connectivity does not depend on neurotransmitter secretion, but its maintenance does. Neurotransmitter secretion probably functions to validate already established synaptic connections.

Every connection is genetically specified

?

Connections sort themselves out through functional validation

Connectomics

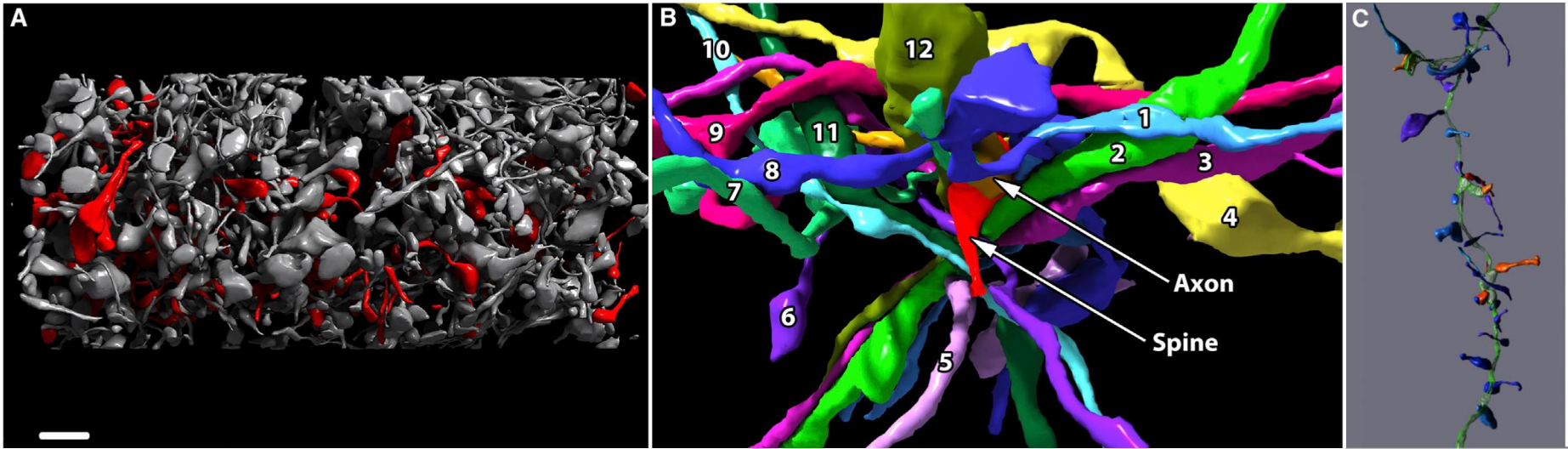


The problem

1500 μm^3

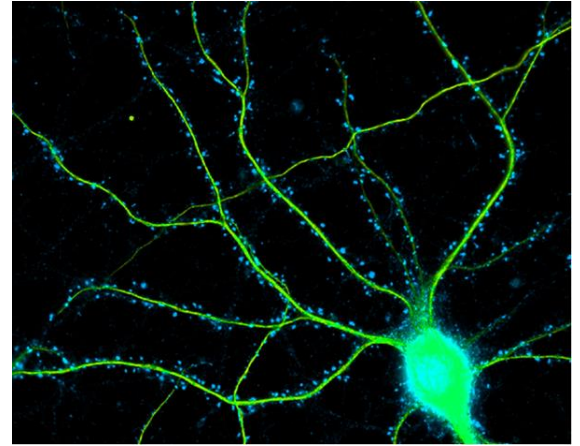
1700 synapses

1.13 synapses/ μm^3



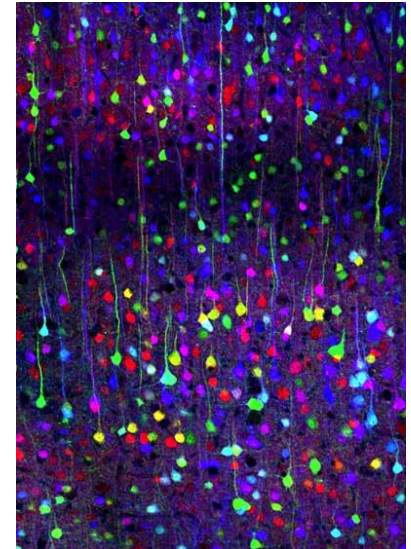
The problem

➤ 1 neuron



➤ 1 mm³ of mammalian cortex

➤ Number of neurons: 10⁵

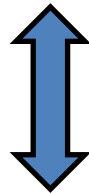


The problem

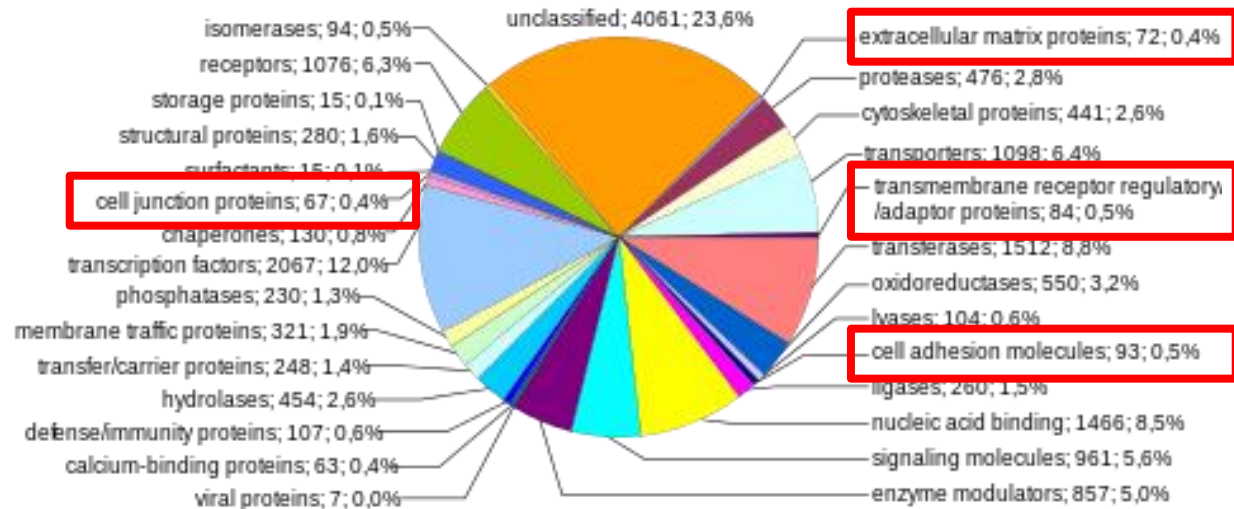
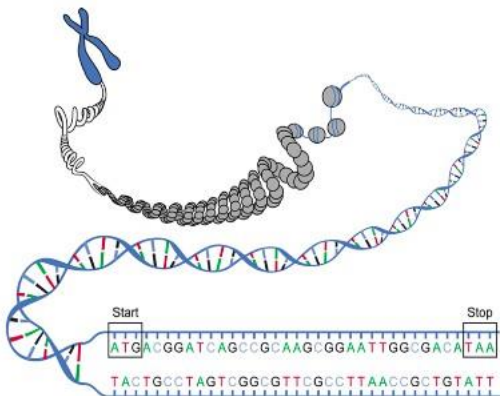
- Human brain: **1 dm³**
- Number of neurons: **10¹¹**
- Number of synapses: **10¹⁵**



???

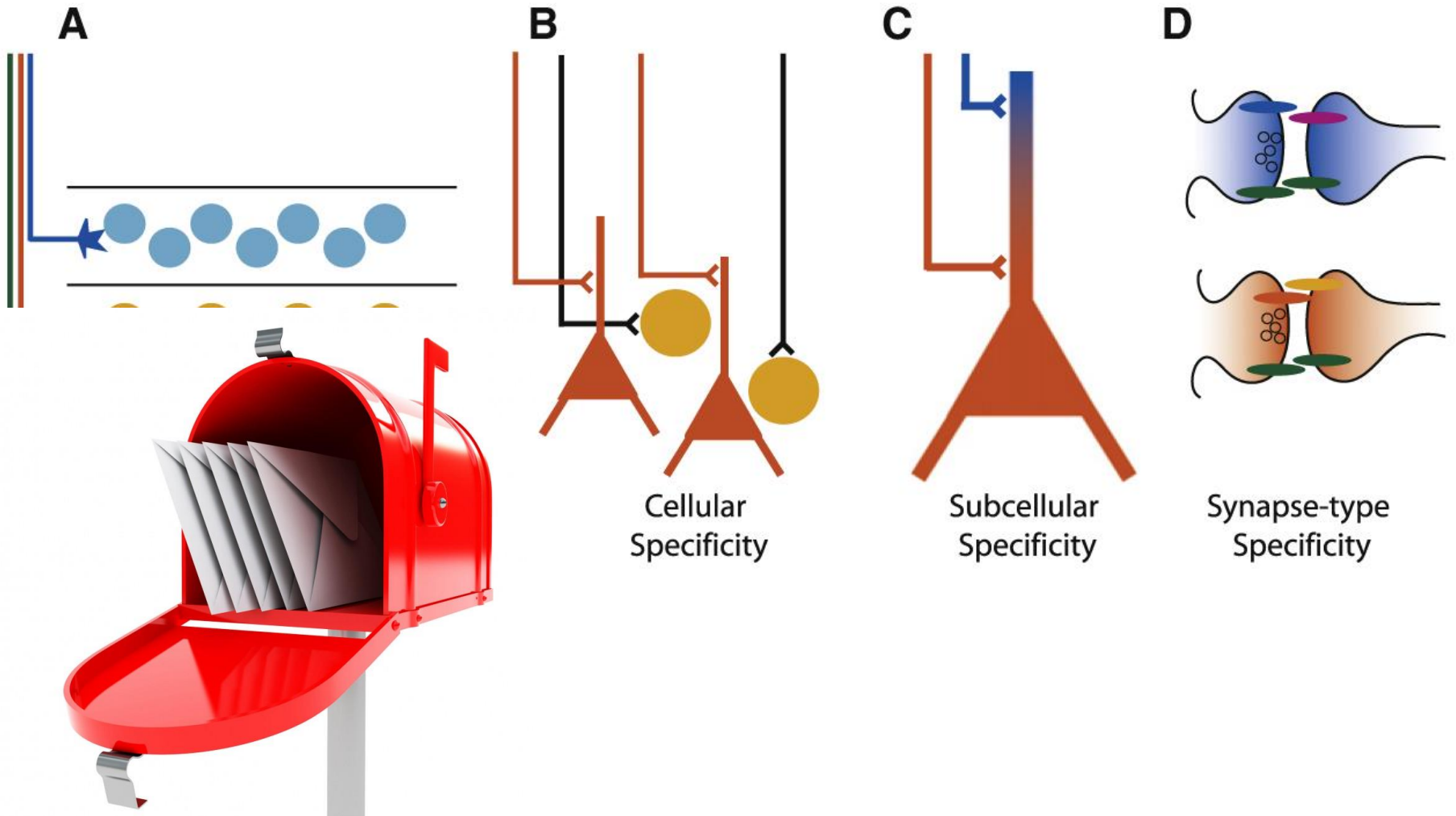


- Number of genes:



**How can 'biochemical tags'
(= synaptic CAMs) contribute
to the molecular code
for synaptic specificity?**

Target selection can be broken down into a number of steps



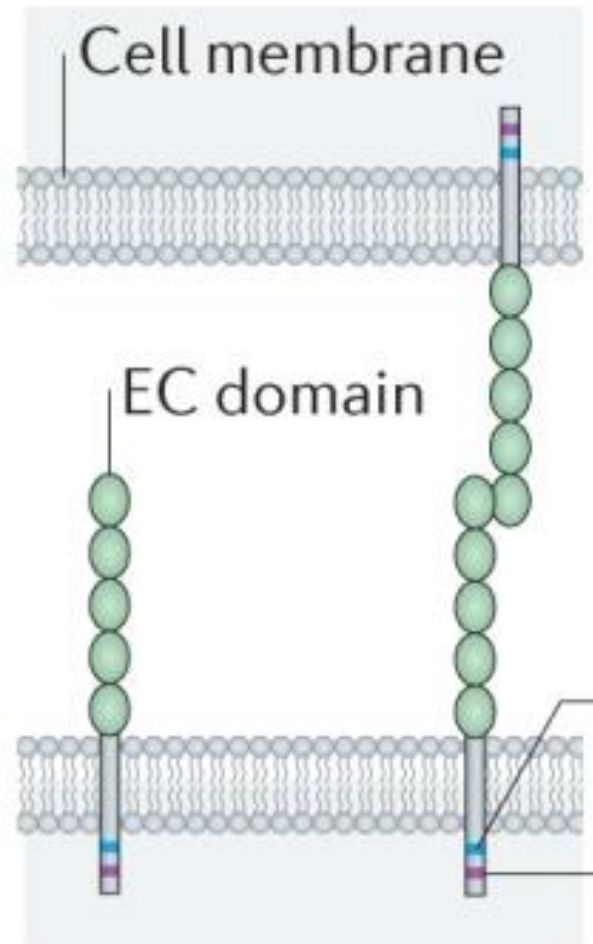
What requirements cell adhesion molecules that regulate synaptic specificity need to have

