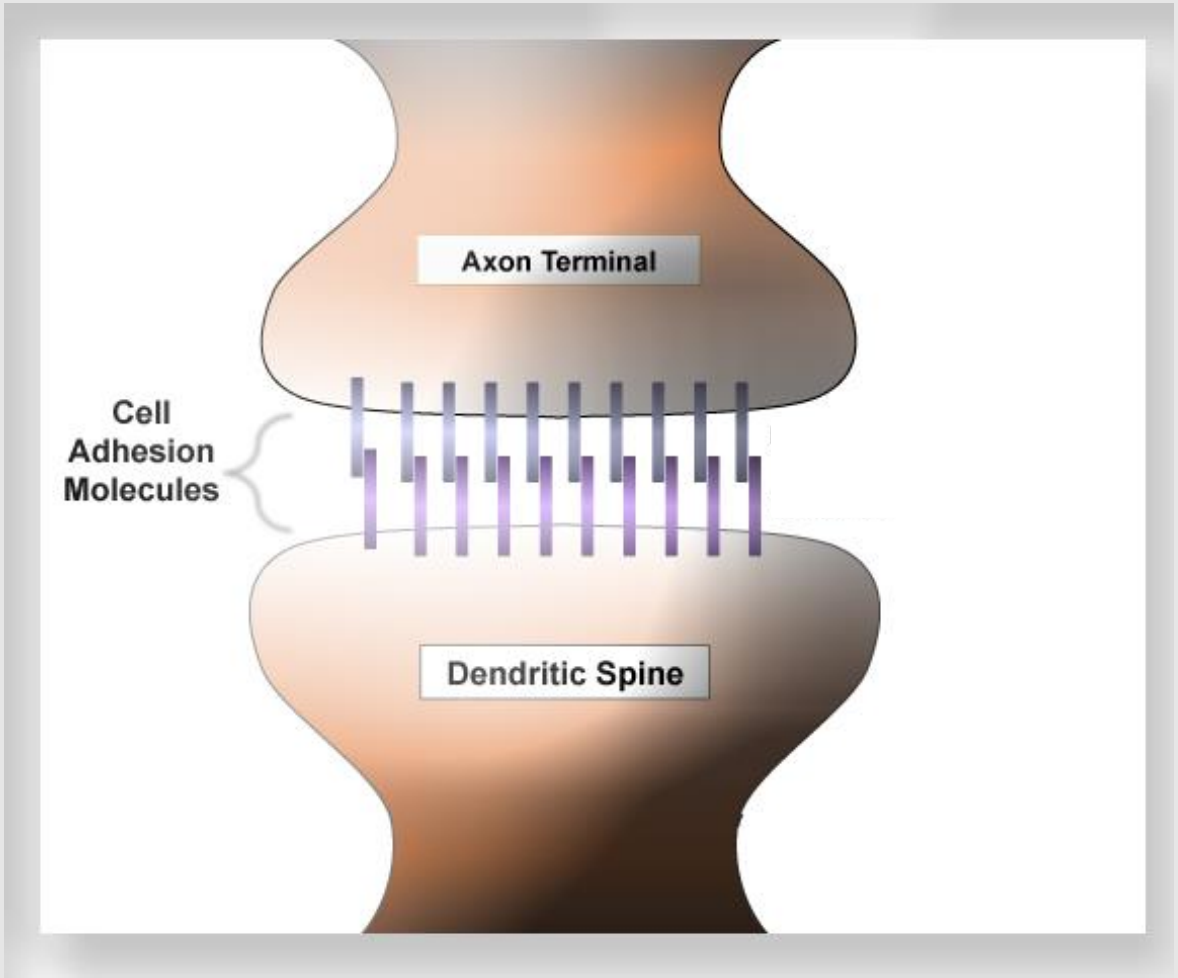
# Molecole di adesione alla sinapsi

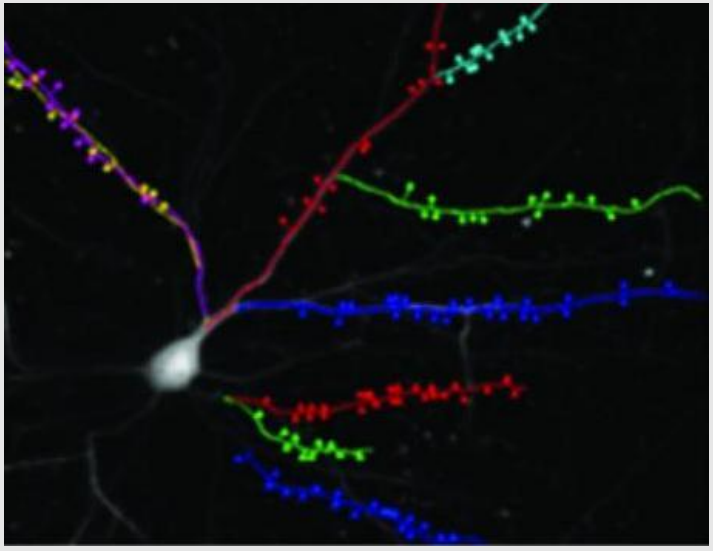


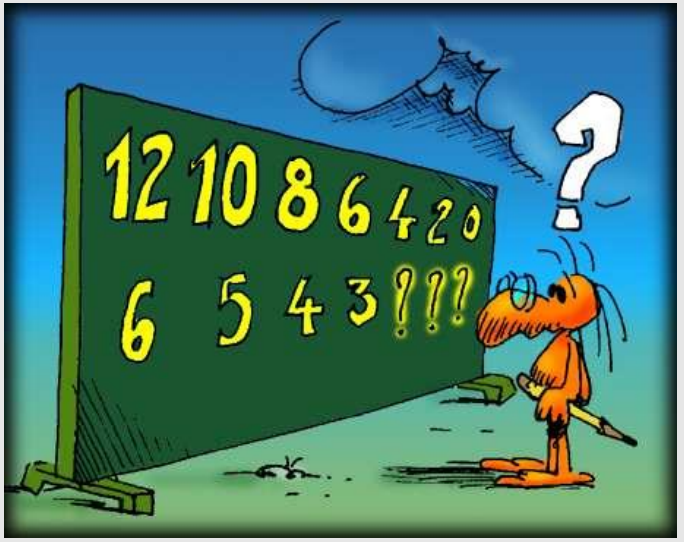
Iniziamo dando un po' di numeri….

1) Si stima che nel cervello umano ci siano centinaia di bilioni di neuroni, connessi l'uno all'altro centinaia di trilioni di punti di contatto chiamati sinapsi.

2) Le sinapsi contengono un gran numero di proteine, da studi di proteomica: circa 2000 proteine si localizzano alla sinapsi.

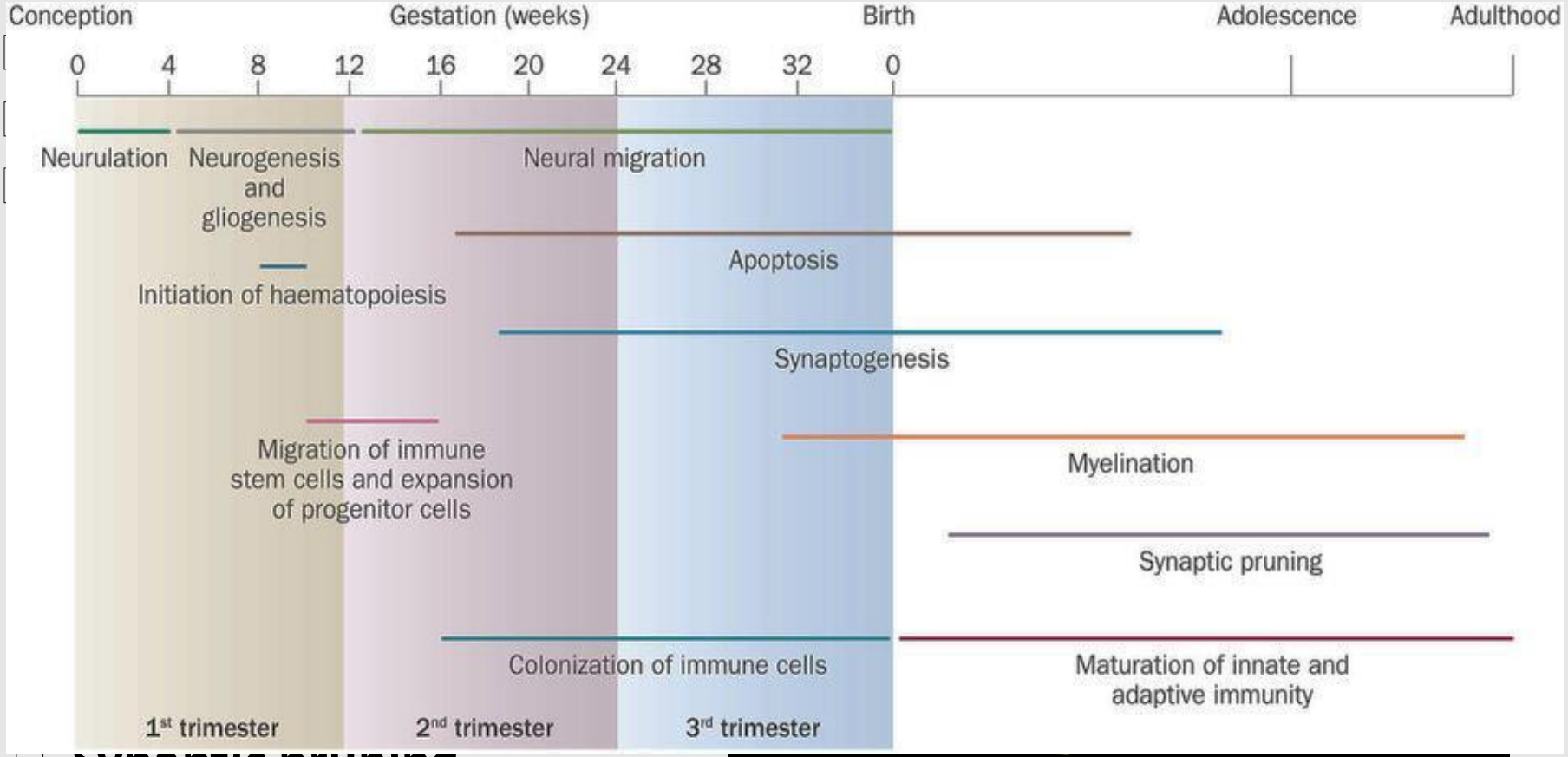
3) Più di 10 anni fa, si è iniziato a capire che difetti alla sinapsi possono essere alla base di disturbi del neuro-sviluppo. Oggi sappiamo che tra i geni implicati con l'autismo ci sono molti che codificano per proteine sinaptiche.





# Lo sviluppo del cervello

## Before birth (mostly)




- Synaptic pruning

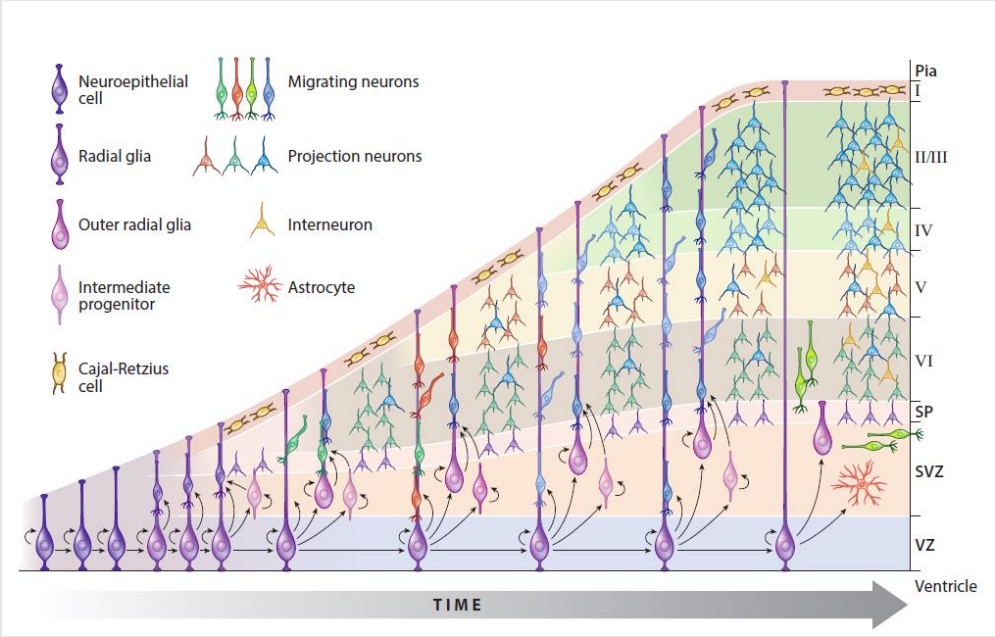
# Lo sviluppo del cervello prima della nascita

- **Proliferazione**
- **Migrazione**
- **Differenziamento**

Prevalentemente prima della nascita (in utero)

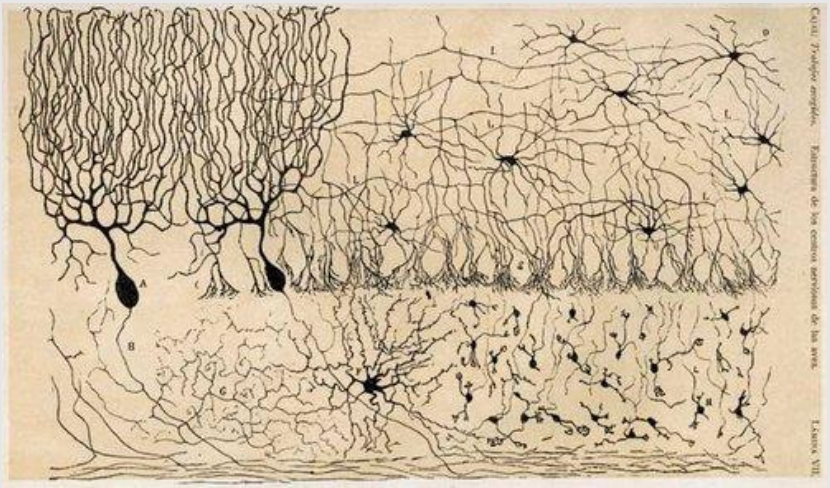
Mutazioni in questo stadio si moltiplicano e si espandono

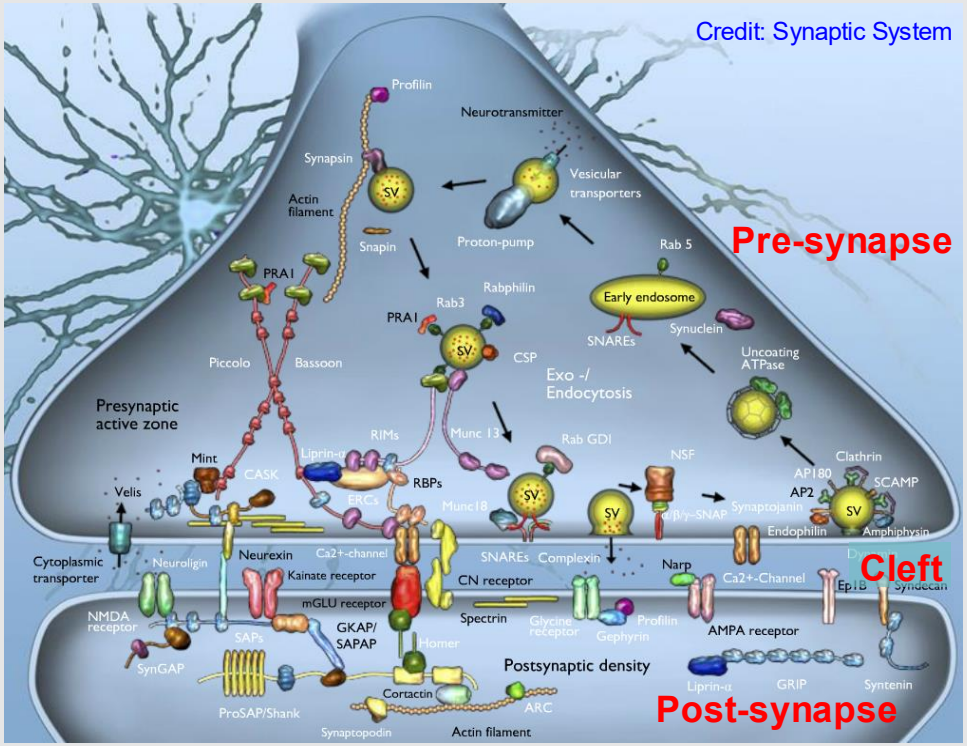
**Diagnosi** è difficile e intervenire anche.



# Lo sviluppo del cervello dopo la nascita

- Formazione dei circuiti
- Formazione della sinapsi
- Eliminazione sinaptica



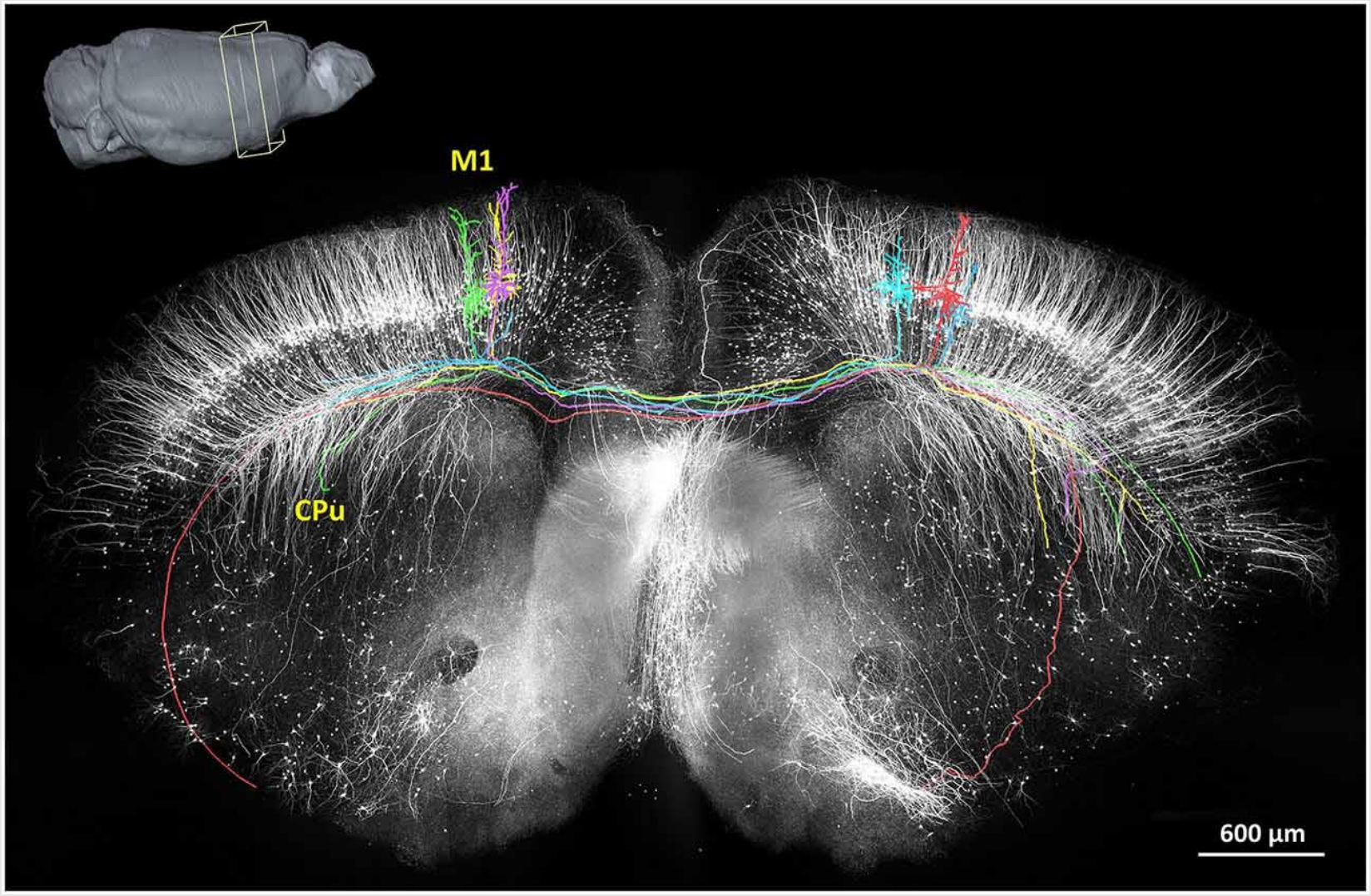



Dopo la nascita

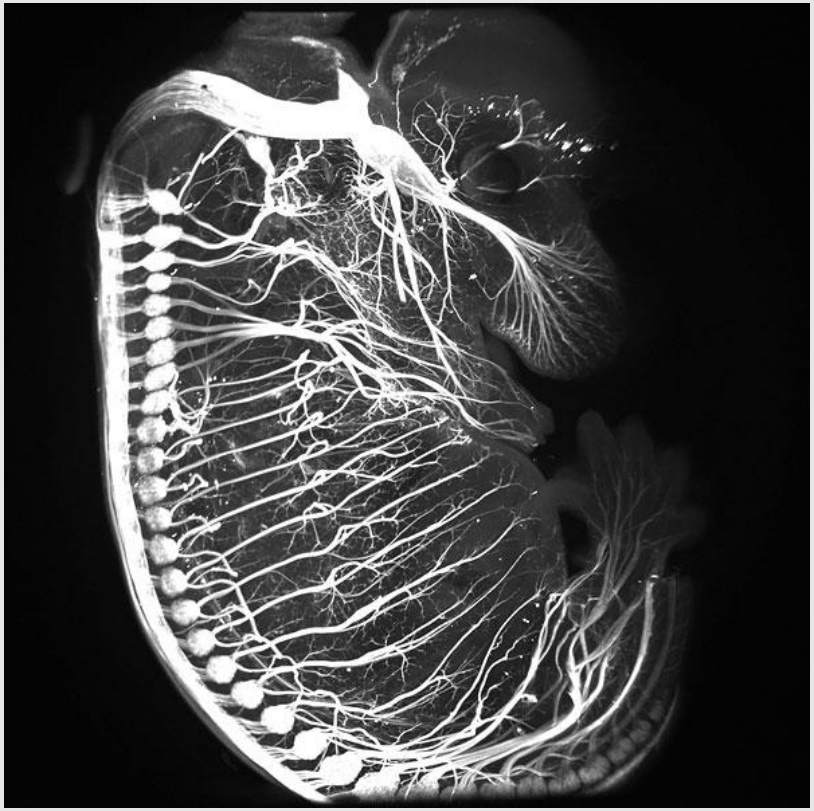
Mutations can avere un effetto su molti di questi processi

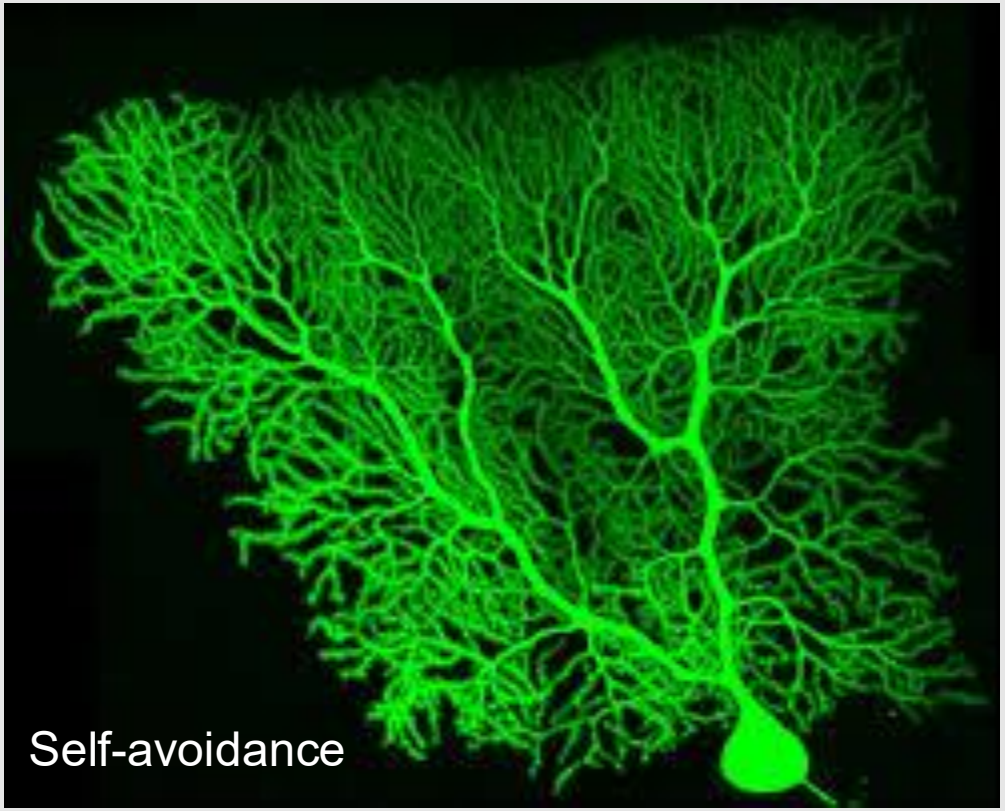
**Diagnosi ed interventi possono Essere tardivi**

# Cosa si intende per connettività?



# Cosa è la connettività sinaptica?

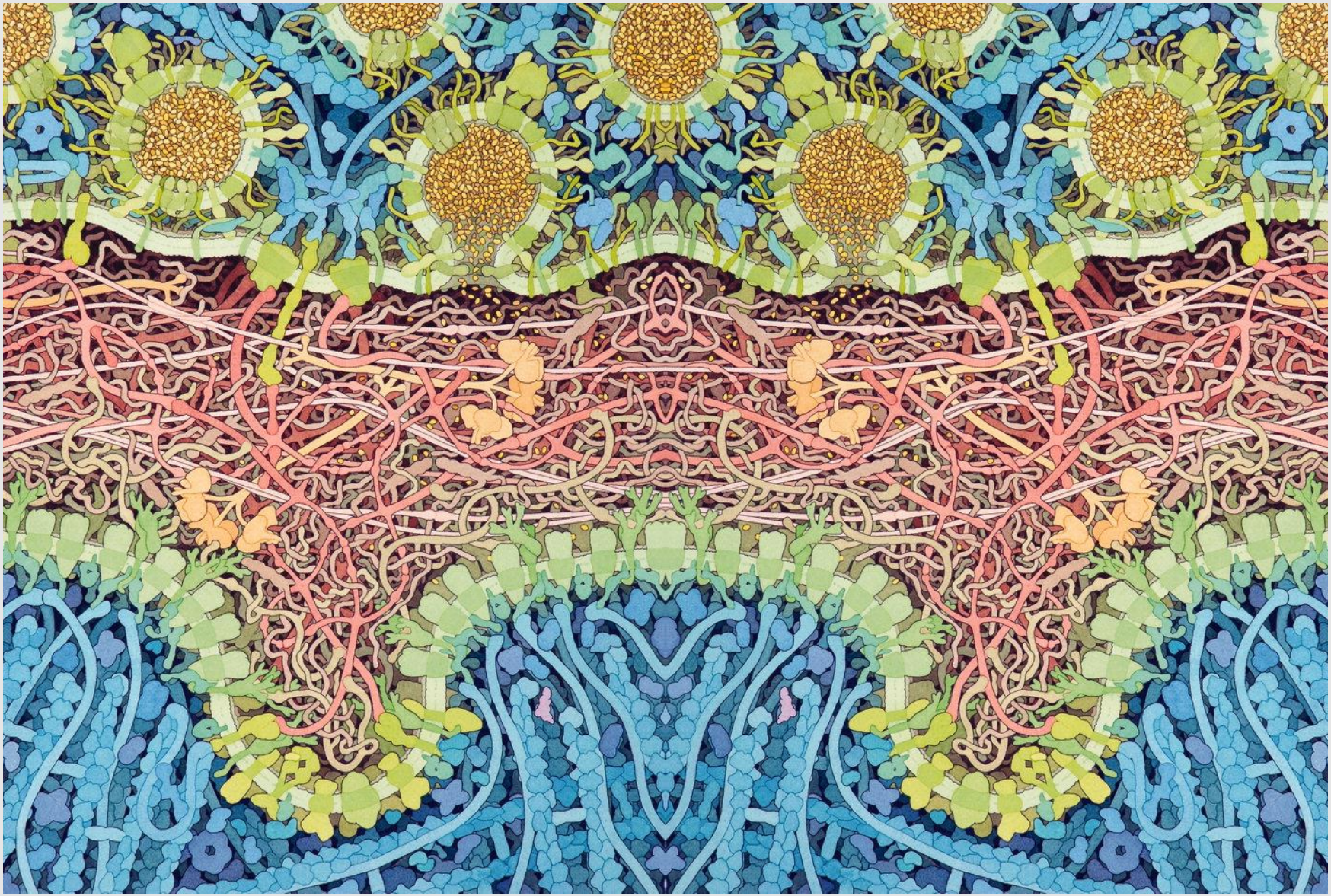





A hallmark of CNS organization is the highly **precise pattern of connectivity** between neurons.

The organization of many brain regions into distinct anatomical layers aids wiring specificity → laminar specificity, limits the range of target cells available for synapse formation.