They should:

- 1) be synaptic
- 2) be expressed in distinct populations of neurons
- 3) be capable of interacting in *trans*, specifically and with high affinity with their binding partner(s)
- 4) provide enough molecular diversity to confer cell type- and synapse type-specific identities

Three examples:

Classic cadherins

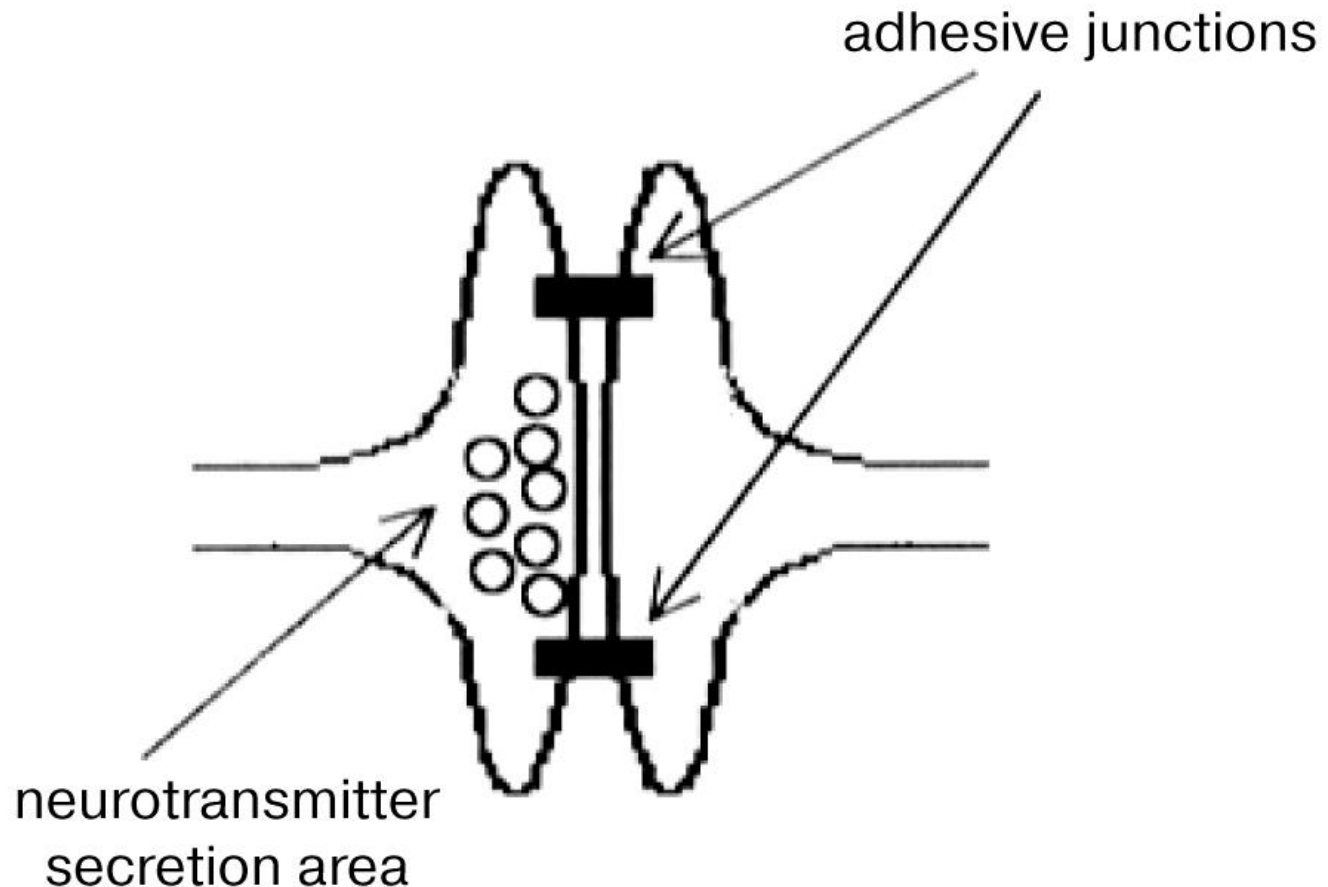


The cadherin superfamily comprises >100 members

Table 1. Members of the cadherin superfamily in human

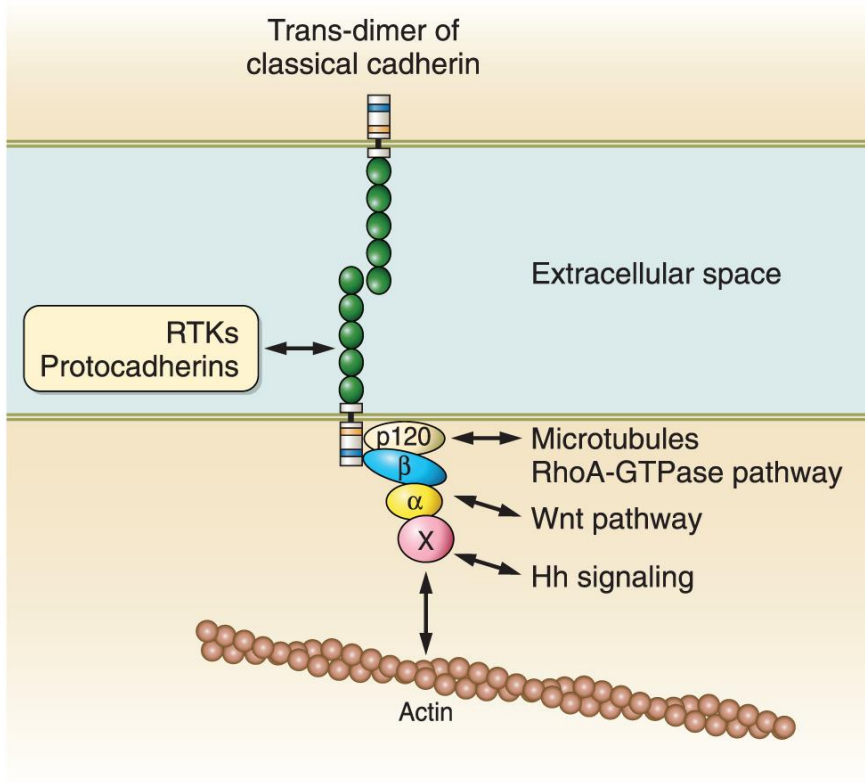
Cadherin Superfamily	Members
Classic cadherin	18 members
Type I: CDH1 (E-cad), CDH2 (N-cad), CDH3 (P-cad), CDH4 (R-cad) CDH15 (M-cad)	(5 members)
Type II: CDH5 (VE-cad), CDH6 (K-cad), CDH7, CDH8, CDH9 (T1-cad) CDH10 (T2-cad), CDH11 (OB-cad), CDH12 (N-cad-2), CDH18, CDH19, CDH20, CDH22, CDH24	(13 members)
Desmosomal cadherin	7 members
Desmocollins: DSC1, DSC2, DSC3	
Desmogleins: DSG1, DSG2, DSG3, DSG4	
CDH13 CDH13 (T-cad)	1 member
7D family CDH16 (Ksp-cad), CDH17 (LI-cad)	2 members
Protocadherins	64 members
Clustering	
Pcdh α : PCDH α 1-13, c1,2	(15 members)
Pcdh β : PCDH β 1-16	(16 members)
Pcdh γ : PCDH γ 1-12, b1-7, c3-5	(22 members)
Nonclustered	
Pcdh δ 1: PCDH1, PCDH7 (BH-Pcdh, NF-Pcdh), PCDH9, PCDH11X, PCDH11Y	(5 members)
Pcdh δ 2: PCDH8, PCDH10 (OL-Pcdh) PCDH17, PCDH18, PCDH19	(5 members)
Others: PCDH20	(1 member)
CDH23-PCDH15	6 members
PCDH15: PCDH15, PCDH21 (PrCAD, CDHR1)	
CDH23: CDH23, PCDH24	
mu-cadherin: MUPCDH, PCDH12(VE-cad 2)	
Fat-Dachsous	6 members
FAT: FAT4	
FAT-like: FAT, FAT2, FAT3	
Dachsous: DCHS1 (PCDH16), DCHS2	
CDH26 CDH26	1 member
CDH28 CDH28(CDHR3), CDH29, LOC100130345	3 members
Flamingo CELSR1, CELSR2, CELSR3	3 members
Calsyntenins CLSTN1, CLSTN2, CLSTN3	3 members
RET RET	1 member
Total	115 members

Classic cadheins are enriched in Puncta adherentia

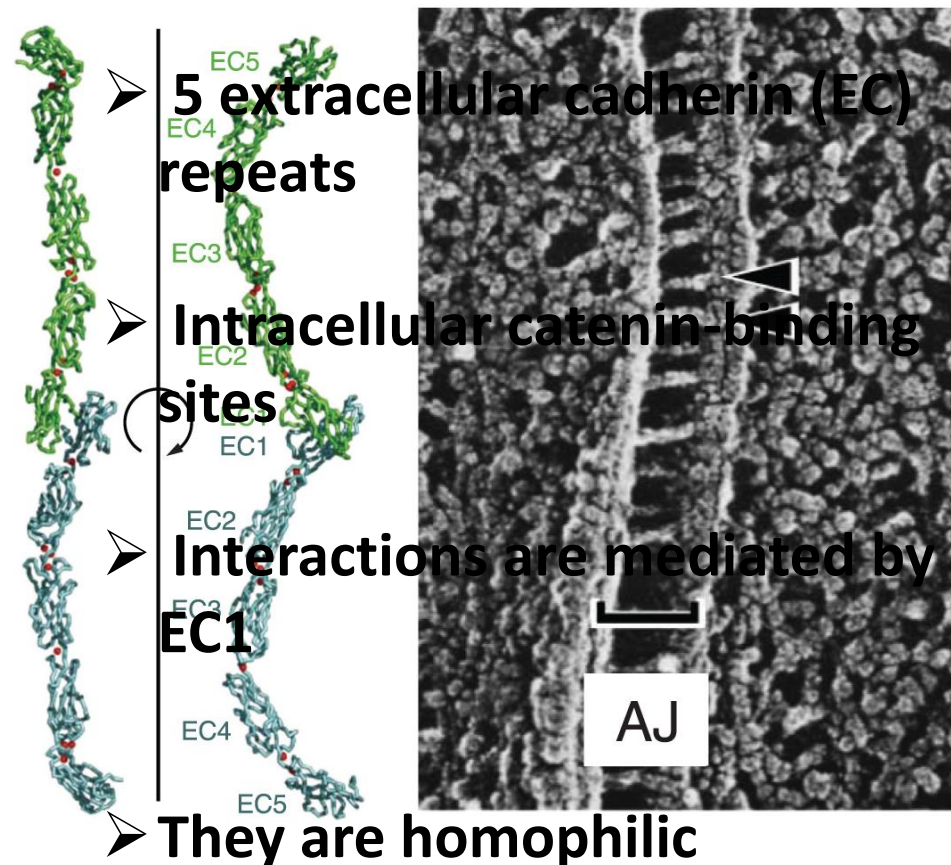


1) be synaptic

Structure of classic cadherins

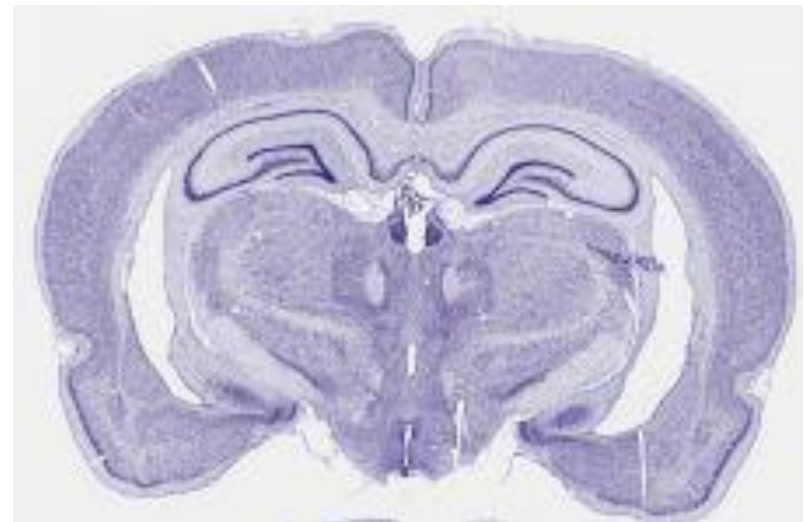
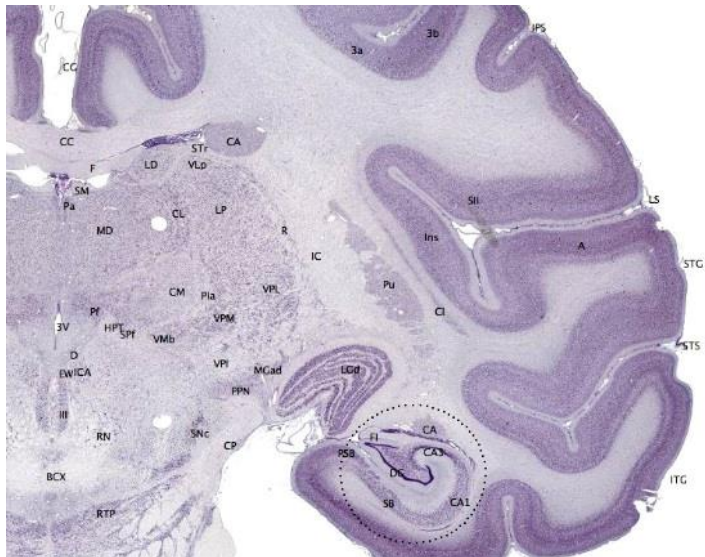
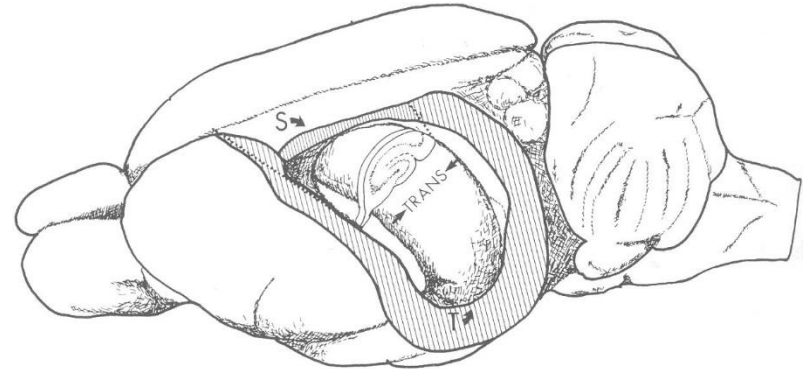


➤ ~ 20 members

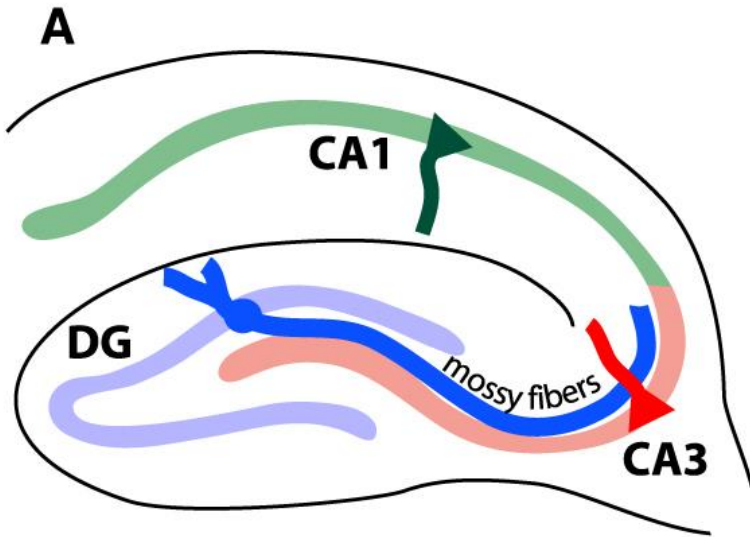


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The hippocampus



Hippocampal circuitry

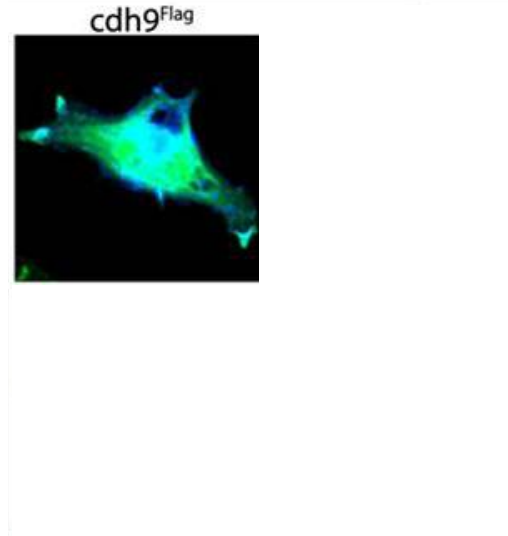


Cdh-9 is expressed preferentially in DG and CA3



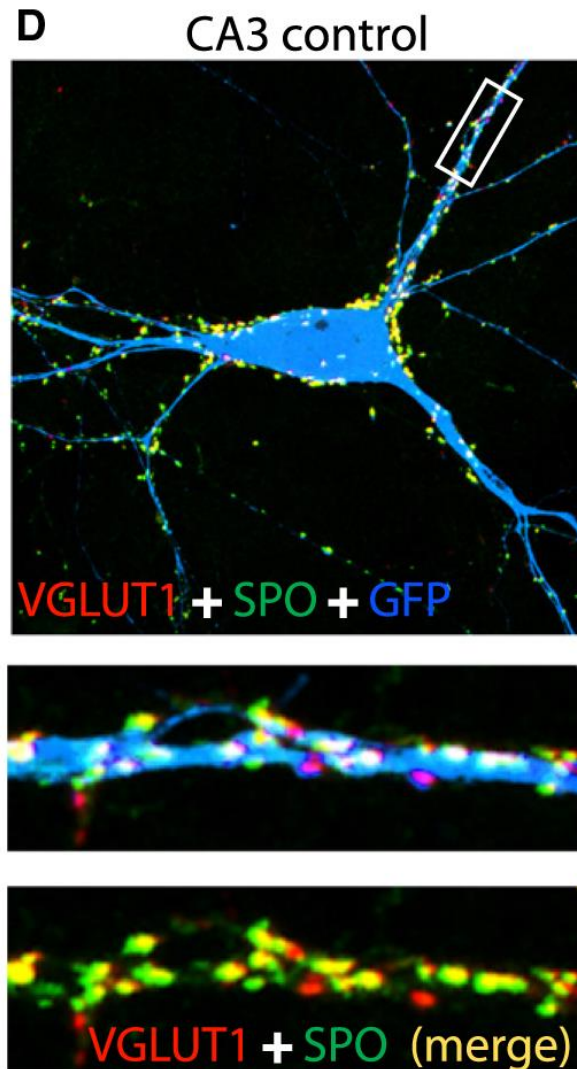
2) be expressed in distinct populations of neurons

Cdh-9 mediates homophilic interactions



- 3) be capable of interacting in *trans*, specifically and with high affinity with their binding partner(s)

Cdh-9 regulates Mossy fiber synapse formation



Summary

What requirements cell adhesion molecules that regulate synaptic specificity need to have

They should:

- 1) be synaptic
- 2) be expressed in distinct populations of neurons
- 3) be capable of interacting in *trans*, specifically and with high affinity with their binding partner(s)
- 4) provide enough molecular diversity to confer cell type- and synapse type-specific identities

Protocadherins

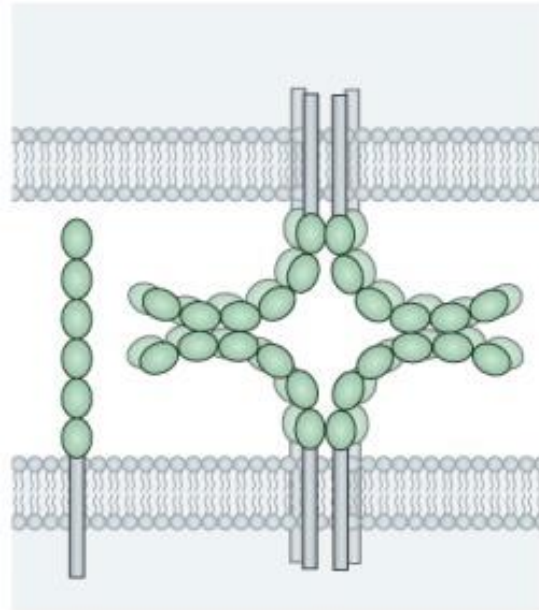
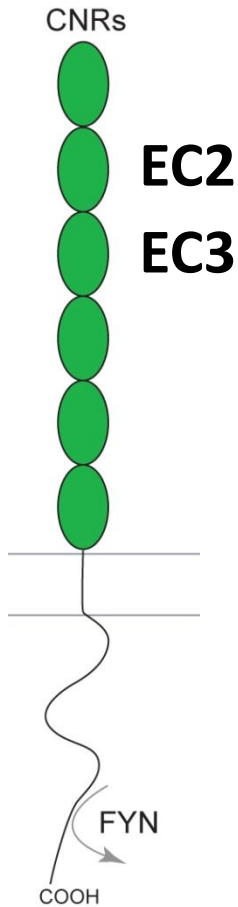


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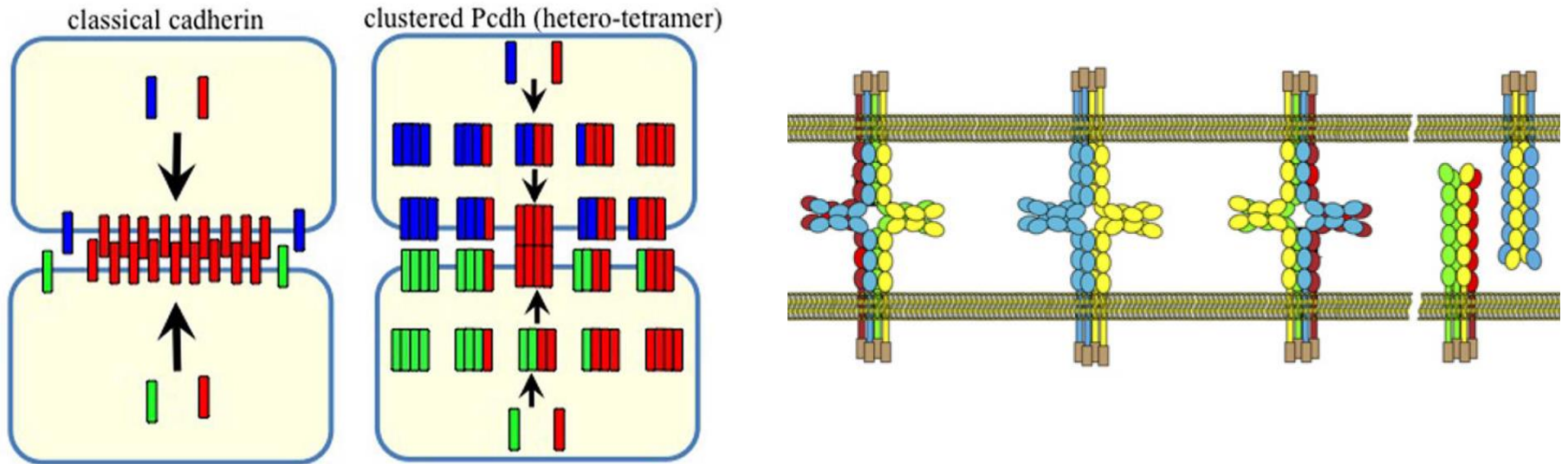
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Total	115 members