# The Synaptic Cleft



David S. Goodsell: Illustrating the Machinery of Life – The Neuromuscular Synapse

# Come funziona e cosa è importante

## Neuronal functions

**Neuronal migration**

**Axon and dendrite branching**

**Axon and dendrite guidance**
ephrins, semaphorins, netrins, etc.

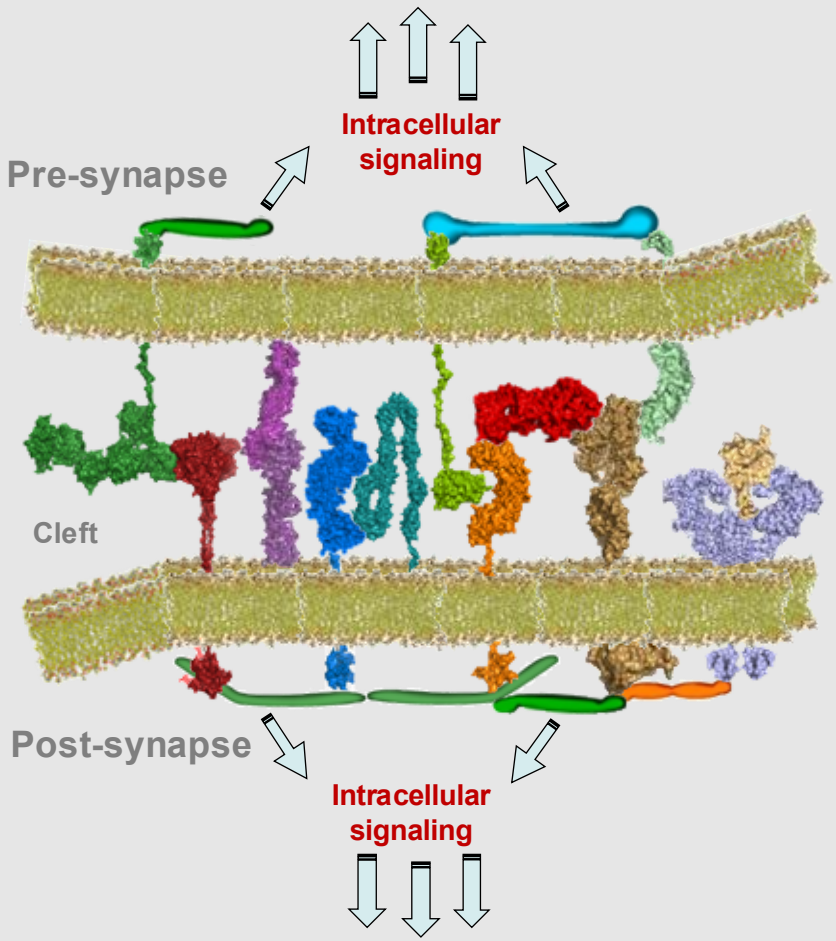
**Target recognition**
Neurexin, Neuroligins, LRRTMS, etc.

**Self-avoidance**
Protocadherins

**Synaptogenesis**
Neurexin, Neuroligins, LRRTMS, SLITRKs, etc.

**Axon and dendrite regeneration**

**Dysfunctions**
ASD, schizophrenia, etc




## Non-neuronal functions

**Vascular patterning**

**Cell migration**

**Immune cell differentiation and function**

## Disease related functions

**Autoimmune activity**

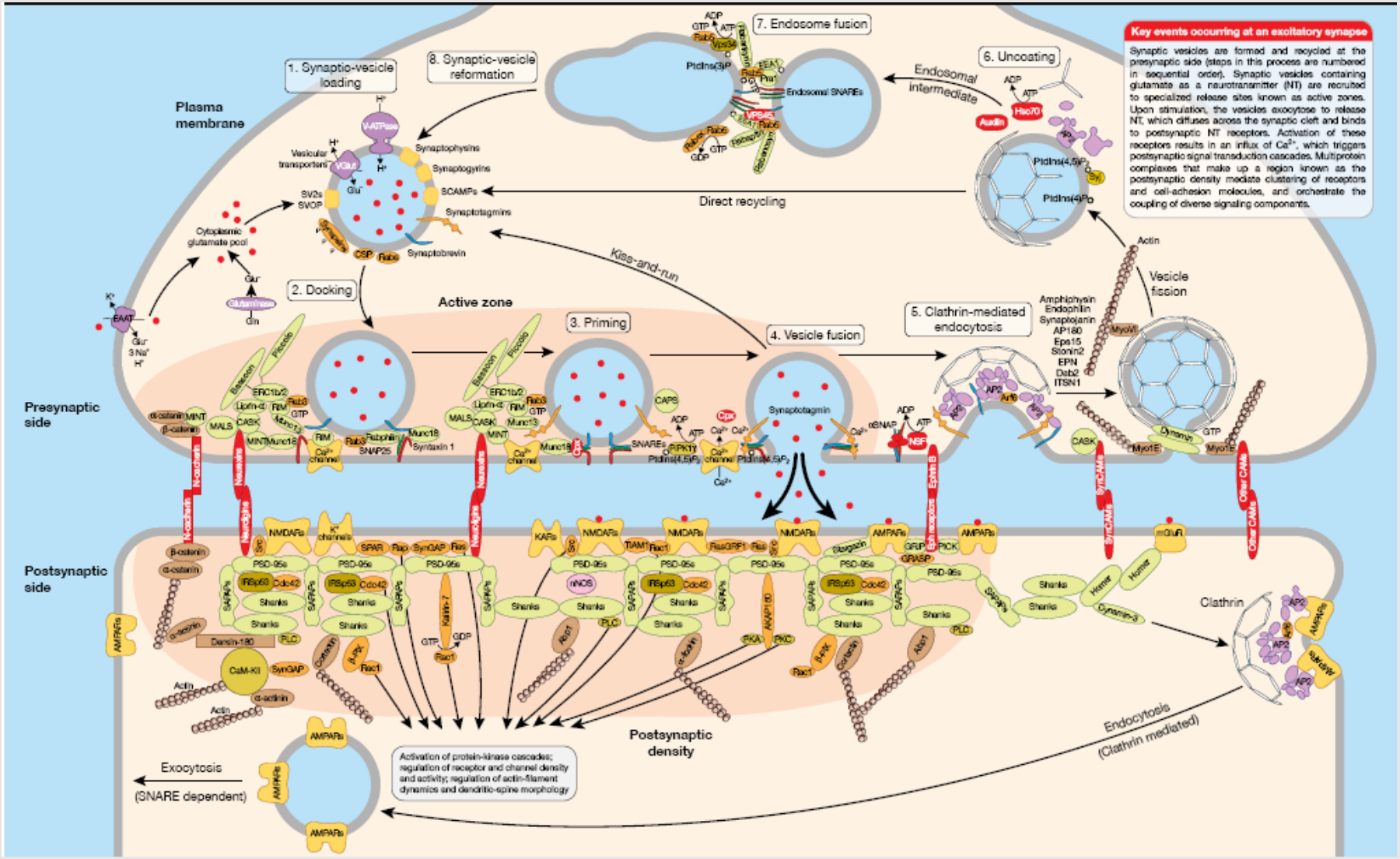
**Tumorigenesis & Metastasis**

**Neurotropic viral receptor**

## Therapeutic targets

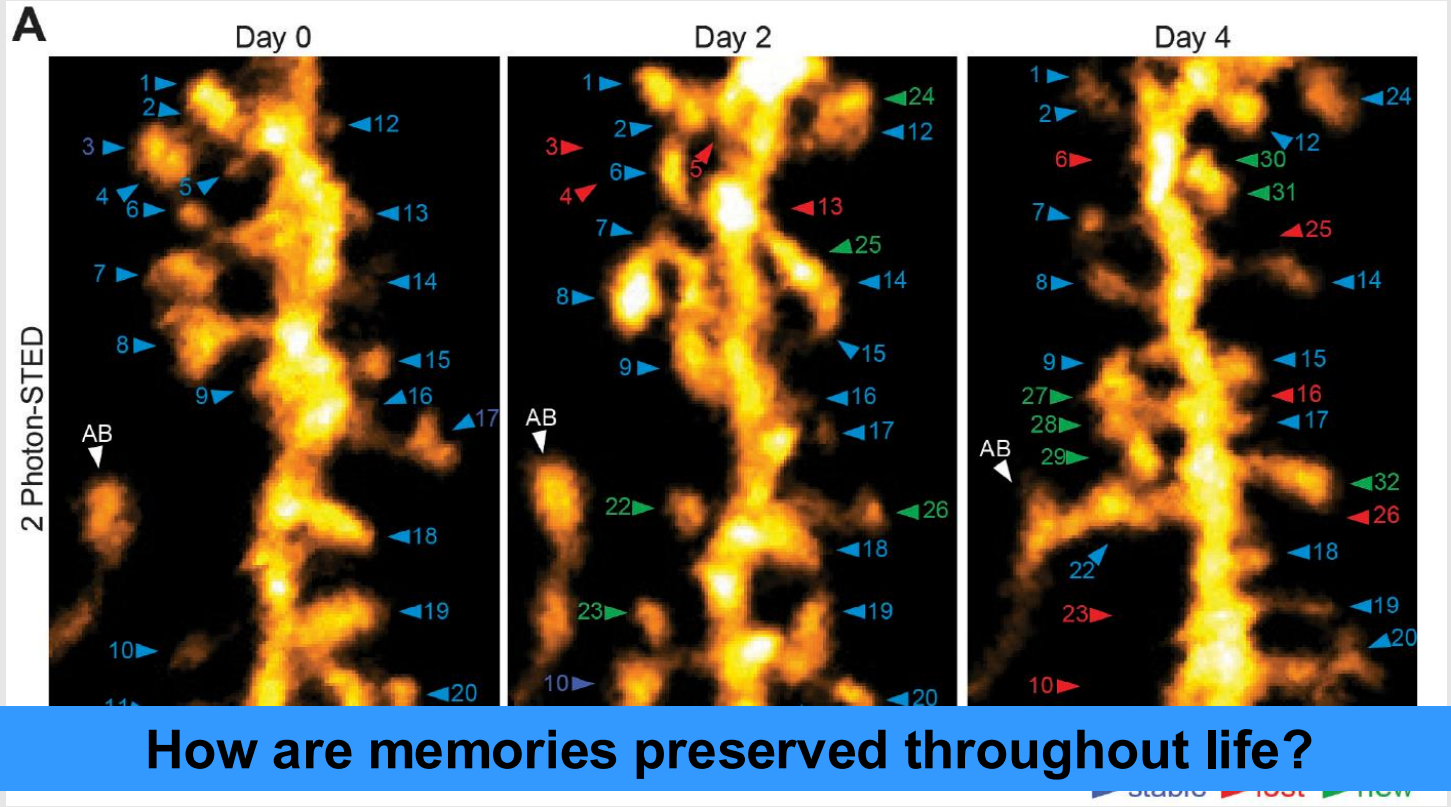
**Rituximab (CD20), Herceptin (EGFR2)**

# LA COMPLESSA ARCHITETTURA DI UNA SINAPSI




**Le molecole di adesione tengono insieme le membrane pre- e post-sinaptiche attraverso lo spazio sinaptico, ma in realtà fanno molto di piu' !**

# Quanto sono stabili le sinapsi?




**How are memories preserved throughout life?**

- ~40% of dendritic spines on pyramidal neurons in the sensory and motor cortex are replaced every 5 d
- in the hippocampus, nearly 100% of spines turn over every 2 weeks

→ most neurons and their long-range axonal and dendritic structures are stable in mature brain, their synaptic connections are often not

# Come funziona e perchè è importante

**By engaging trans-cellular interactions, SAMs can:**

1. nucleate nascent synapses,
2. drive synapse maturation,
3. control the properties of synapses,
4. regulate synapse elimination

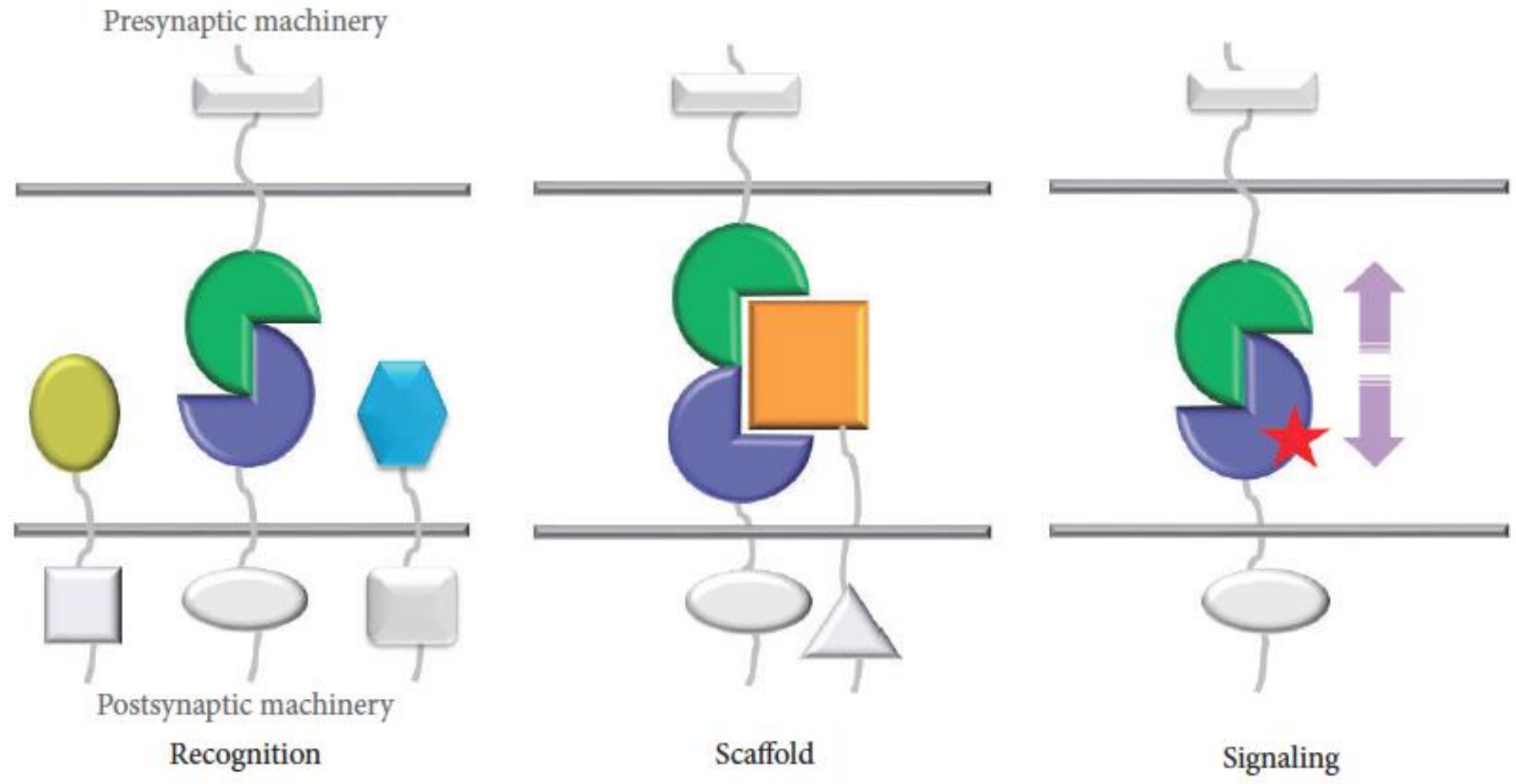
But: **No single "master"** SAM likely controls everything; instead, an **orchestra of SAMs** mediates assembly of diverse synaptic junctions

**Presynaptic** SAMs are mostly "hub" molecules that are present in excitatory and inhibitory synapses, like Neurexins

**Postsynaptic** SAMs are more diverse as ligands for these hub molecules and are often specific for excitatory or inhibitory synapses.