Protocadherins



- **6 EC domains**
- **Conserved cytoplasmic domains**
- **Homophilic interactions involving EC2 and EC3**
- **Expression largely restricted to the nervous system**
- **Enriched at synapses**

Protocadherin binding specificity

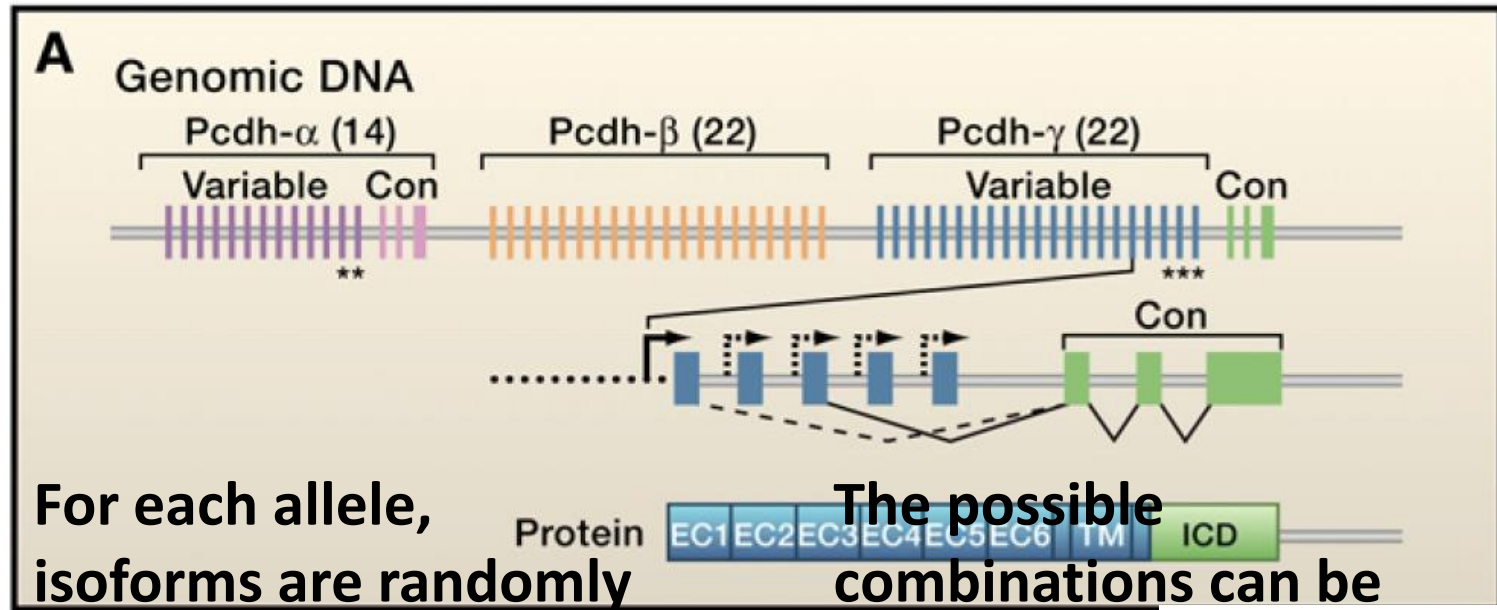


- Pcdhs form *cis*-tetramers in an isoform-independent fashion
- They bind homophilically in *trans*



Only cells expressing the same tetramers can interact

The Protocadherin Gene Cluster



1 out of 12 for Pcdh-α

2 out of 22 for Pcdh-β

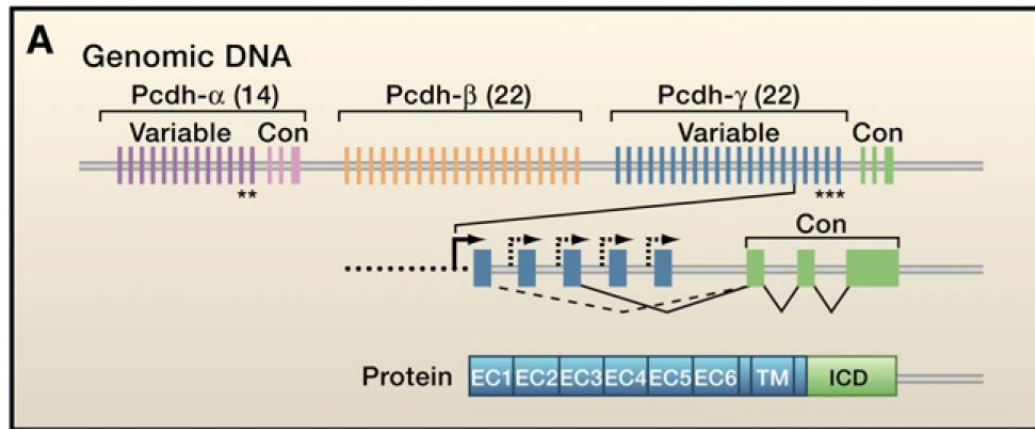
2 out of 19 for Pcdh-γ

$$\alpha \binom{12}{1} = 12$$

$$\beta \binom{22}{2} = 231$$

$$\gamma \binom{19}{2} = 171$$

The Protocadherin Gene Cluster



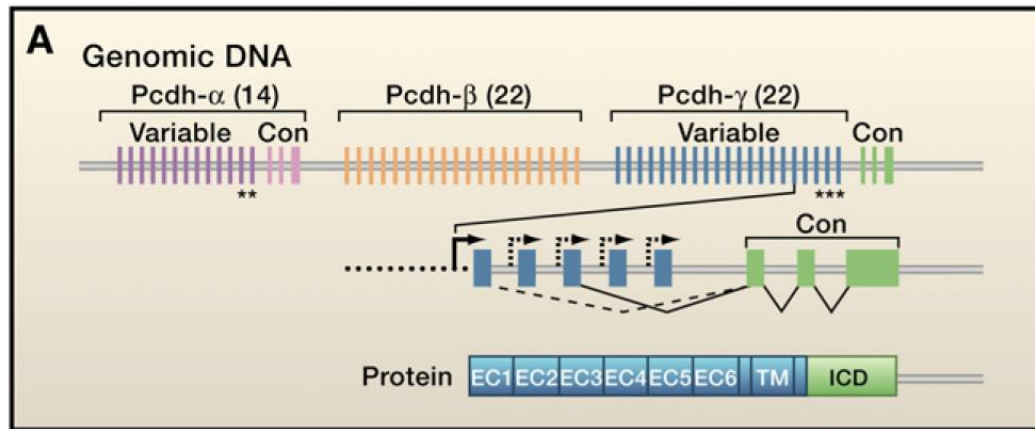
The number of combinations with repetition from both alleles is $\binom{m+2-1}{2}$, where m is the number of permutations in each allele and 2 is the number of alleles

$$\alpha \binom{12 + 2 - 1}{2} = \binom{13}{2} = 78$$

$$\beta \binom{231 + 2 - 1}{2} = \binom{232}{2} = 26,796$$

$$\gamma \binom{171 + 2 - 1}{2} = \binom{172}{2} = 14,706$$

The Protocadherin Gene Cluster

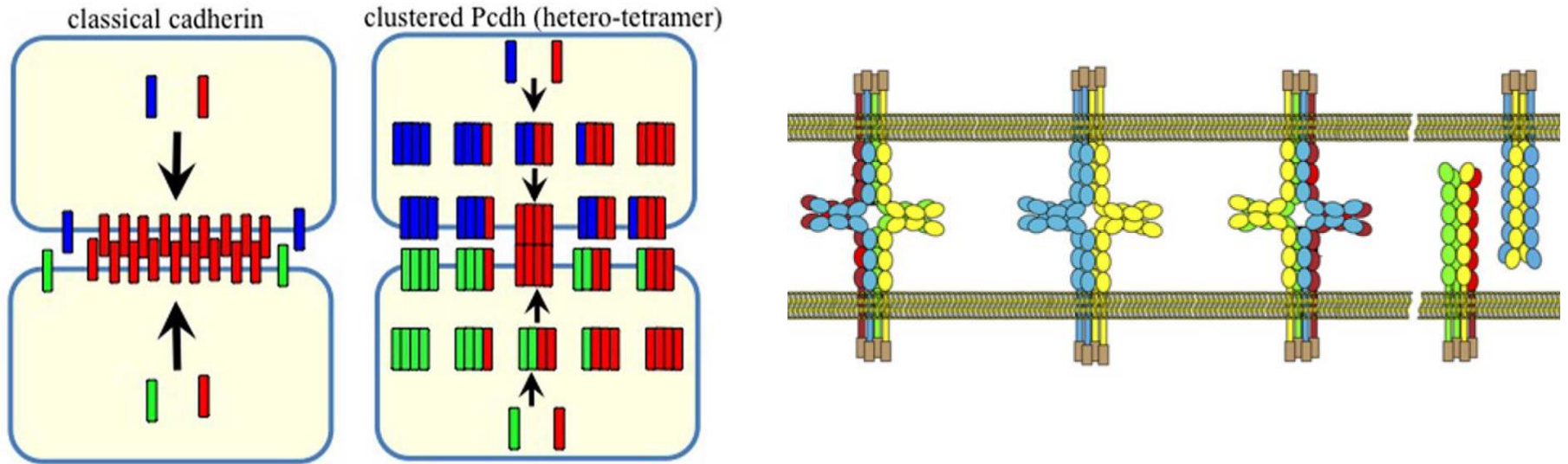


Therefore, a total of

$$78 \times 26\,796 \times 14\,706 = 30\,736\,834\,128 (\sim 3 \times 10^{10})$$

variations are possible for each neurons.

Protocadherin binding specificity



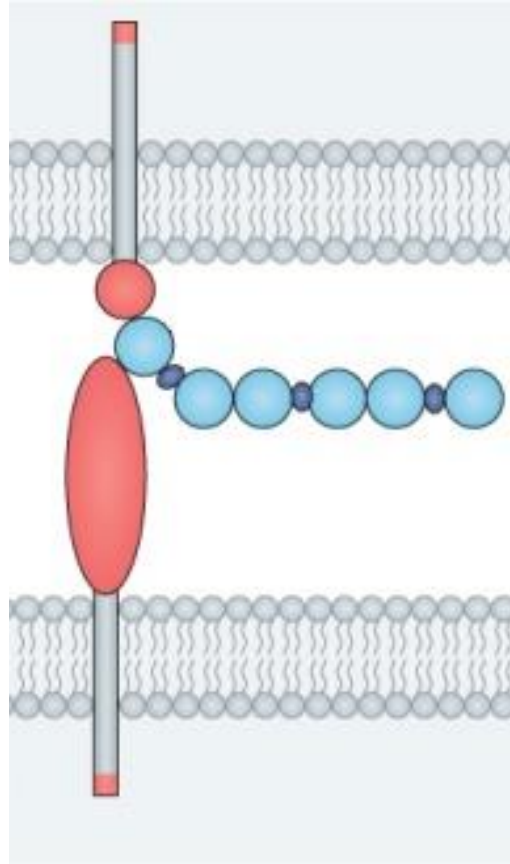
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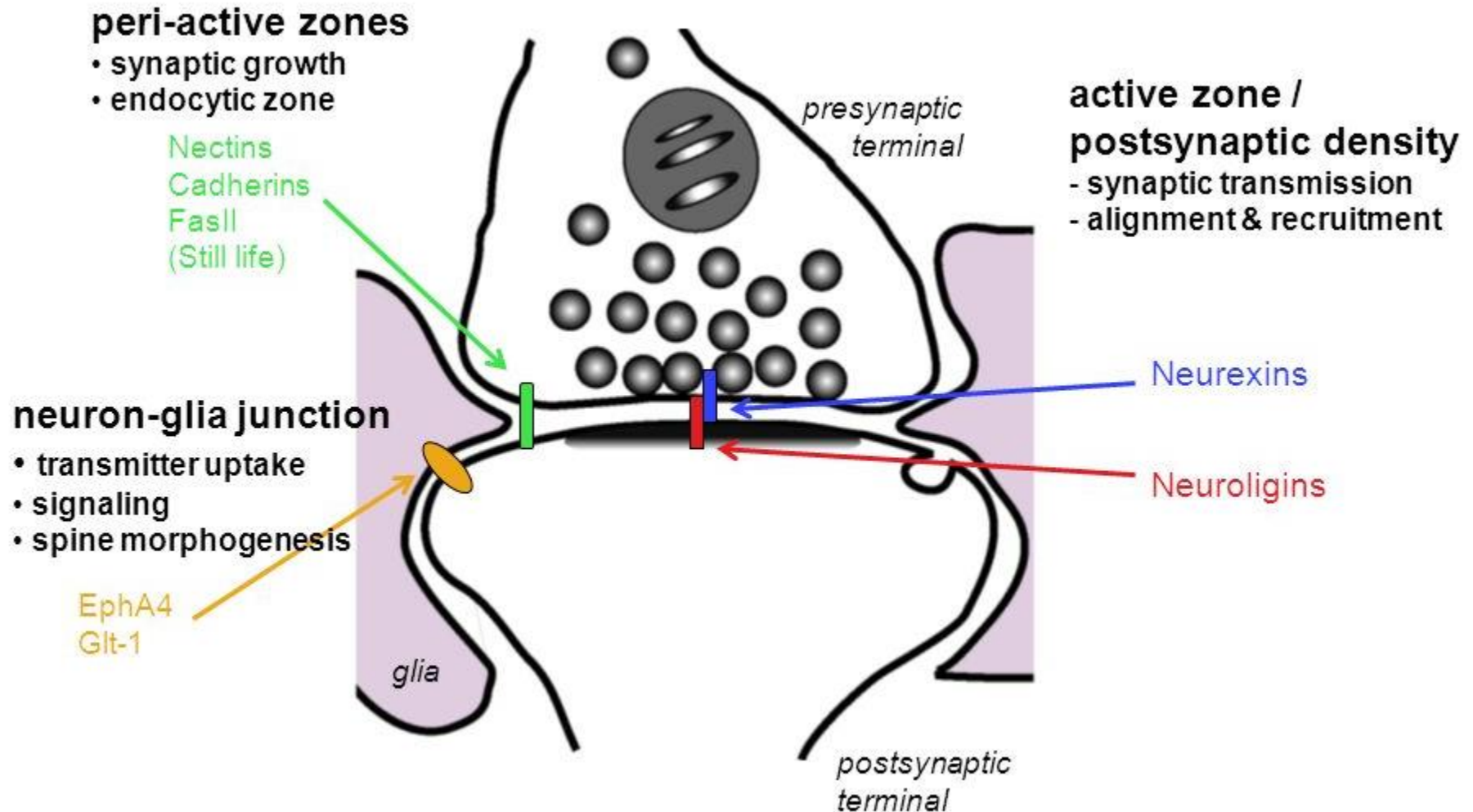
Only cells expressing the same tetramers can interact

Summary

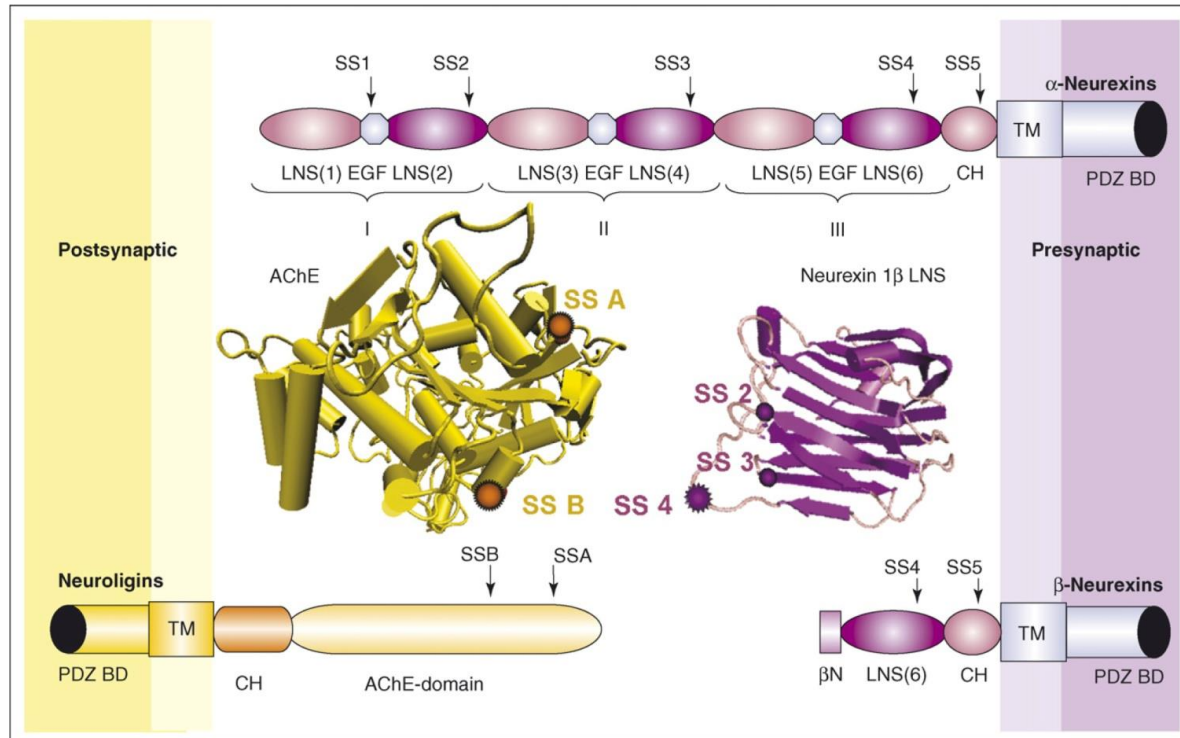
Neuroligins - neurexins



Neuroligins and neurexins are enriched at the center of the synapse



Neuroligins - neurexins



➤ Postsynaptic

➤ 4 - 5 genes

➤ 1 acetylcholinesterase domain

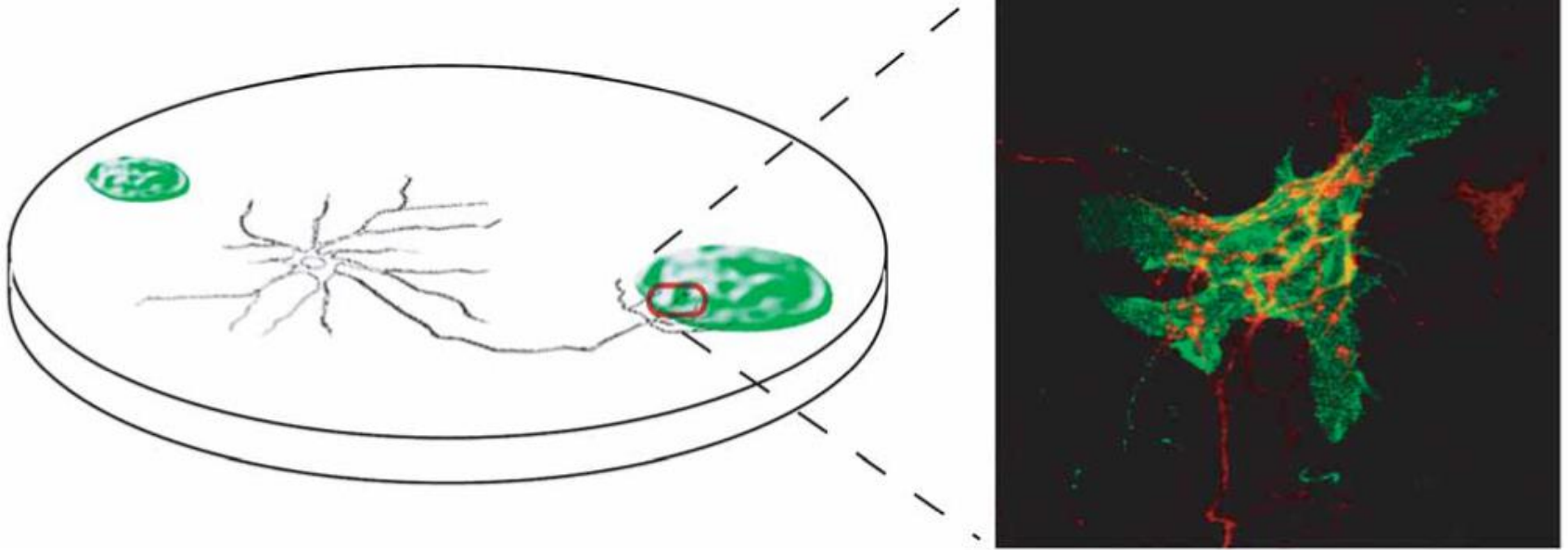
➤ Presynaptic

➤ 3 genes

➤ 2 forms each: α (long) and β (short)

➤ 6 LNS, 3 EGF domains

Mixed culture assay



Neurotrophin Expressed in Nonneuronal Cells Triggers Presynaptic Development in Contacting Axons

Peter Scheiffele,^{*‡} Jinhong Fan,^{*} Jenny Choih,^{*} Richard Fetter,[†] and Tito Serafini^{*‡}

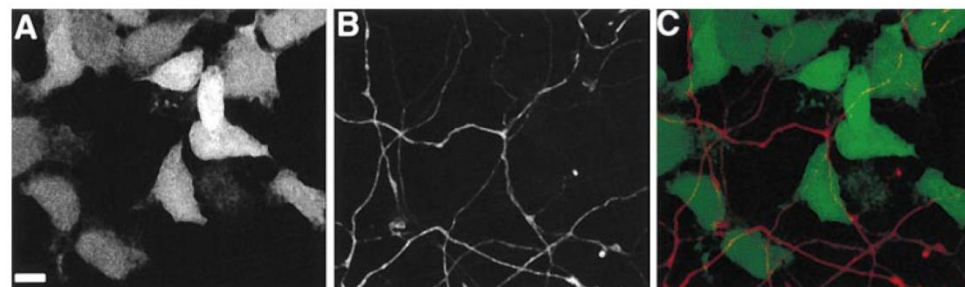
^{*}Department of Molecular and Cell Biology