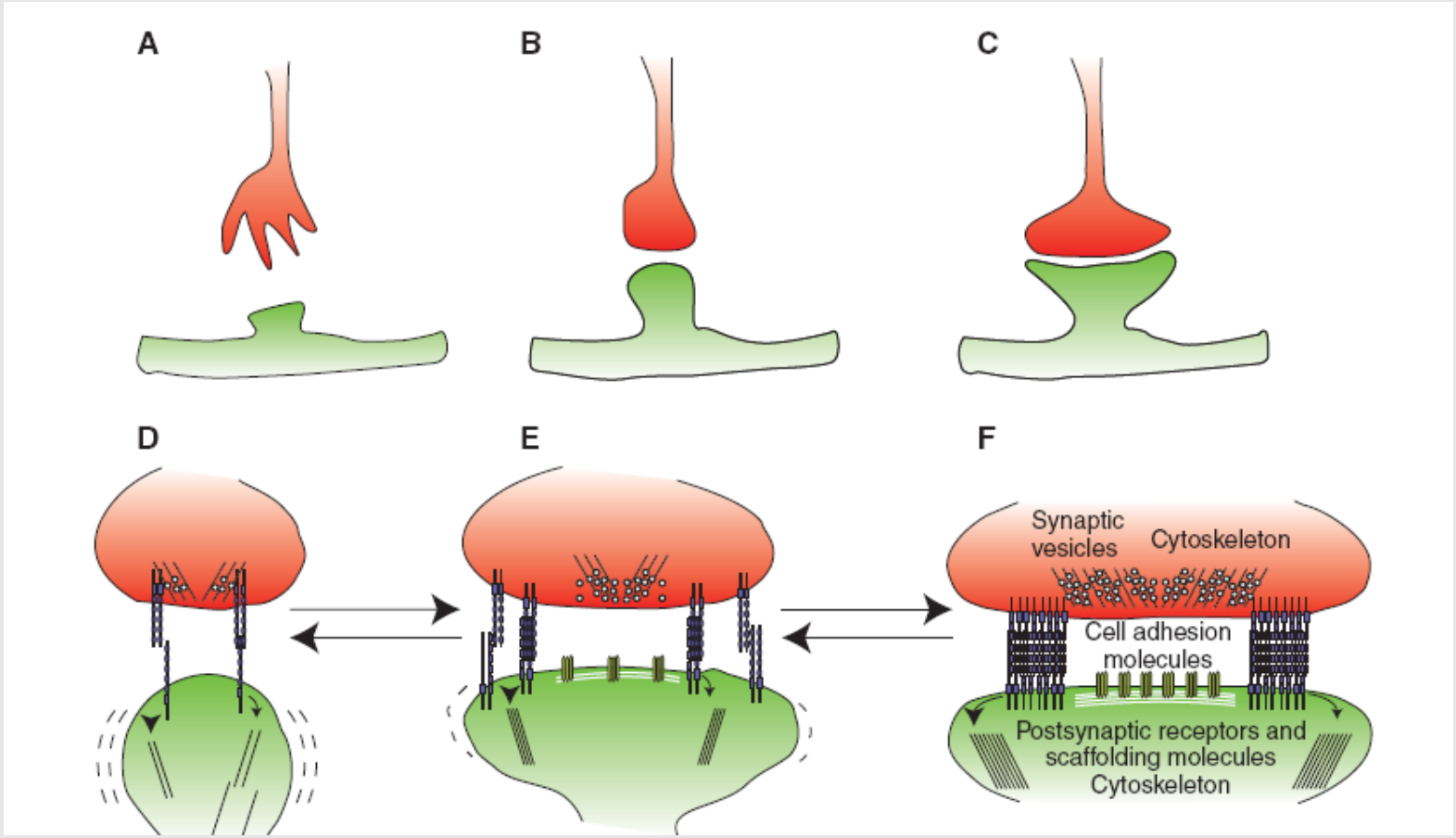
**Post-synaptic sites have receptors, more diverse than pre-synaptic**

# Funzioni delle molecole di adesione sinaptica e meccanismi




SAMs/CAMs possono reclutare e organizzare una rete di interazioni proteiche nella fessura sinaptica: (a) tramite meccanismi di riconoscimento di specifici partners ma non di altri, attraverso legame diretto b) legando altre molecole per generare una impalcatura in cui una terza proteina può interagire (c) legando un partner ed inducendo un evento di segnalazione attraverso un meccanismo per esempio allosterico.

# …INFATTI PARTECIPANO AI VARI STADI DURANTE LA FORMAZIONI DI UNA SINAPSI



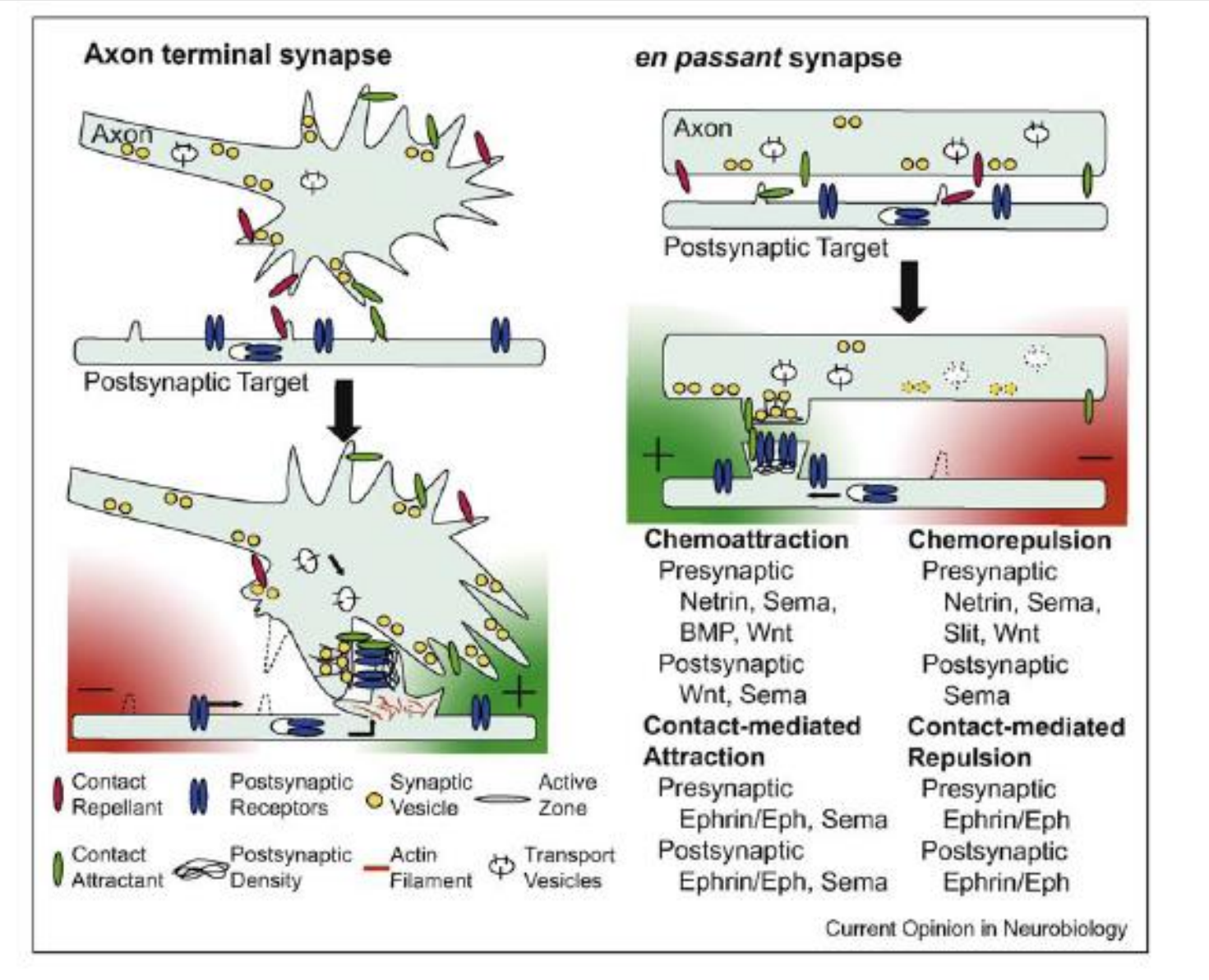

Selezione del bersaglio

Formazione della sinapsi
Reclutamento delle specializzazioni pre e post sinaptiche

Maturazione e stabilizzazione
Plasticità sinaptica

# GUIDA SINAPTICA

Recettori Eph/Efrine sono molecole di guida e partecipano nei fenomeni di attrazione/repulsione mediati da contatto



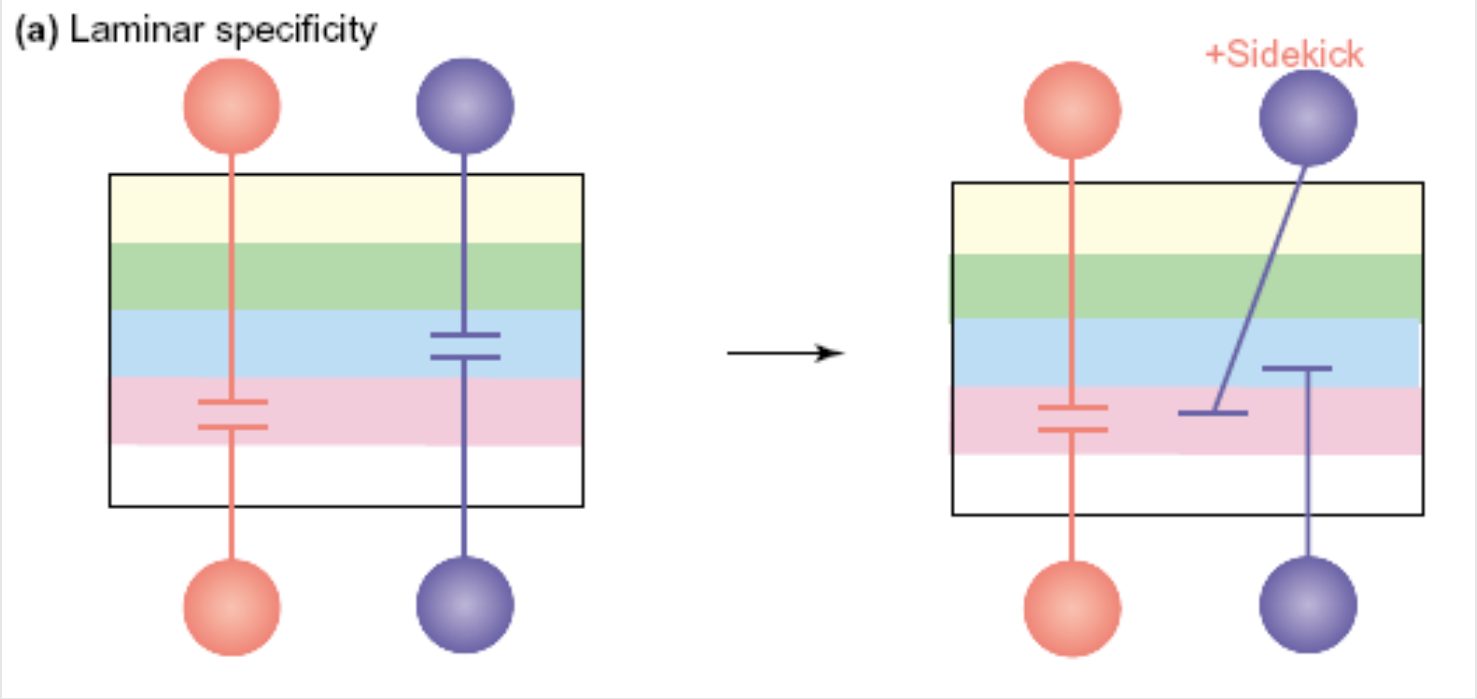
# RICONOSCIMENTO DEL TARGET

Il preciso indirizzamento degli assoni dipende dall'espressione di molecole di adesione. Esempi:

Eph/ephrins

NEPH-1/nephrin (SYG-1 Droso)

SIDEKICK (famiglia delle immunoglobuline): formazione di sinapsi selettive in zone stratificate (specificità laminare) nella retina di pollo




La sovraespressione di sidekick altera la connettività

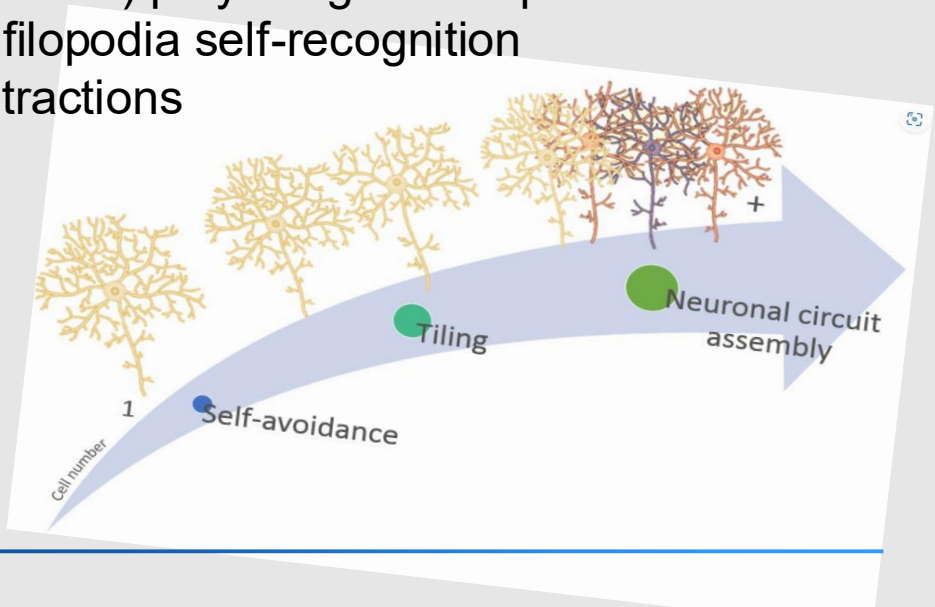
# Dendrite Self-Avoidance

Sister branches from the **same neuron** recognize and repel each other → a) preventing overlap and, b) ensuring uniform coverage of the receptive field.

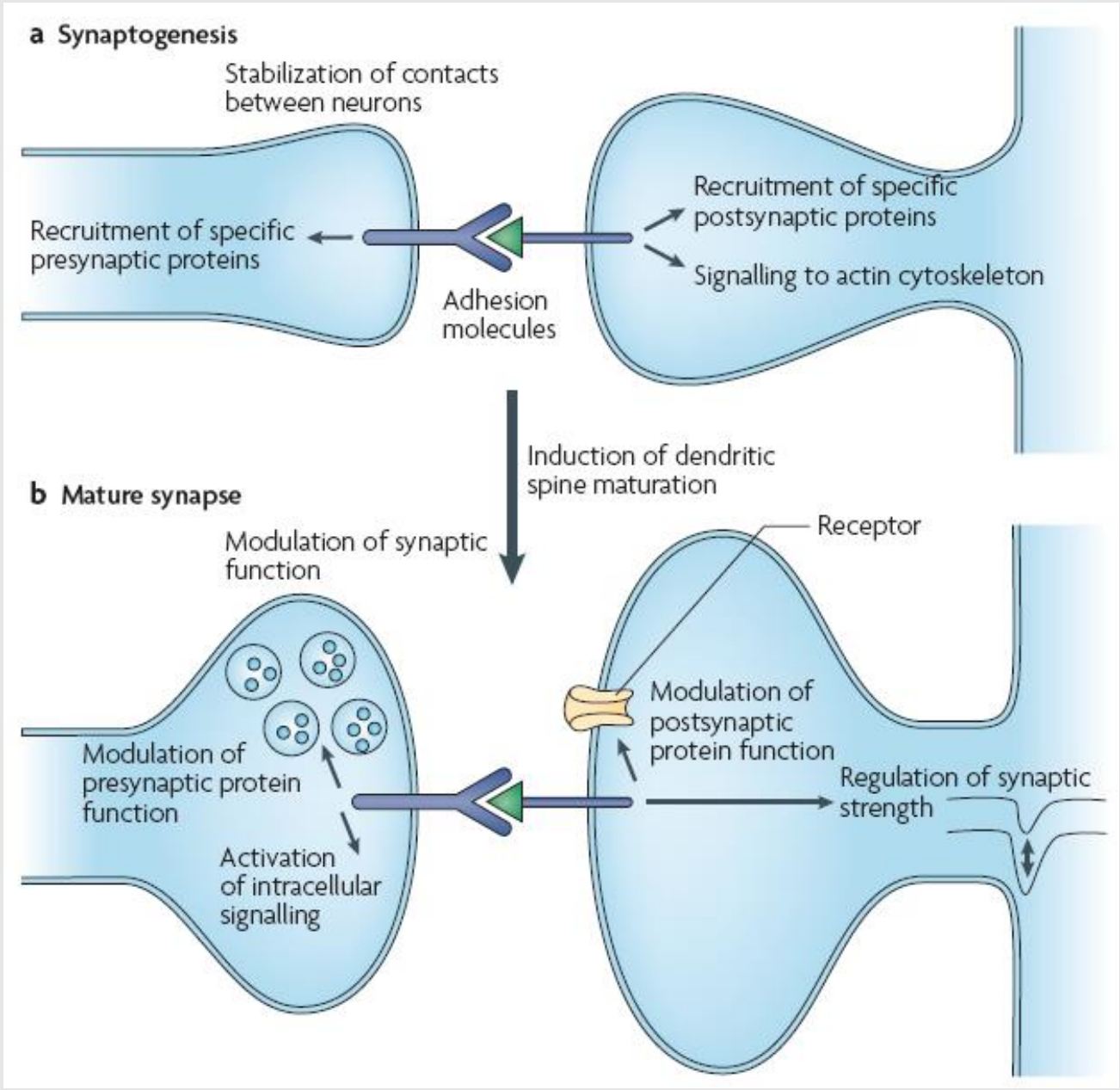
**Molecular Mechanisms**
**In Drosophila**, Dscam1 (Down Syndrome Cell Adhesion Molecule 1) provide most of the dendritic self-avoidance
Homotypic repulsion is mediated by **binding of identical Dscam1 isoforms** on opposing sister branches
Dscam1 has huge molecular diversity (thousands of isoforms)

**In mammals,** Clustered Protocadherins (cPcdhs) play a significant part
Regulate contact-induced retractions upon filopodia self-recognition
Selectively mediate self-contact-induced retractions
Not enough molecular diversity?

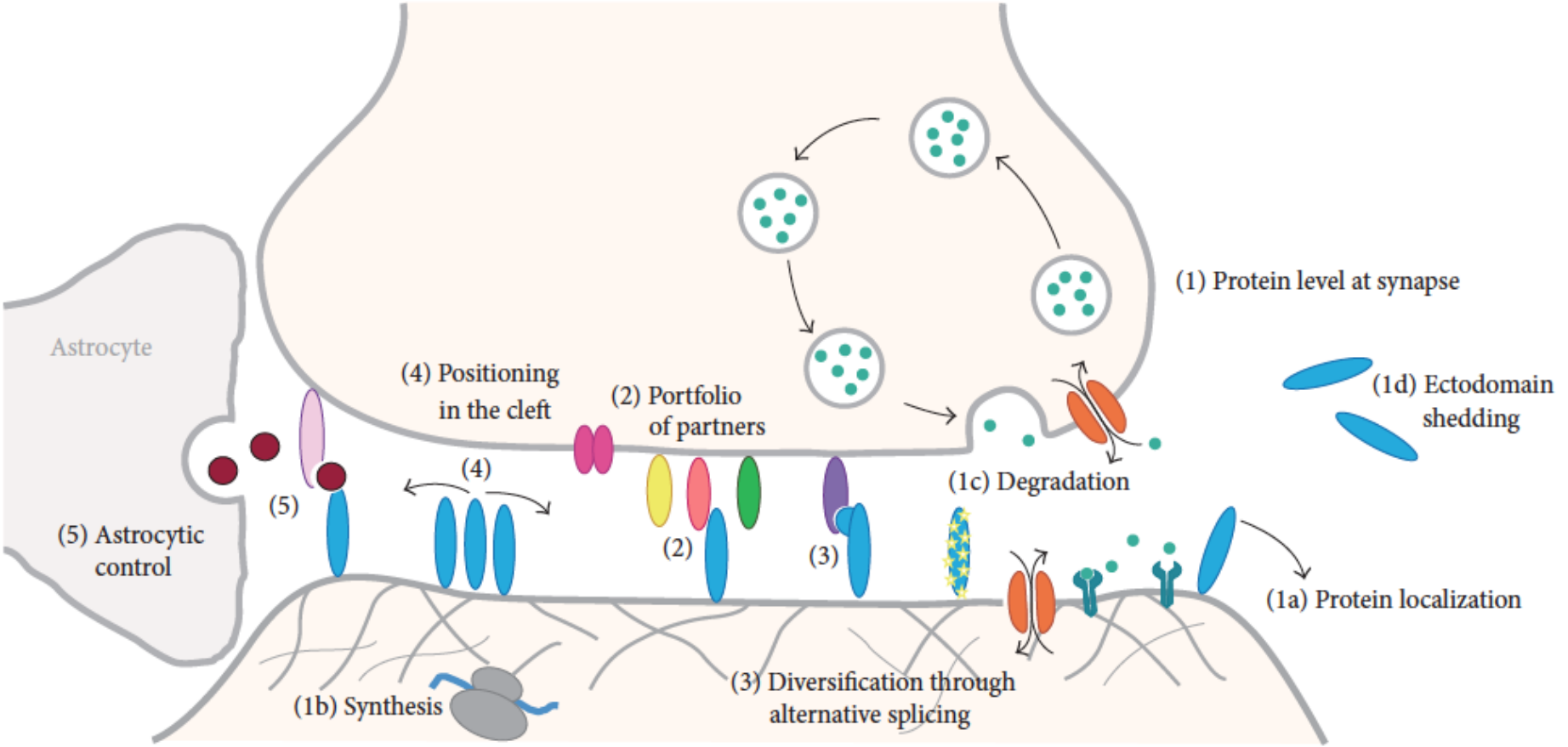
Other molecular players are likely involved
Other mechanisms involved?



# SINAPTOGENESI E MATURAZIONE SINAPTICA

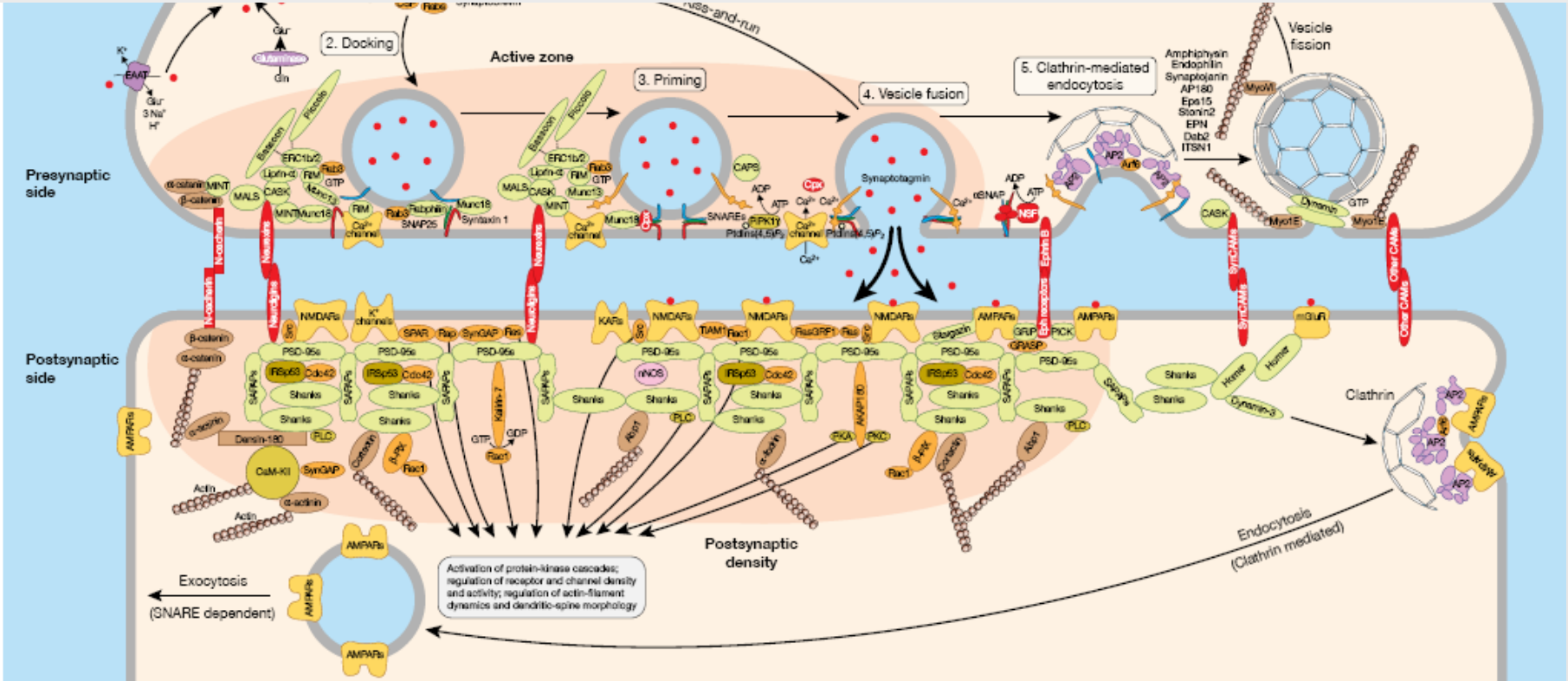


SAMs sono strategicamnte posizionate per contribuire alla plasticità sinaptica, infatti possono modificare la struttura della sinapsi e la sua funzione attraverso la loro capacità di scolpire e regolare la rete delle interazioni proteiche alla sinapsi.

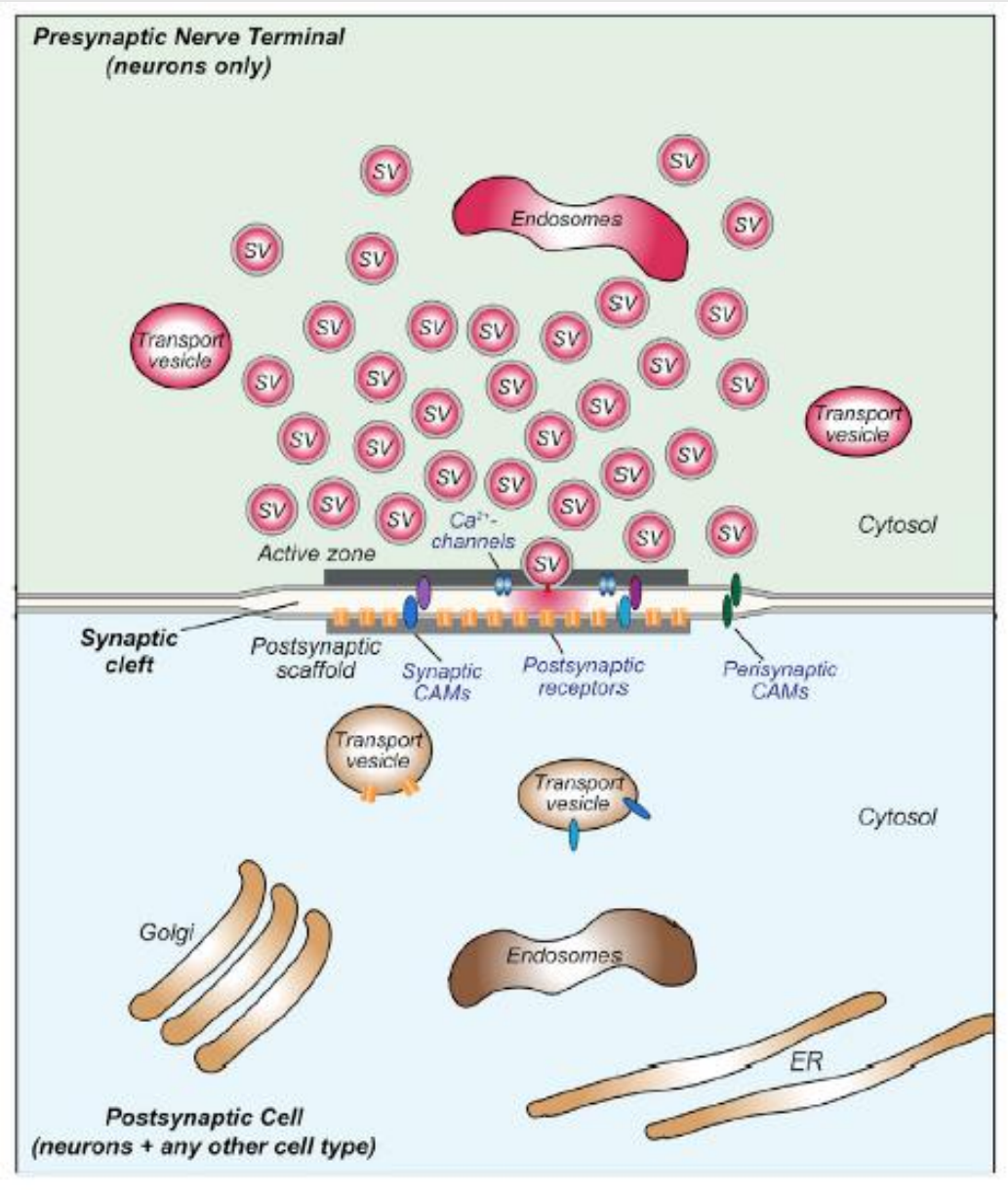


# Ancoraggio e segnalazione intracellulare delle molecole di adesione sinaptiche: impalcature multimolecolari

-Interazione fra proteine tramite domini citoplasmatici e/o proteine scaffold
-Caderine: interazione con catenine e col citoscheletro di actina
-Integrine: interazione col citoscheletro di actina tramite vinculina e spectrina
-Segnalazione via attività enzimatica propria o tramite interazione con altre proteine (kinasi, fosfatasi)



# RAPPRESENTAZIONE CLASSICA DELLA SINAPSI

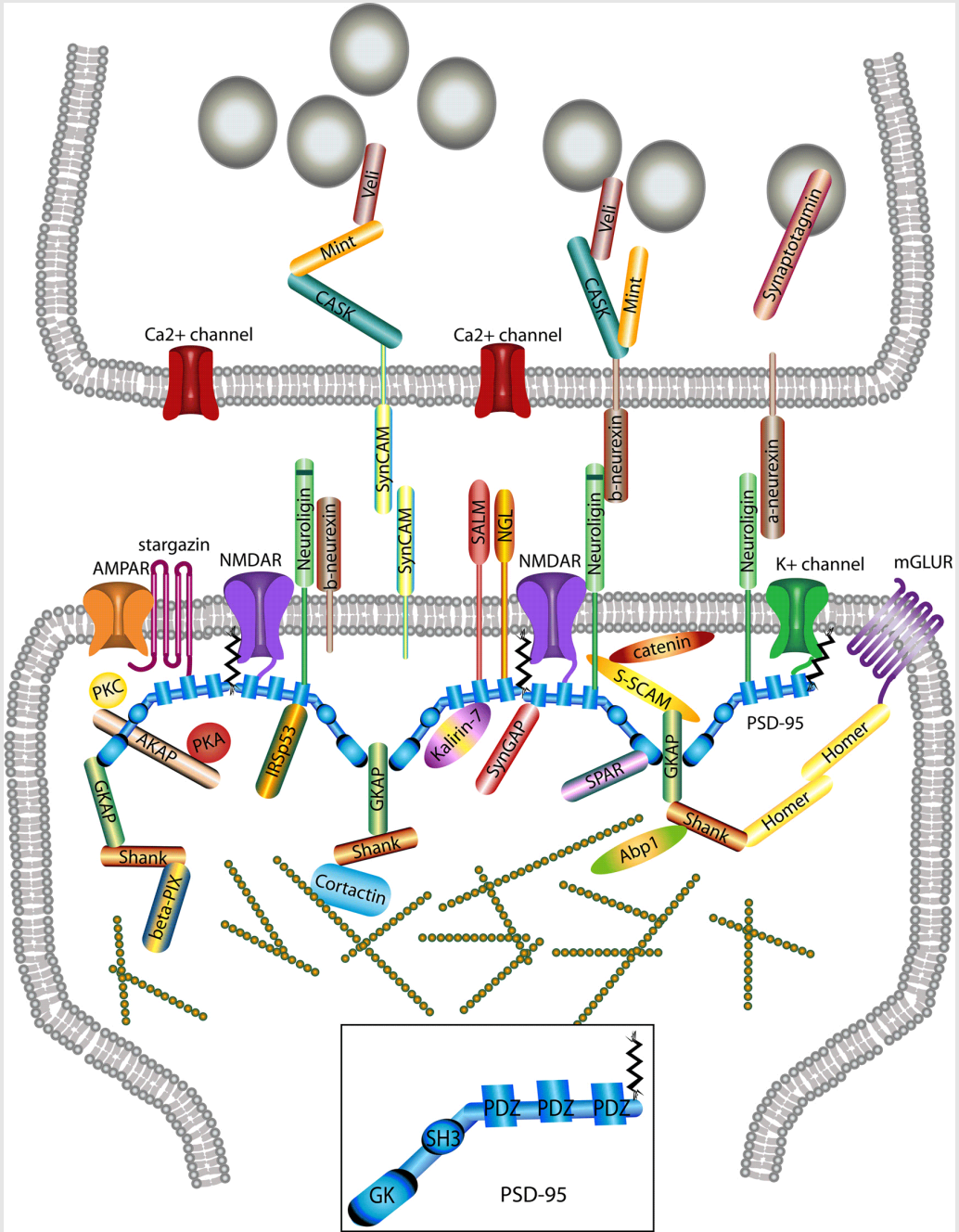



Tutte le sinapsi contengono componenti pre-sinaptici simili independentemente dal tipo cellulare, sebbene isoforme specifiche possono variare.