[†]Howard Hughes Medical Institute

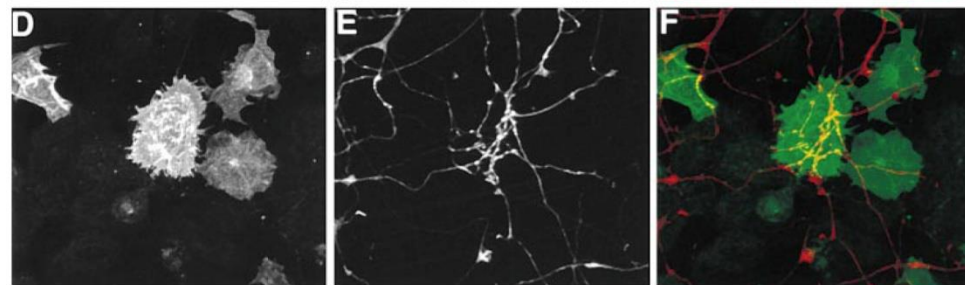
University of California, Berkeley

Berkeley, California 94720

GFP

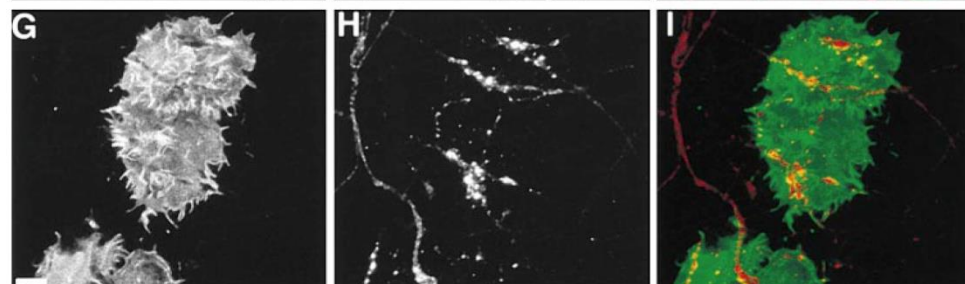


Neurotrophin1

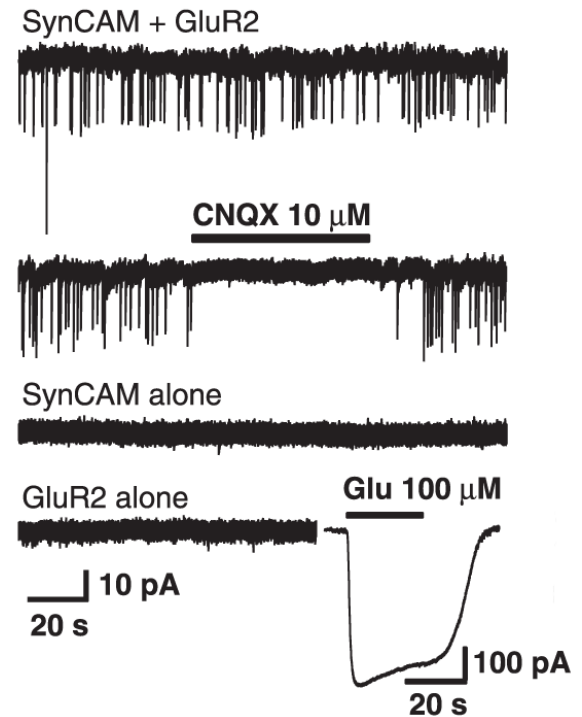
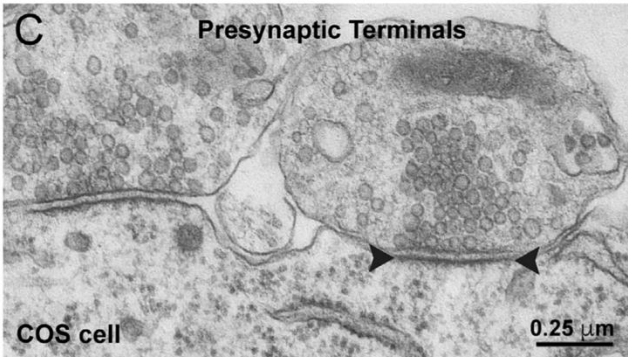
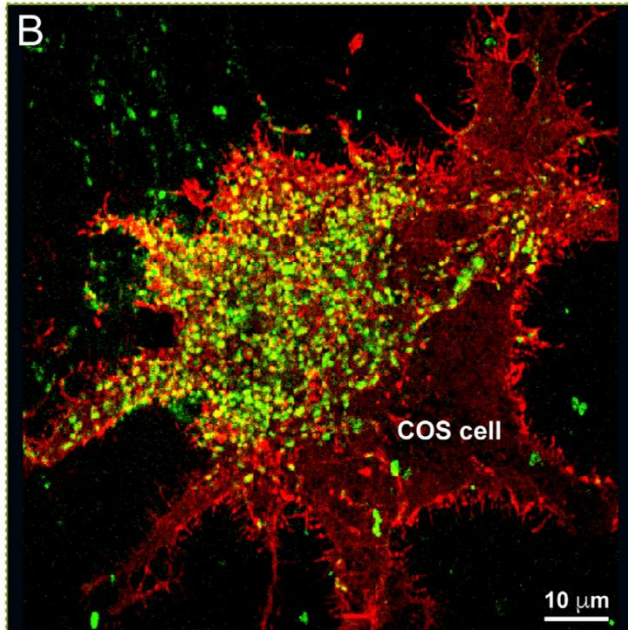


Neurotrophins are synaptogenic

Neurotrophin2



Artificial synapse formation assay

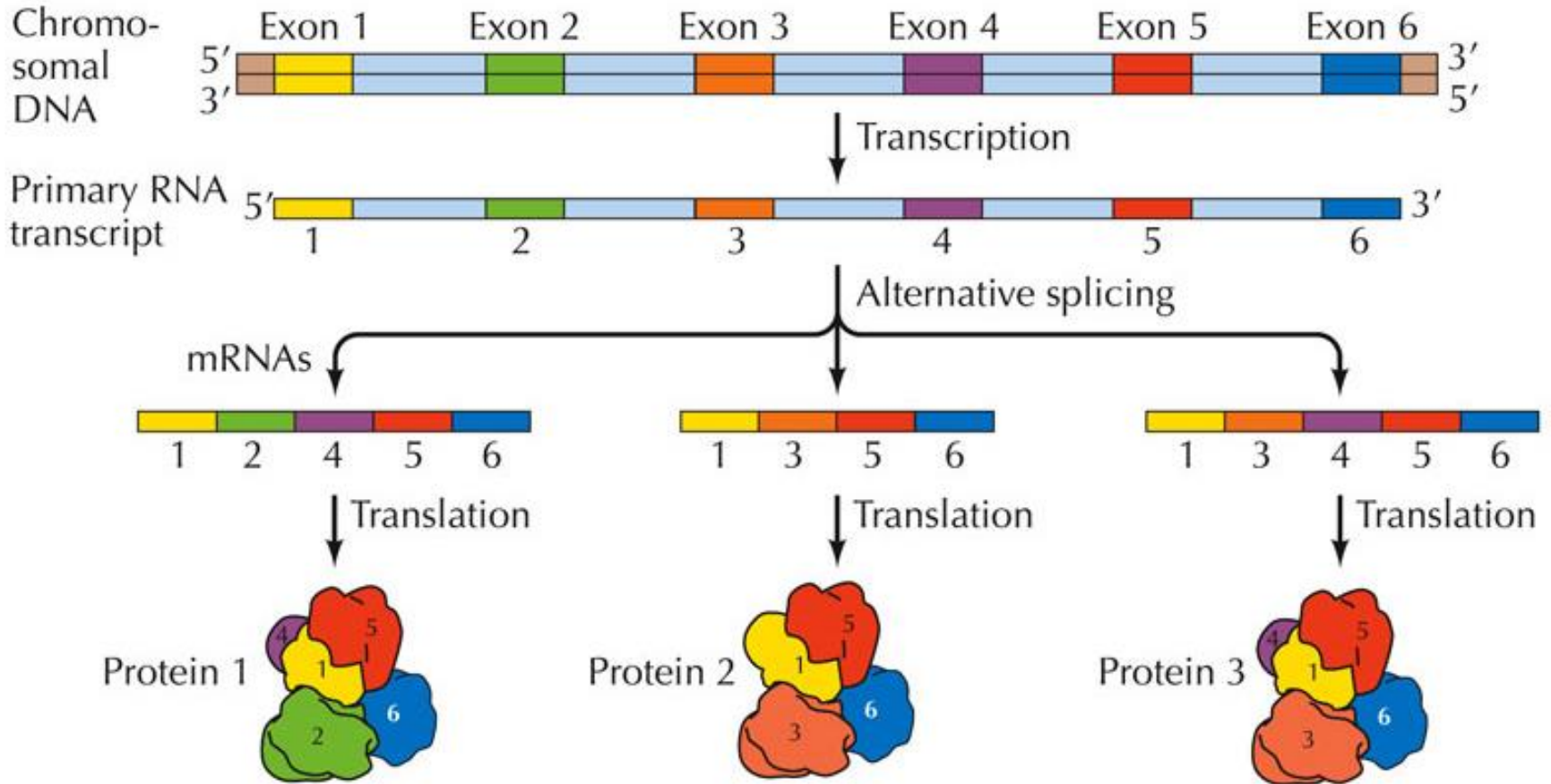


A

<i>Protein expressed in non-neuronal cell</i>	<i>Synaptic feature induced in co-cultured neuron</i>
Neuroligins	→ Presynaptic
LRRTMs	
SALMs	
SliTrks	
SALMs	

Neurexins	→ Postsynaptic
Neur. pentraxin receptor	
Teneurins	
PTPRs	

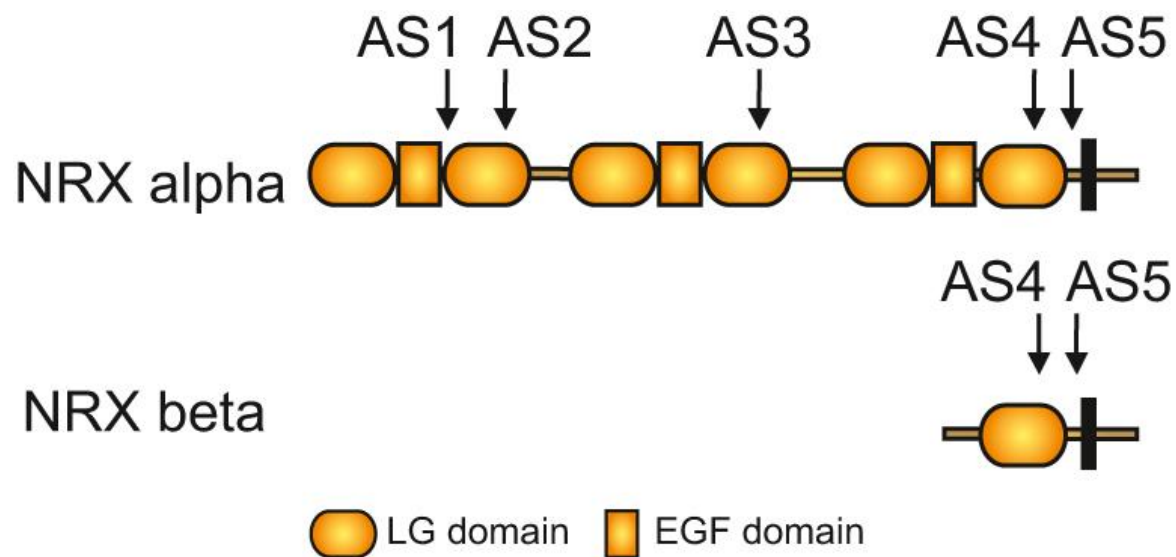
Alternative RNA splicing



Cartography of Neurexins: More Than 1000 Isoforms Generated by Alternative Splicing and Expressed in Distinct Subsets of Neurons

Beate Ullrich, Yuri A. Ushkaryov,*
and Thomas C. Südhof

Department of Molecular Genetics
Howard Hughes Medical Institute
University of Texas Southwestern Medical School
Dallas, Texas 75235