Mentre la componente post-sinaptica ha meno omologie

Le specializzazioni pre-sinaptiche sono formate da neuroni mentre quelle post-sinaptiche possono essere formate da cellule anche diverse.

# DOMINII DI INTERAZIONE INTRACELLULARE: IL DOMINIO PDZ



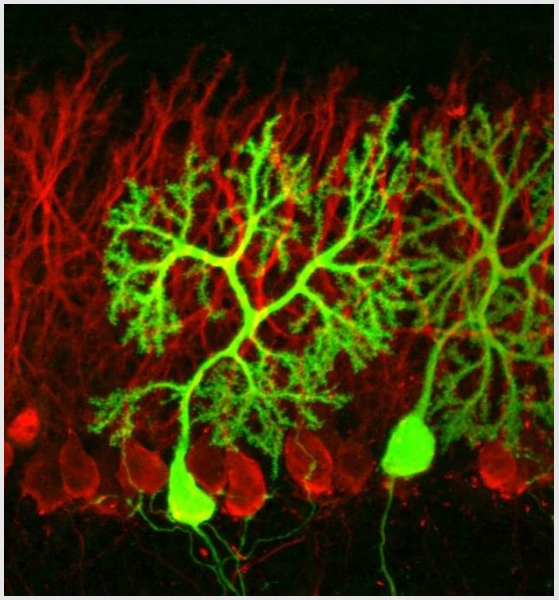

Domini PDZ: lunghi 90 residui, identificati come regioni omologhe in diverse proteine di segnalazione

Il nome PDZ deriva dalle prime 3 proteine in cui il dominio è stato identificato: PSD-95 (proteina di 95 kDa nella densità post-sinaptica; Dlg (the *Drosophila* discs large protein), and ZO1 (proteina della zonula occludente).

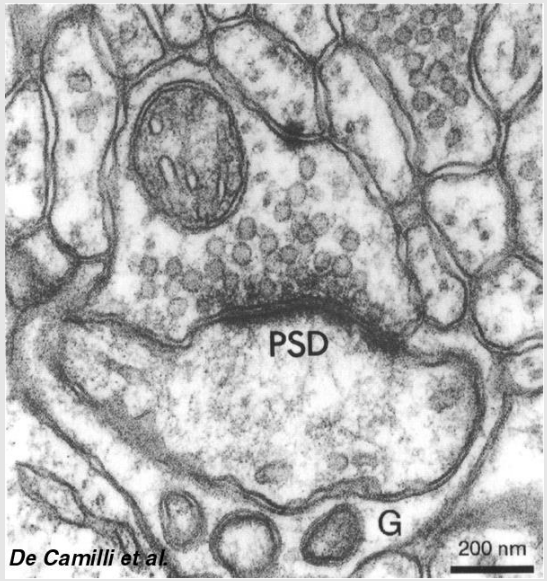
# DALLE MOLECOLE DI ADESIONE AI COMPLESSI SINAPTICI

## REGOLAZIONE DELLA STRUTTURA E FUNZIONE

- Le molecole di adesione sono i regolatori centrali della polarizzazione cellulare, della migrazione e dell'organizzazione tri-dimensionale della sinapsi

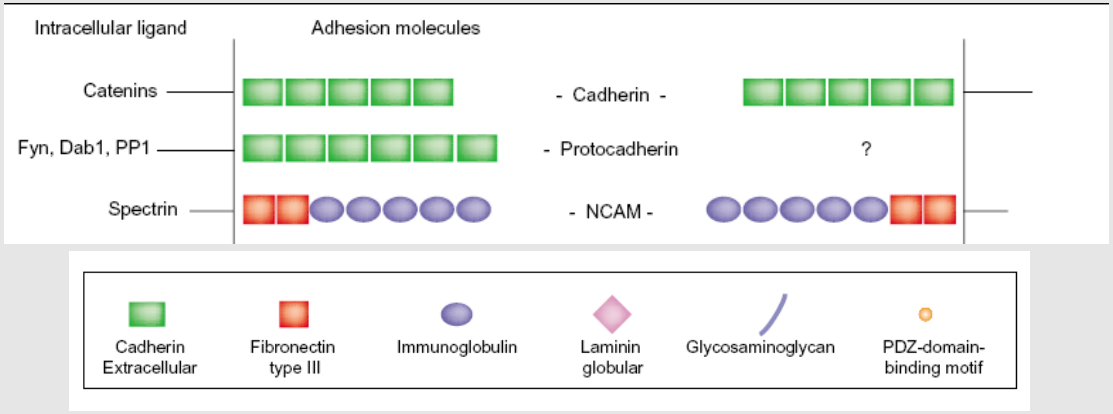


- I complessi sinaptici composti da molecole di adesione cellulare ed altre molecole sinaptiche sono regolati in maniera dinamica per modificare la architettura e la funzione cellulare.



Si stima che nell'uomo ci siano 470 molecole di adesione sebbene non sia noto Quante di queste siano espresse nel cervello e quante siano quelle sinaptiche.

Come è raggiunta la diversità:
-La maggior parte ha una regione extracellulare con un'organizzazione modulare che alterna differenti moduli strutturali.
-In più, la maggior parte delle famiglie di molecole di adesione sinaptica contengono diversi membri che pur presentando strutture conservate hanno un certo grado di diversità nella sequenza aminoacidica.
- possono assumere funzioni diverse durante fasi diverse dello sviluppo mentre le connessioni si stanno formando e nel cervello maturo:
Es. in fasi precoci dello sviluppo: neuroligins and LRRTMs si compensano;
Ma una volta formate le sinapsi, hanno effetti diversi sulla trasmissione di tipo eccitatoria



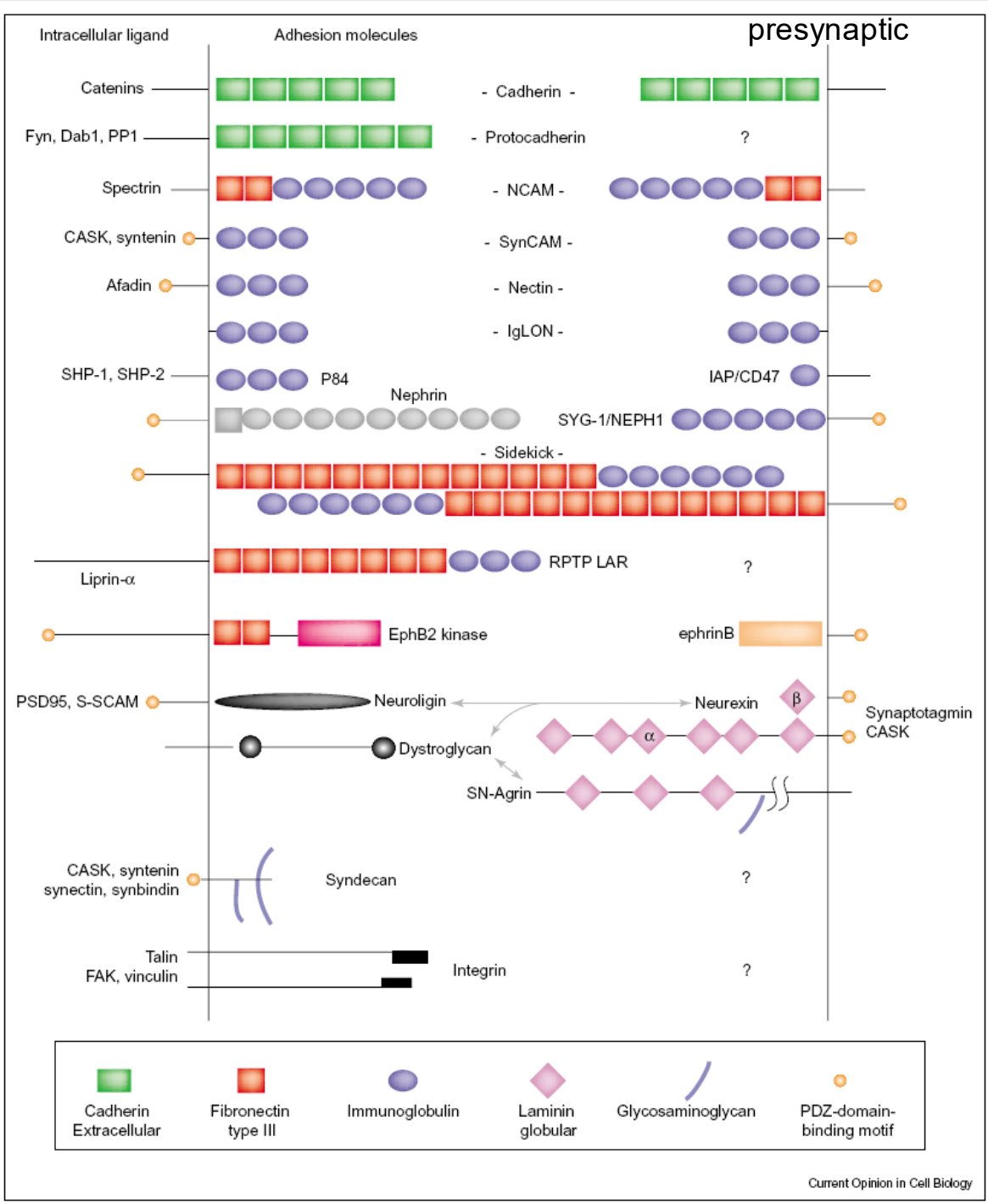

Sebbene ad oggi si conoscano molte molecole di adesione sinaptica, di molte di loro ancora non si conosce l'esatto ruolo funzionale….

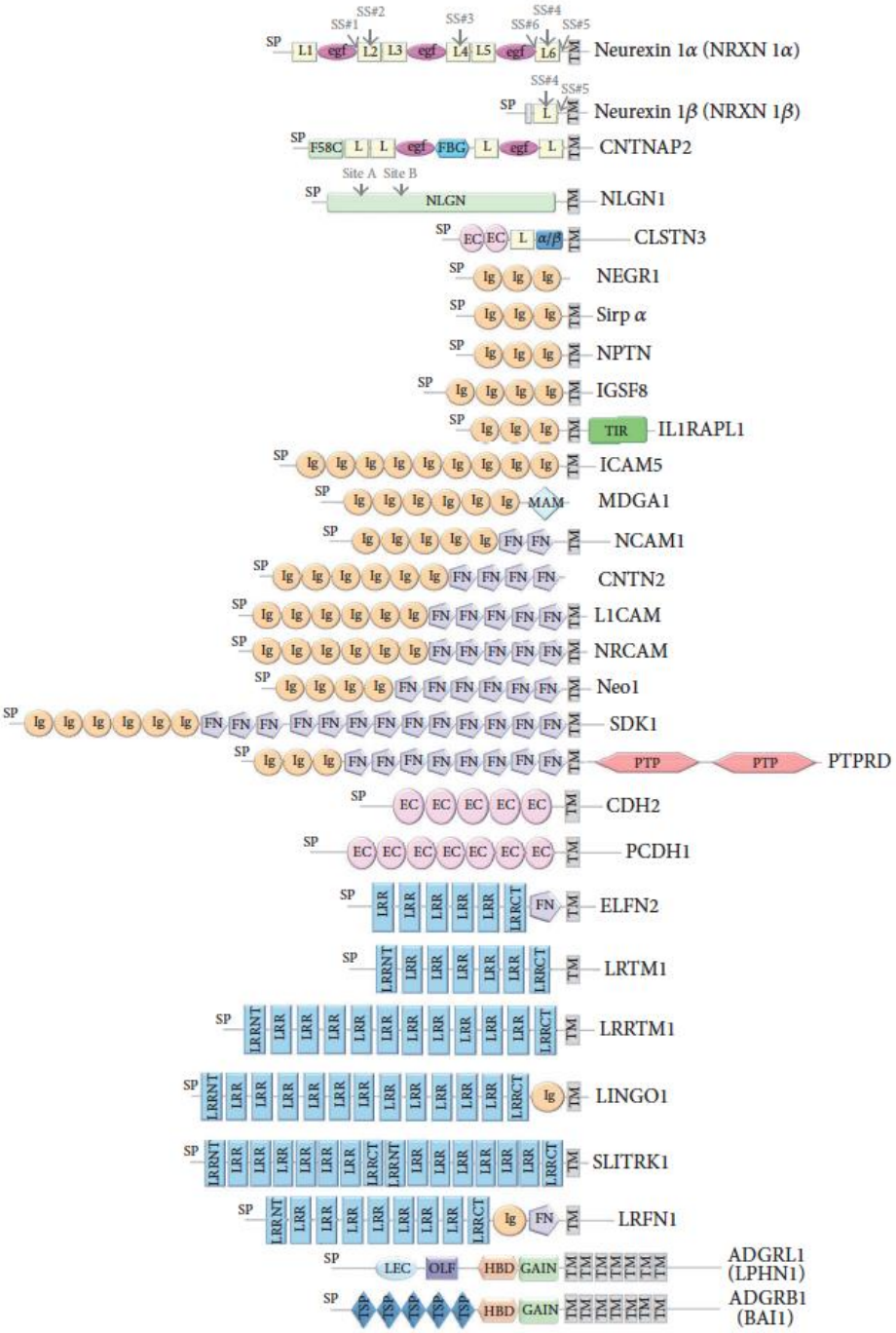
MOLECOLE DI ADESIONE (CAM/SAM) CONCENTRATE ALLE SINAPSI NEURONE-NEURONE

è possibile raggrupparle in base alla loro struttura in sottofamiglie.
Quelle principali sono:

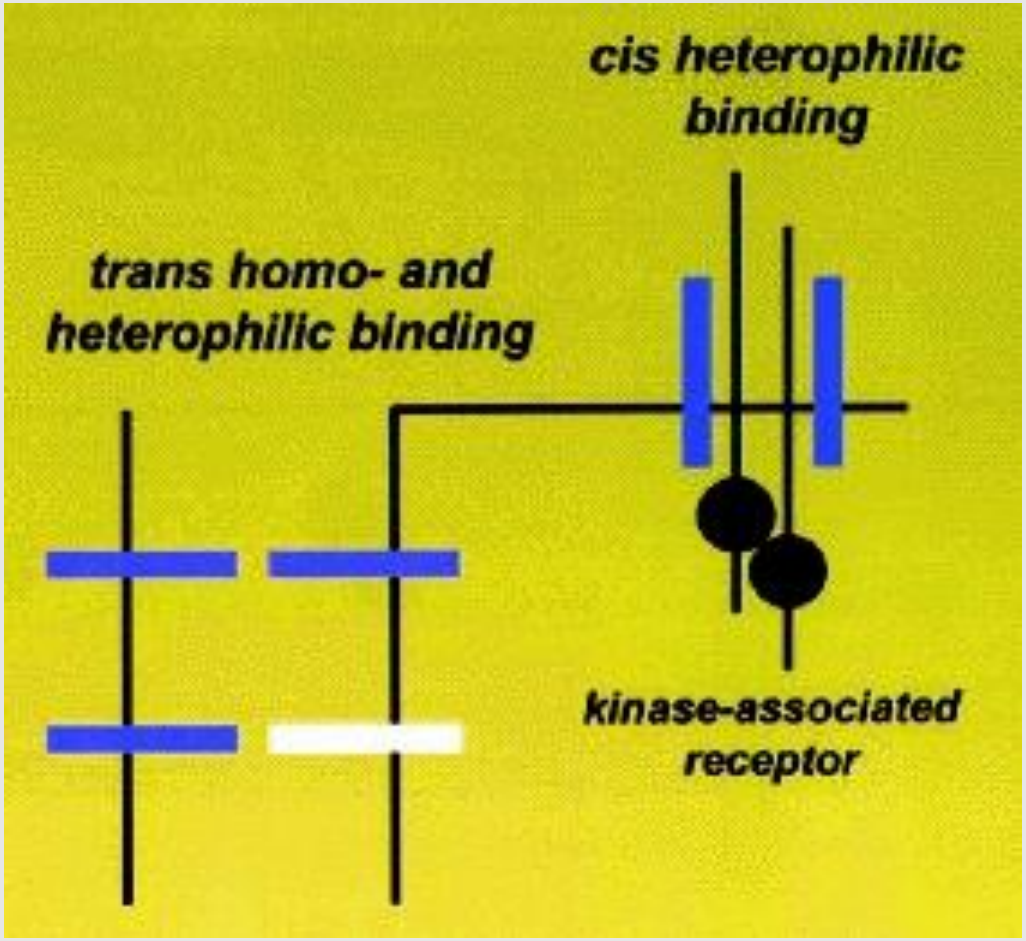
- Caderine
- Immunoglobuline CAM
- Recettori Eph-efrine
- Neurexine-neurolighine

La classifica delle famiglie di molecole di adesione puo' avvenire in base a diversi criteri, uno dei quali e' il tipo di legame che i componenti di ogni famiglia mediano




SS#1
SS#2
SS#3
SS#4
SS#5
SS#6
SP
L1
egf
L2
L3
egf
L4
L5
egf
L6
TM
Neurexin 1α (NRXN 1α)
L
Neurexin 1β (NRXN 1β)
F58C
FBG
CNTNAP2
Site A
Site B
NLGN
NLGN1
EC
α/β
CLSTN3
Ig
NEGR1
Sirp α
NPTN
IGSF8
TIR
IL1RAPL1
ICAM5
MAM
MDGA1
FN
NCAM1
CNTN2
L1CAM
NRCAM
Neo1
SDK1
PTP
PTPRD
CDH2
PCDH1
LRR
LRRCT
ELFN2
LRRNT
LRTM1
LRRTM1
LINGO1
SLITRK1
LRFN1
LEC
OLF
HBD
GAIN
ADGRL1
(LPHN1)
TS
ADGRB1
(BAI1)

# LEGAME OMOFILICO ED ETEROFILICO



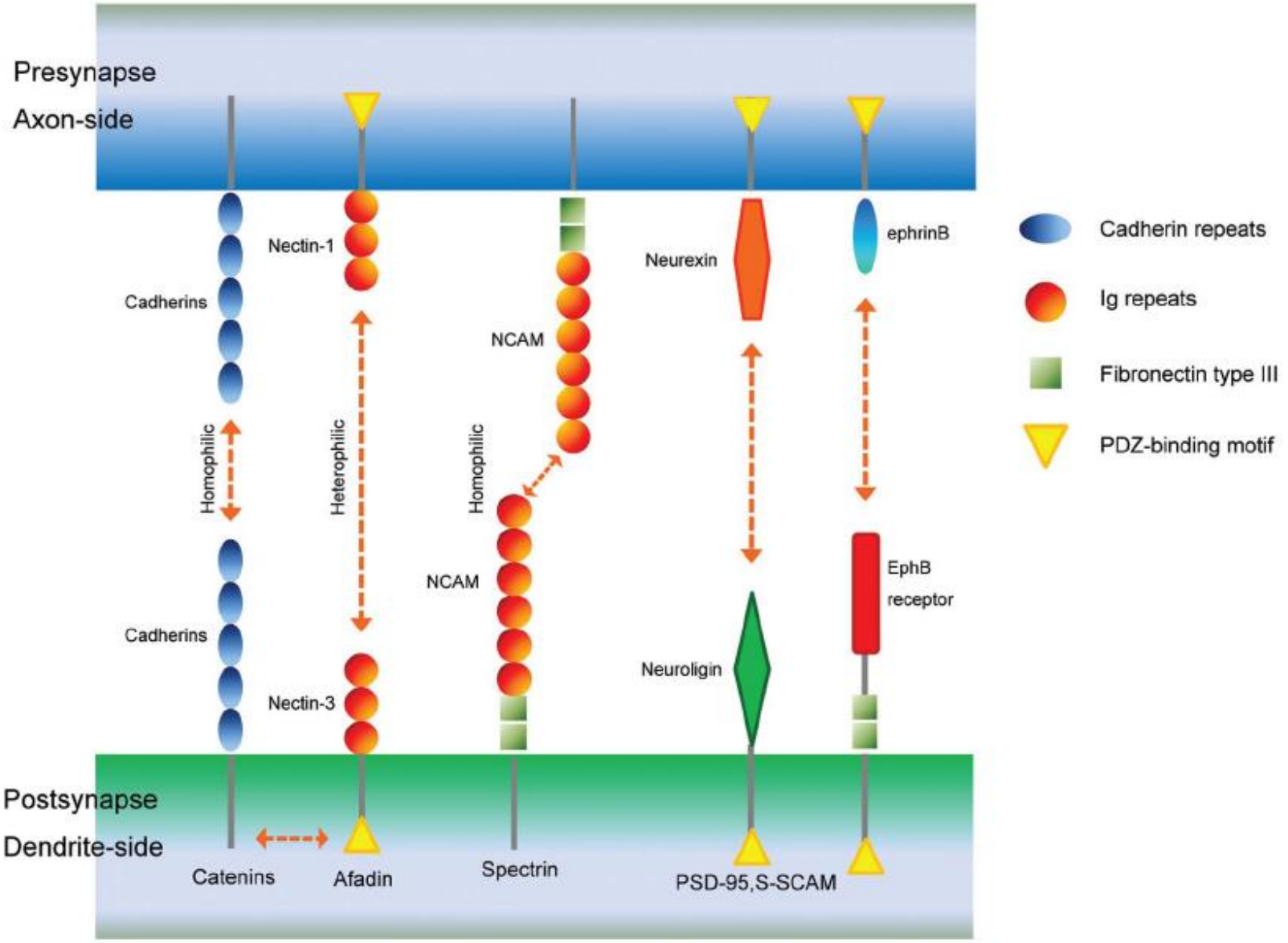

**Omofilico**: legame con la stessa molecola sulla membrana della cellula adiacente

**Eterofilico**: legame con una molecola differente sulla membrana della cellula adiacente

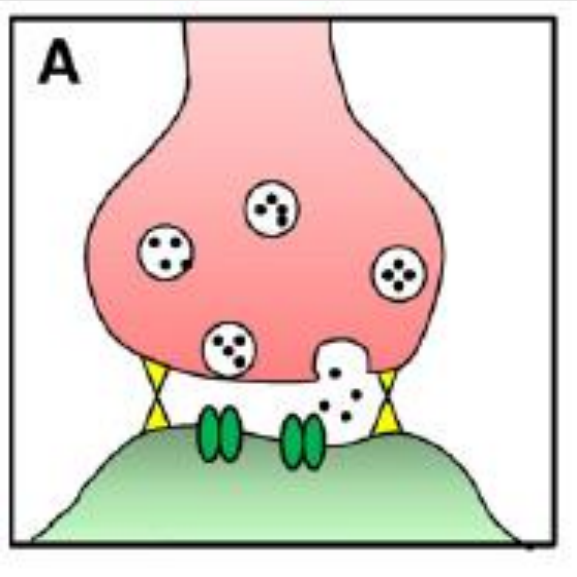
Le molecole di adesione possono interagire in maniera etero- o omofilica tra due cellule diverse (interazione in trans) o sullo stesso piano della membrana di una singola cellula (interazione in cis)

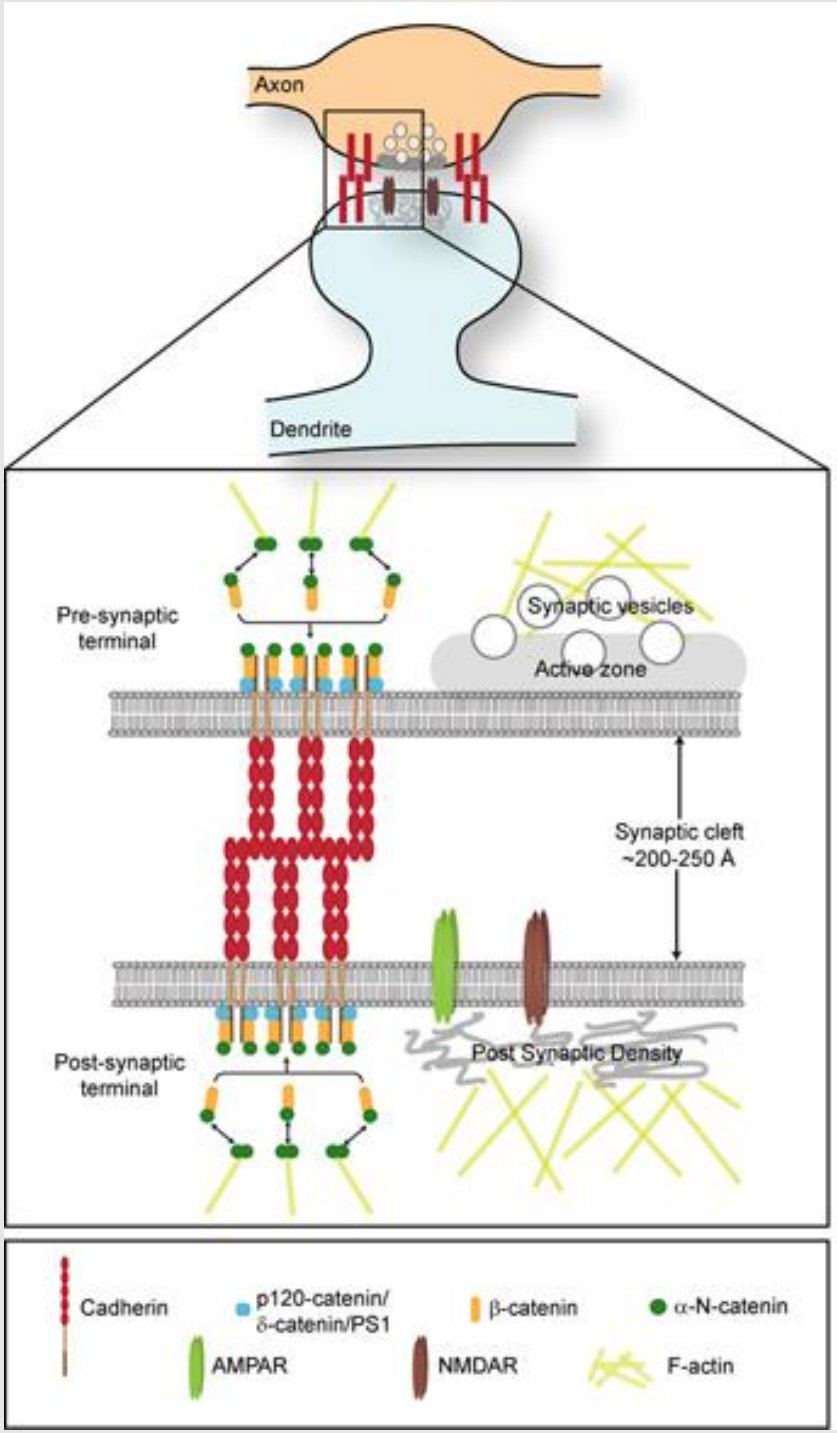
Legame omofilico: caderine, famiglia delle immunoglobuline (NCAM)