Neurexin isoform landscape

Nrxn1 α max: 576

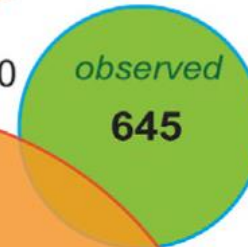
Nrxn2 α max: 384

predicted 576

predicted 384



Nrxn3 α max: 11,520



predicted 5760

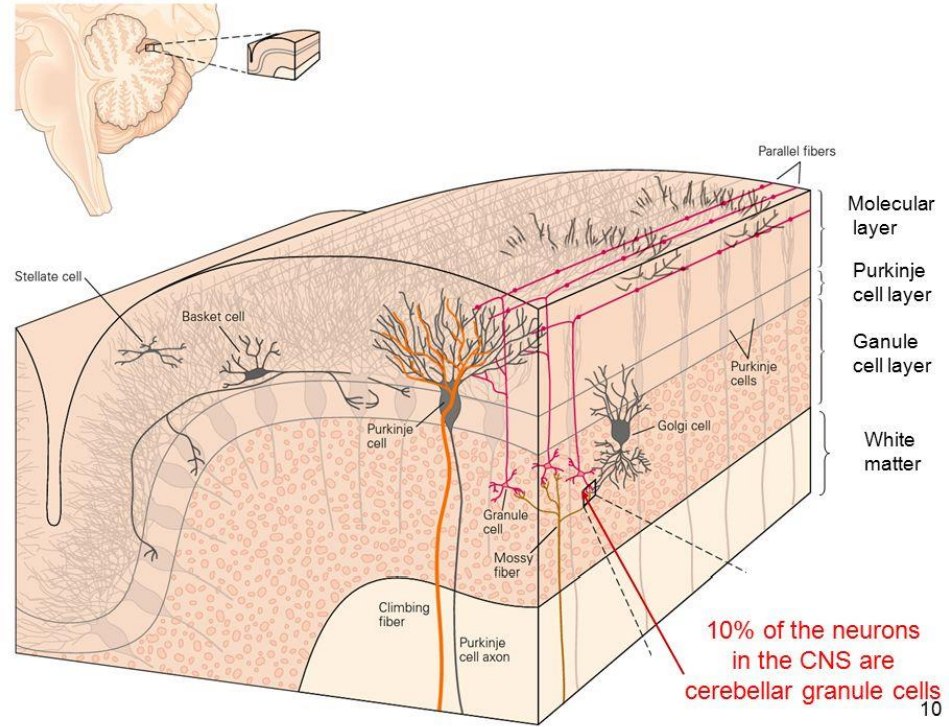
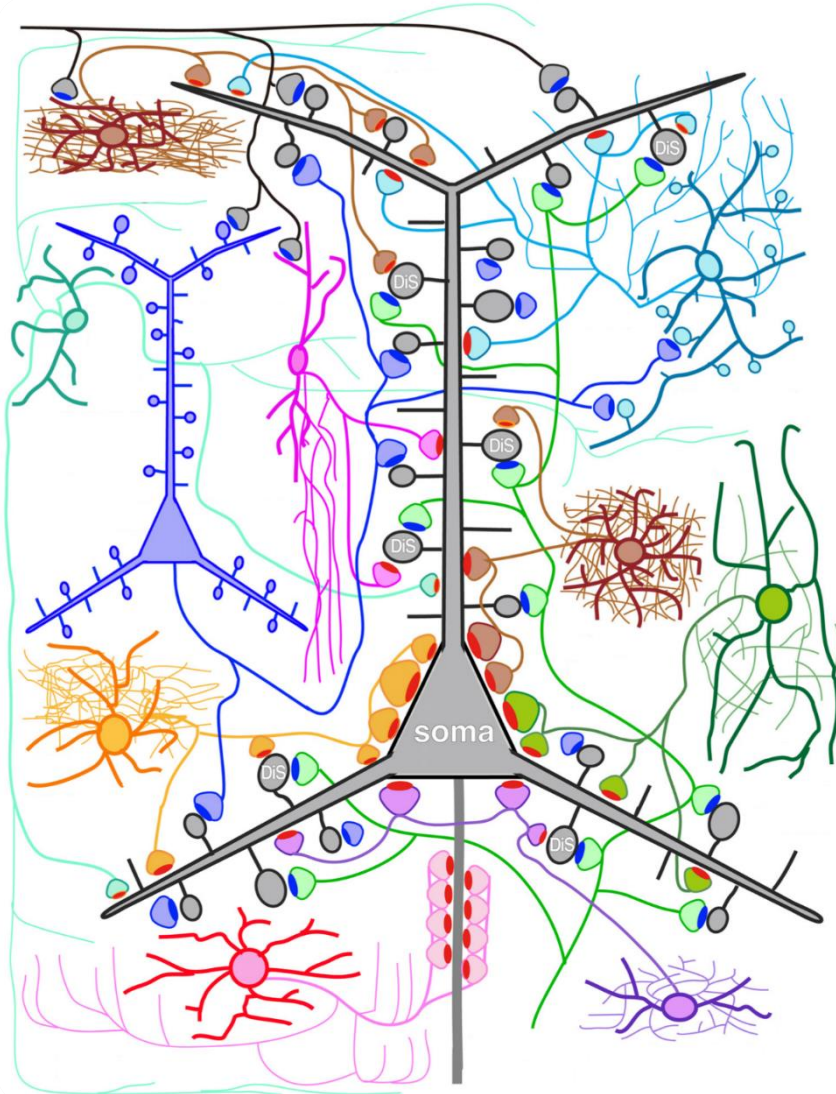
Cortex vs. Cerebellum



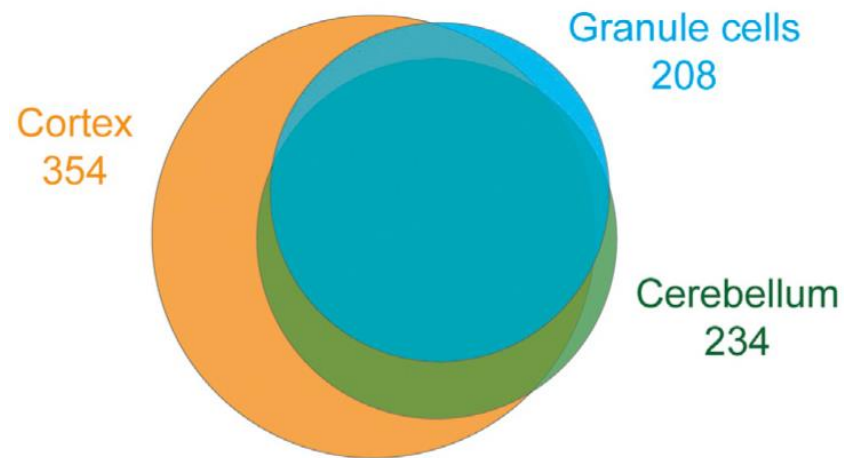
Cortex

vs.

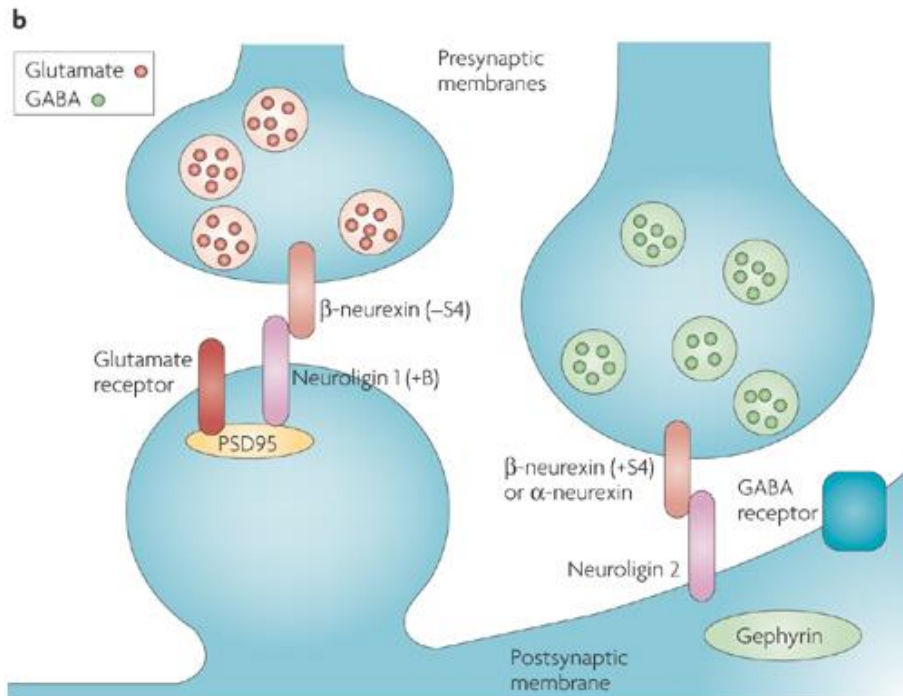
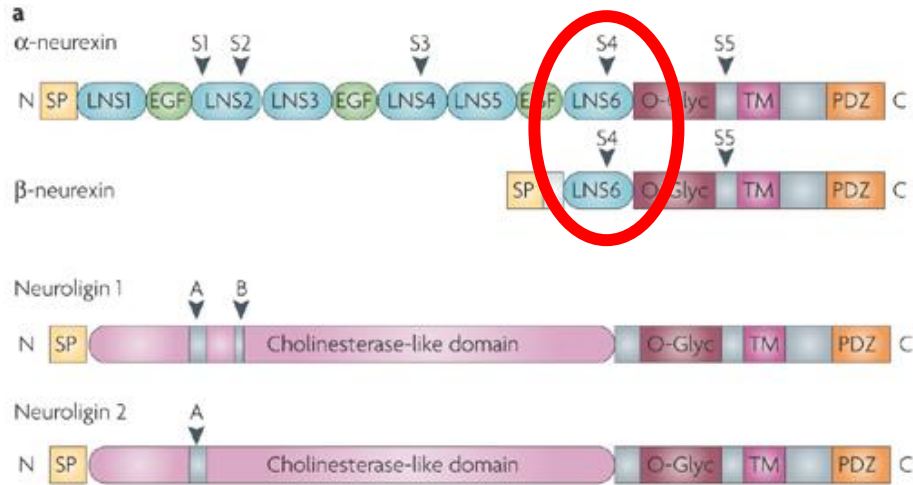
Cerebellum



Nrxn1 α diversity correlates with neuronal cell type complexity



Neurexin - neuroligin splicing code



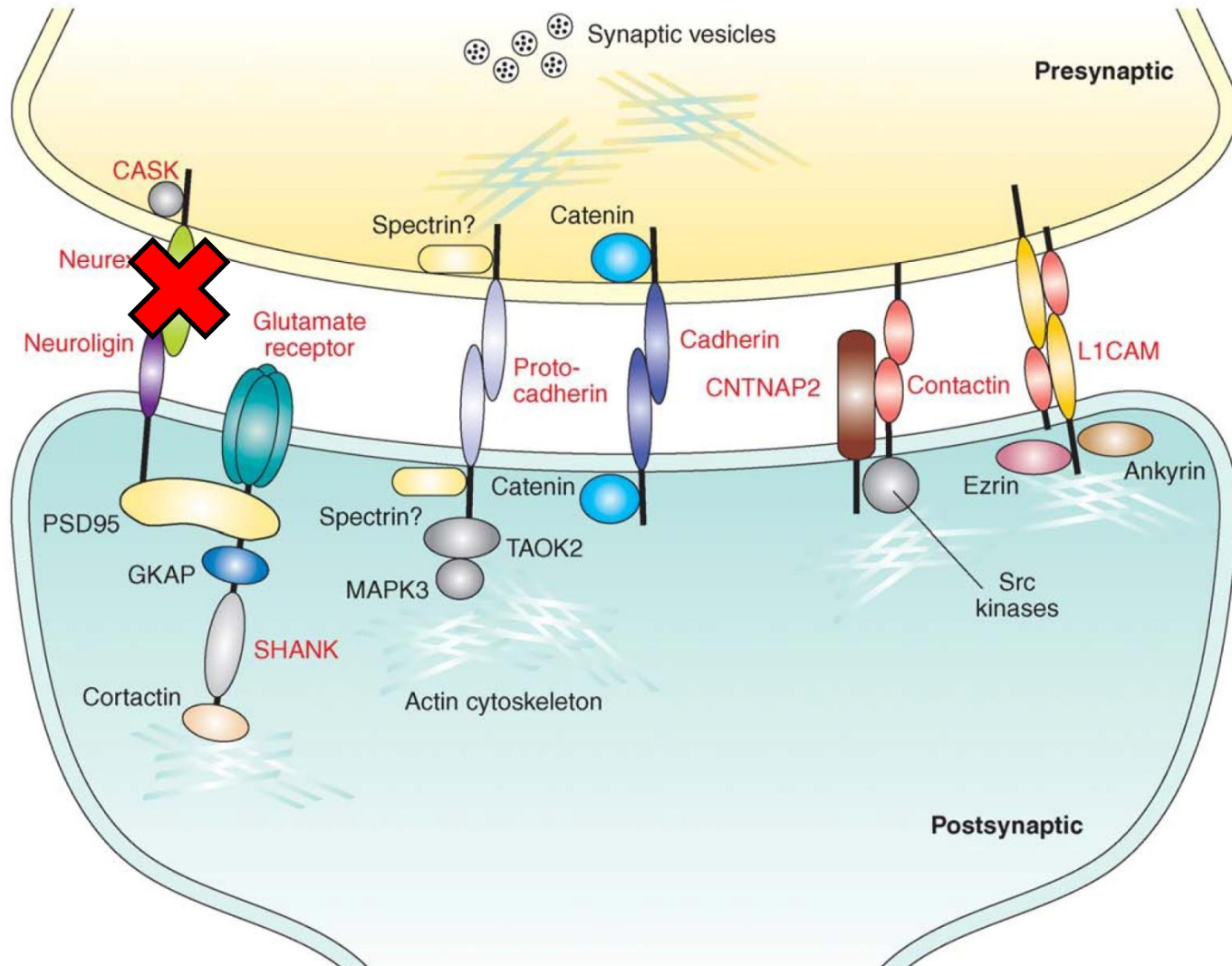
Neuroligins and neurexins can determine if a synapse will be excitatory or inhibitory

Summary

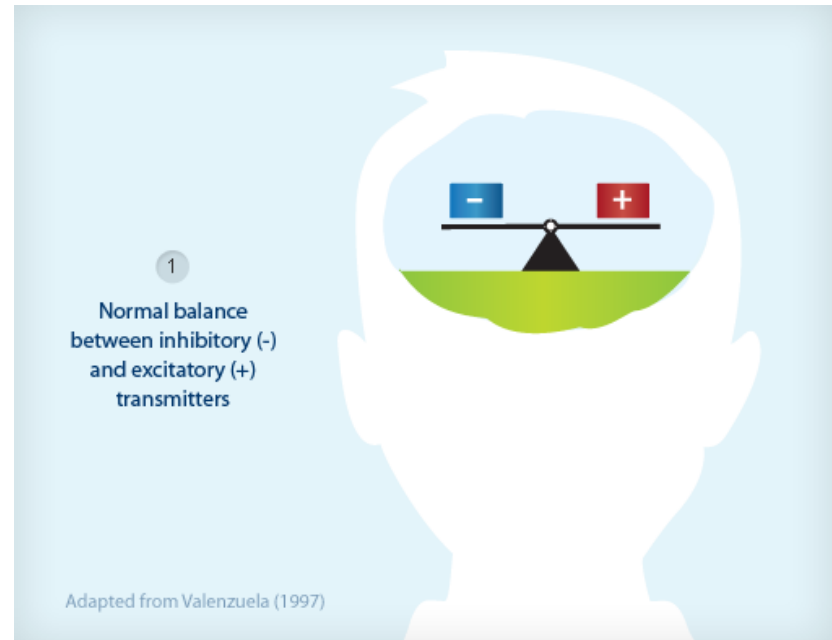
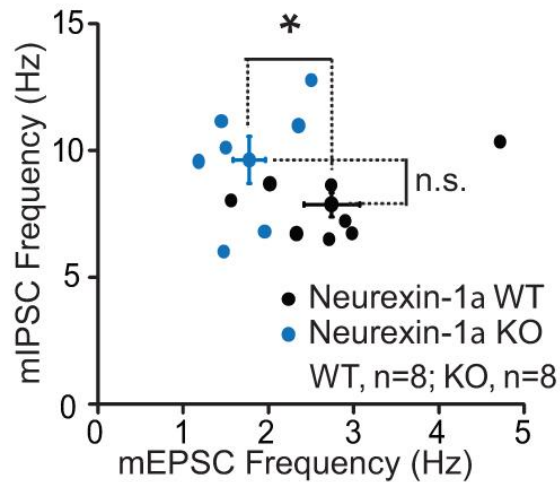
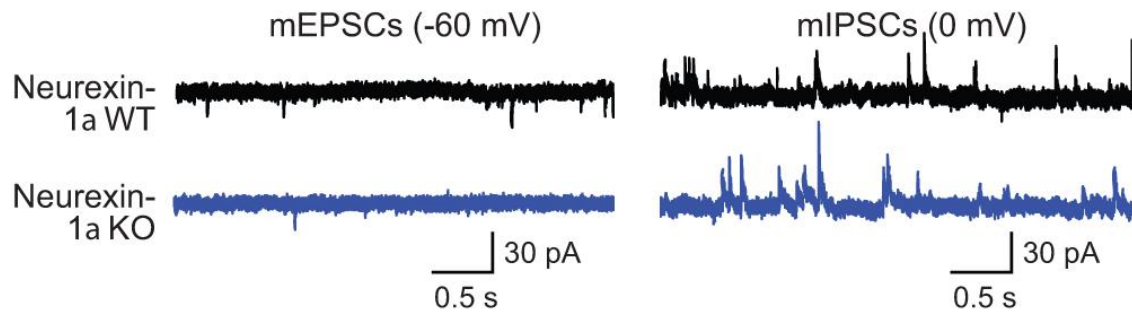
Autism spectrum disorder (ASD)



Synaptic CAMs involved in ASDs



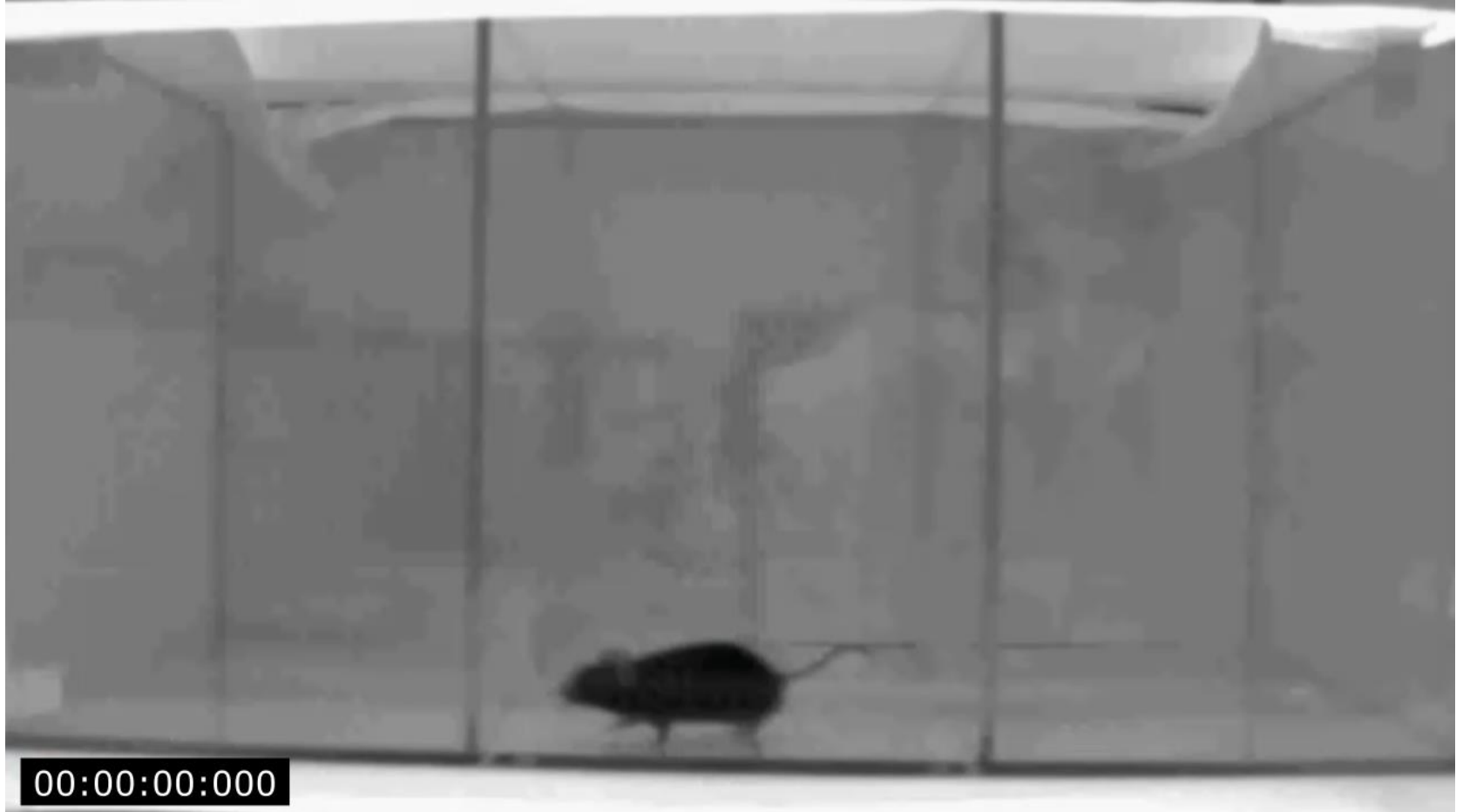
Deletion of Neurexin-1 α reduces the excitatory/inhibitory ratio



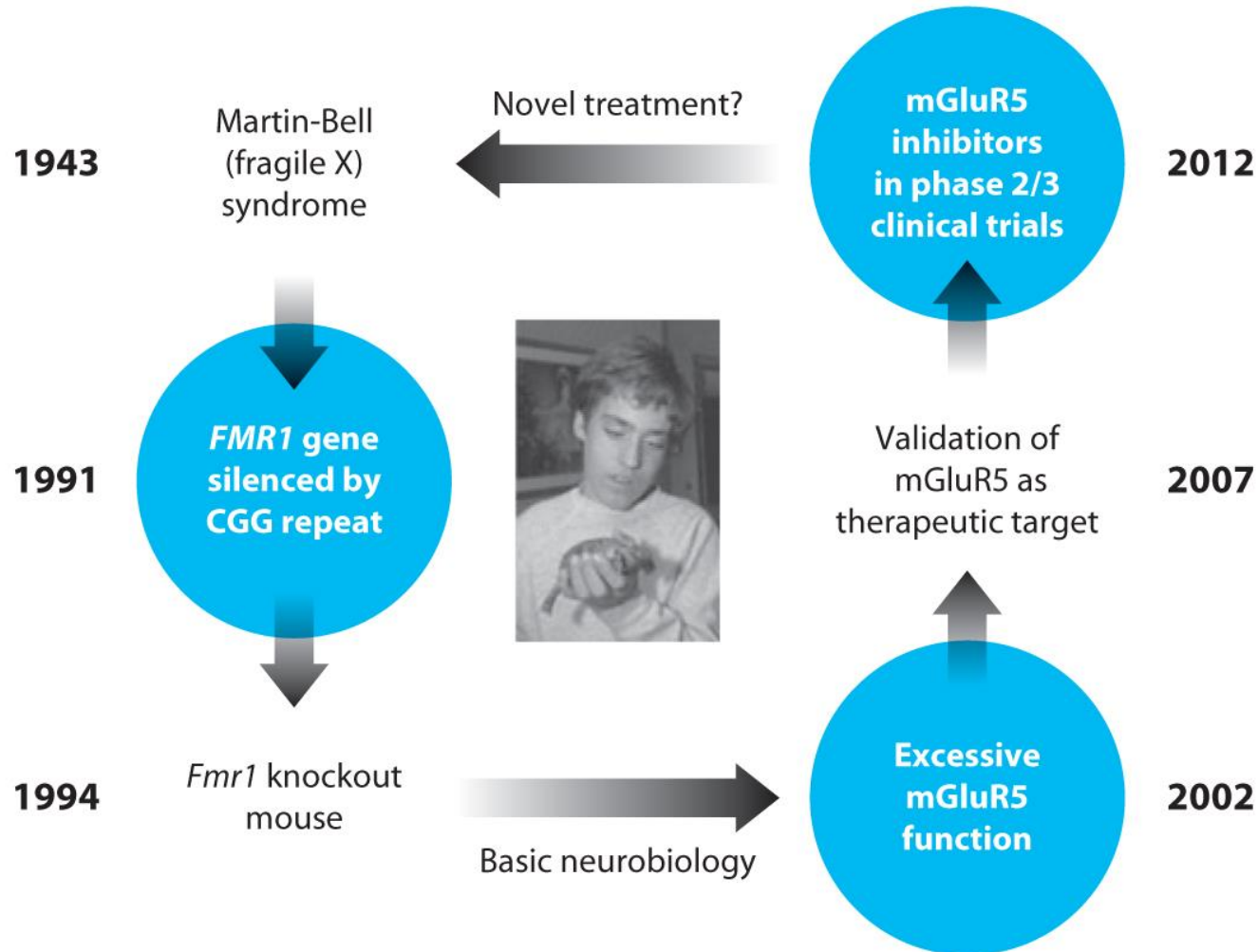
Excessive grooming in mice as a proxy for repetitive behavior in humans



How to evaluate social interaction in mice

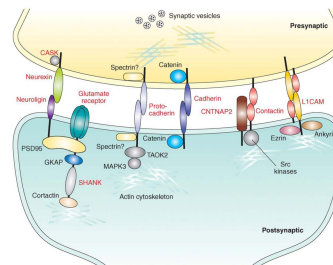


How mouse models can lead to therapies in humans



Summary

- Synaptic CAMs are involved in ASDs



- Mouse models can be used to study the neurobiology of ASDs



- The road to therapies in humans is long but feasible