Legame eterofilico: recettori Eph/ Efrine, neurolighine/neurexine



# LEGAME OMOFILICO






# LA SUPERFAMIGLIA DELLE CADERINE

Presenti sia ai terminali presinaptici che post-sinaptici

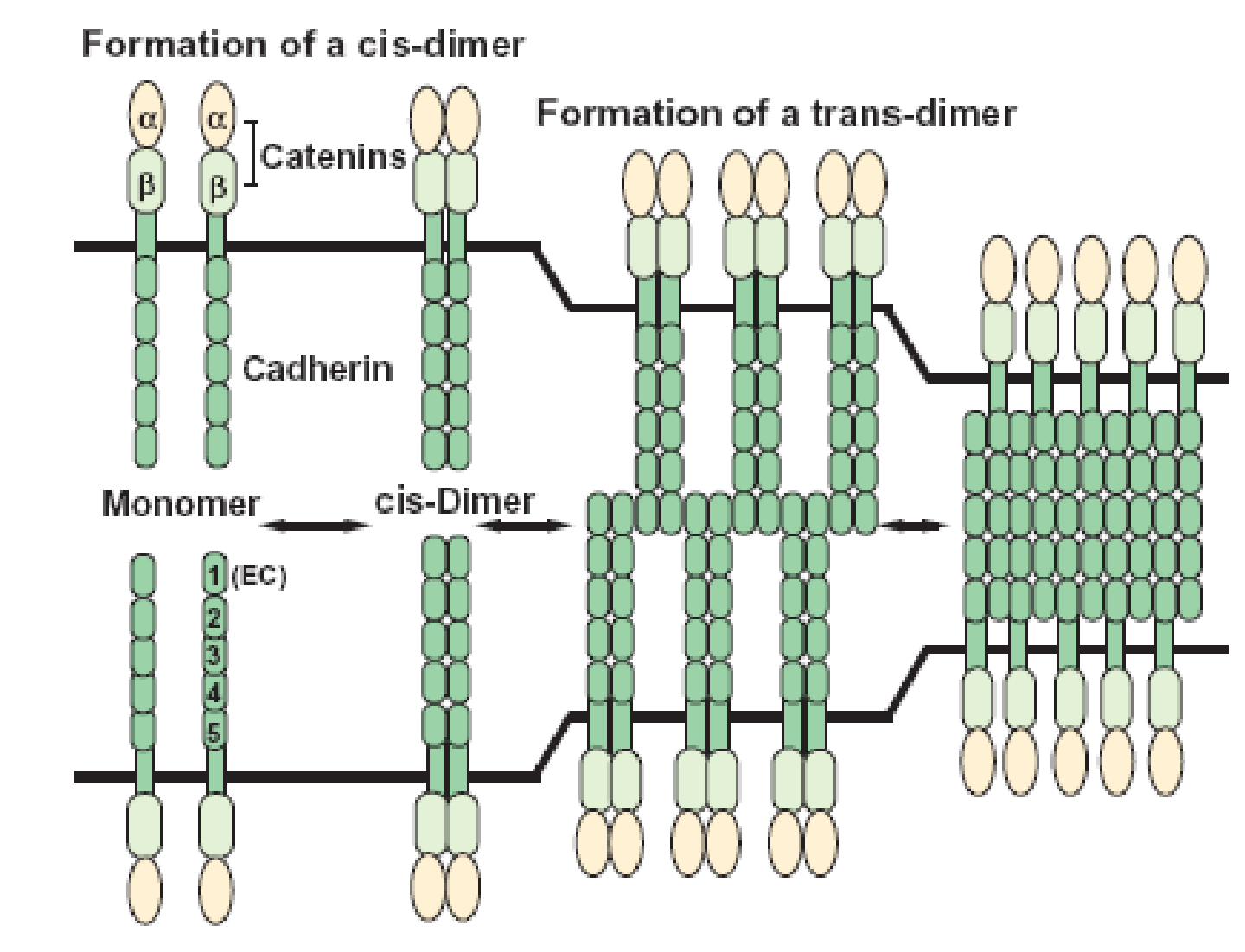
Legano altre Caderine
sia in cis che in trans

Legame calcio dipendente

Dimeri in cis che poi associano con altri dimeri in cis nello spazio sinaptico per formare complessi di adesione in trans (legame a cerniera)

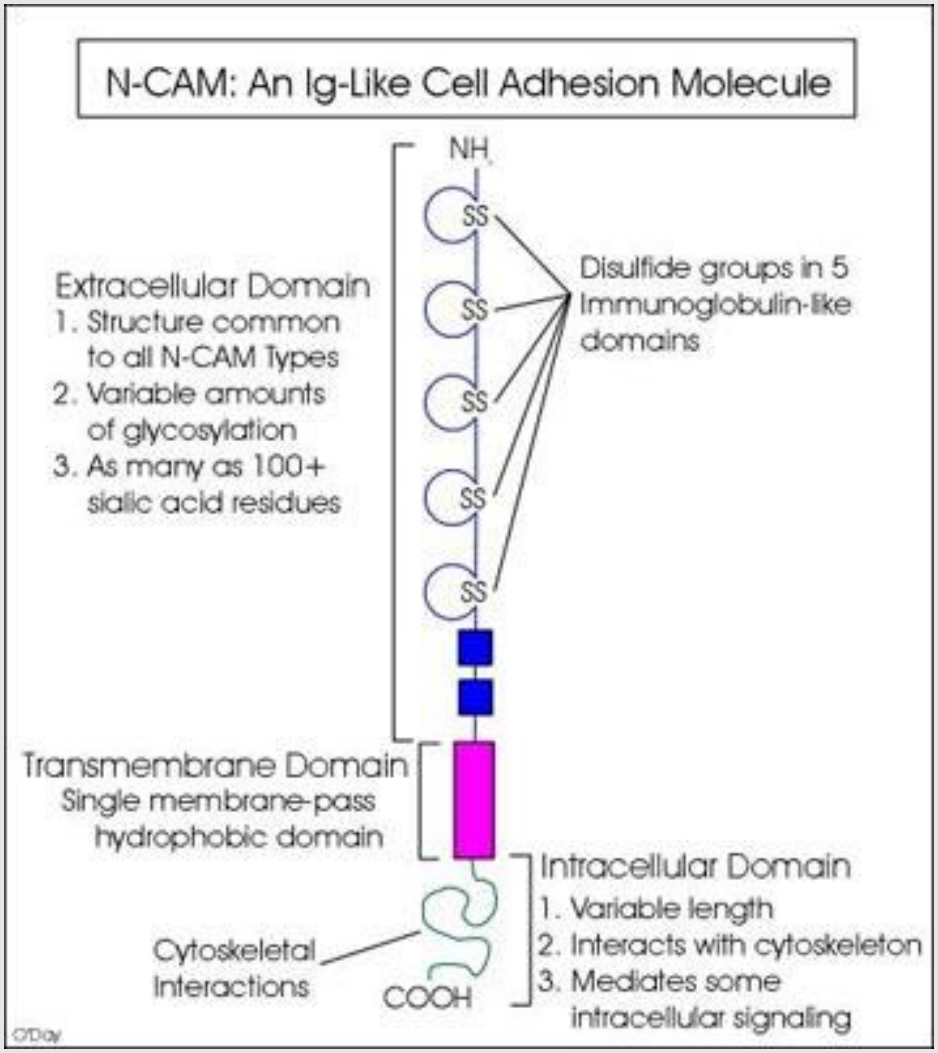
Interazione con il citoscheletro tramite catenine

Si formano dimeri in *cis* che associano con altri dimeri in *trans* nello spazio sinaptico per formare complessi di adesione altamente resistenti



# MOLECOLE DI ADESIONE SUPERFAMIGLIA IMMUNOGLOBULINE

Maggiori rappresentanti: NCAM, L1, Sidekick, SynCAM, Nectina




Legami di tipo omofilico sia in trans che in cis

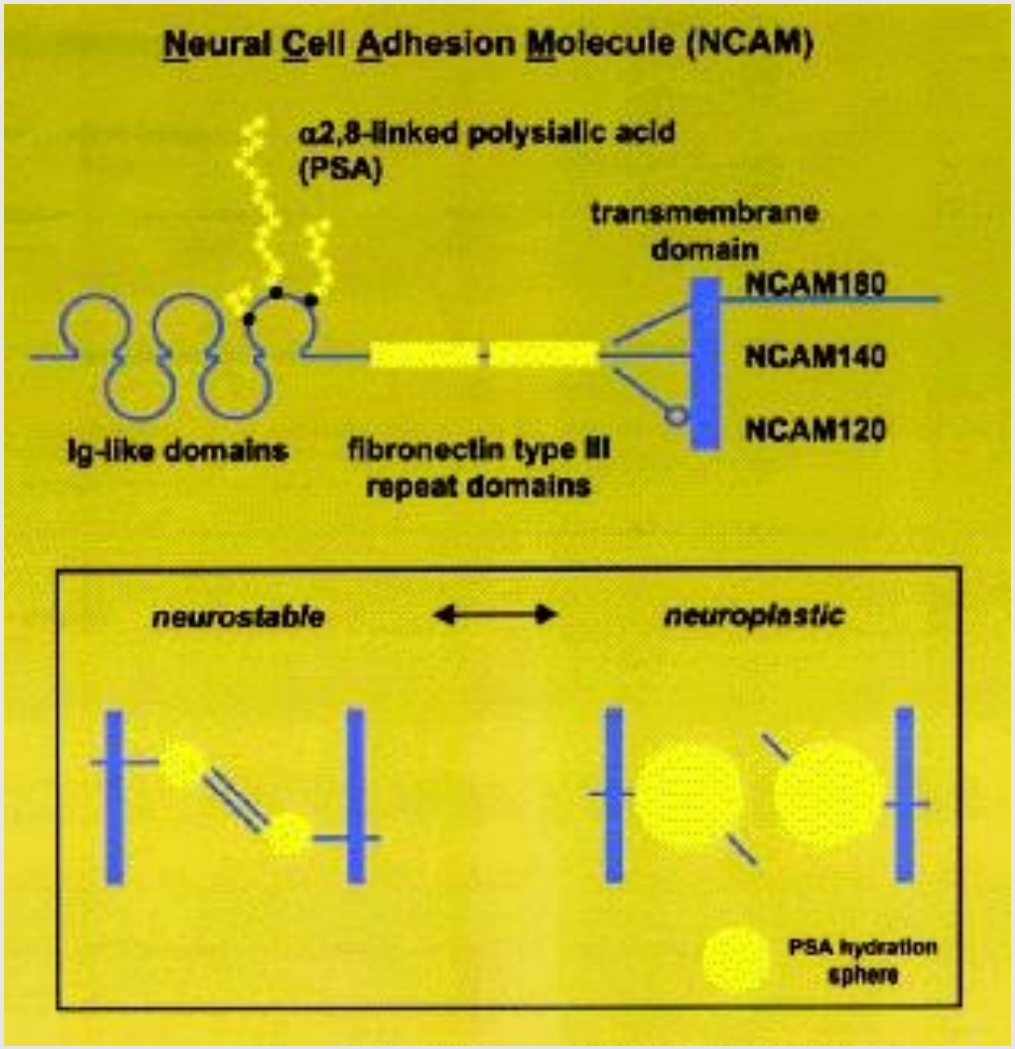
Legame indipendente dal calcio

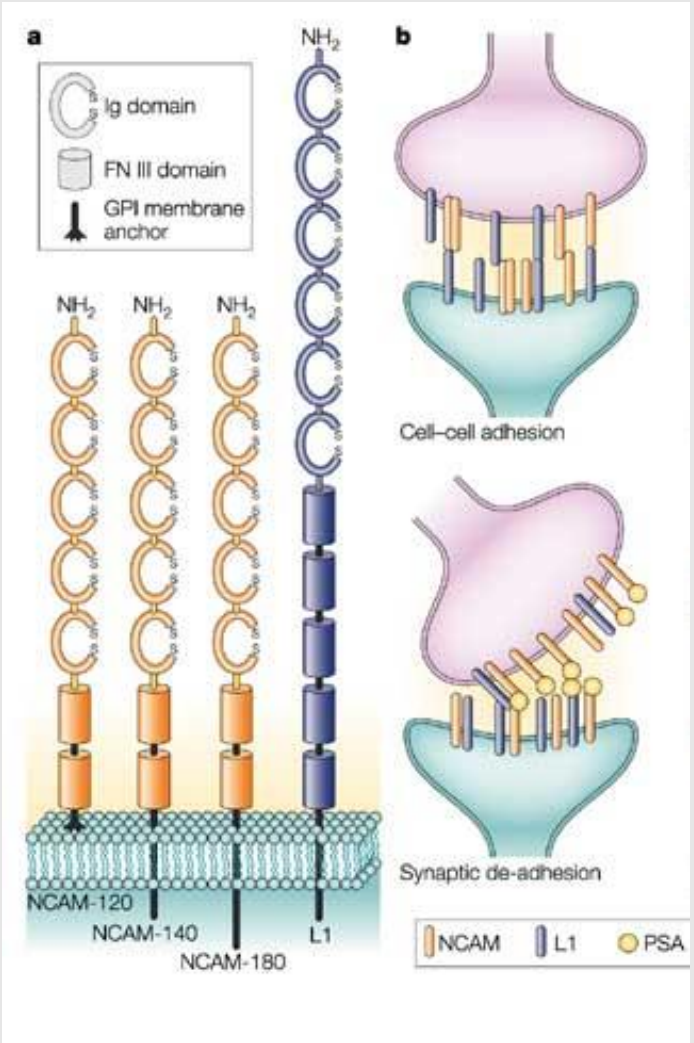
Il dominio extracellulare ha 5 dominii tipici delle immunoglobuline seguiti da dominii tipici della fibronectina (in blu)

Dominio Ig media legame omofilico

# L'acido polisialico (PSA) regola il legame omofilico di NCAM

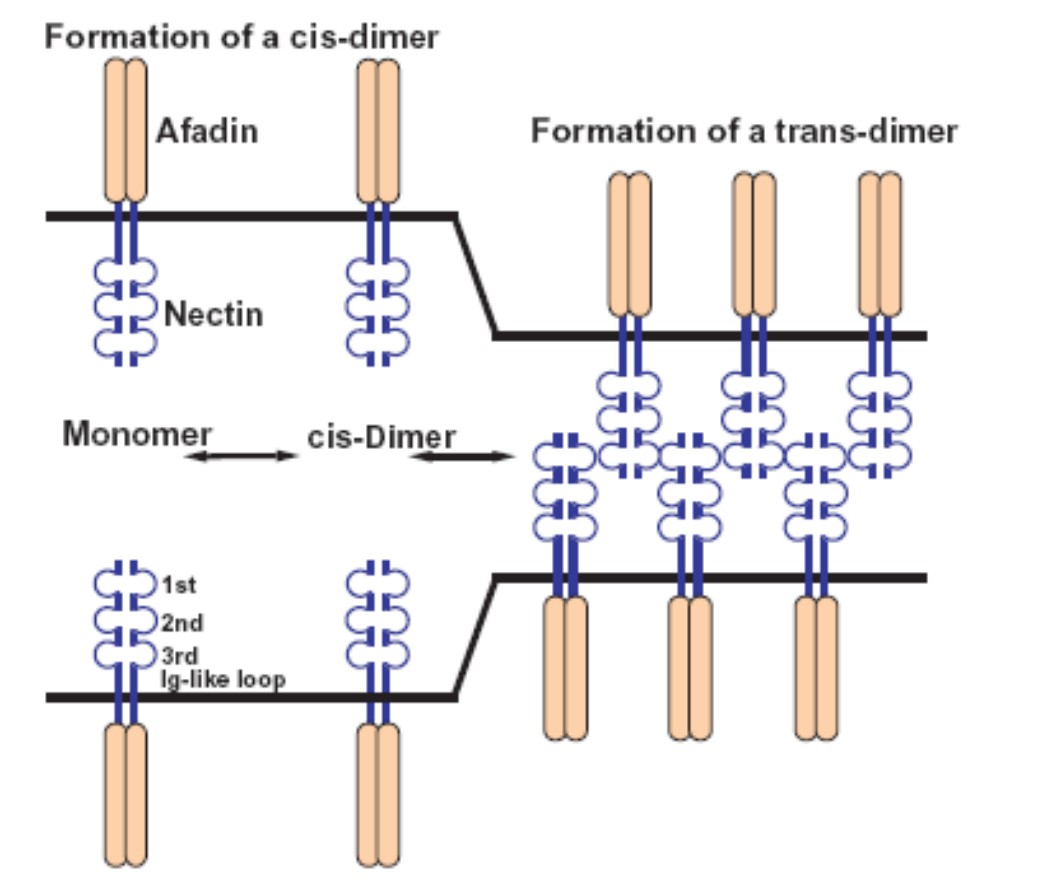
NCAM è glicosilata e viene modificata post-traduzionalmente per aggiunta di acido polisialico al quinto dominio Ig, questo sembra ridurre la sua capacità di adesione in eventi di migrazione cellulare



# “Con-nectin axons and dendrites”

Le nectine fanno parte della famiglia delle immunoglobuline e si associano principalmente tramite legami di tipo omofilico che ricordano le interazioni tra le caderine, ma possono anche legarsi in maniera eterofilica con nectine di tipo diverso (principalmente nectina 1 con nectina 3).

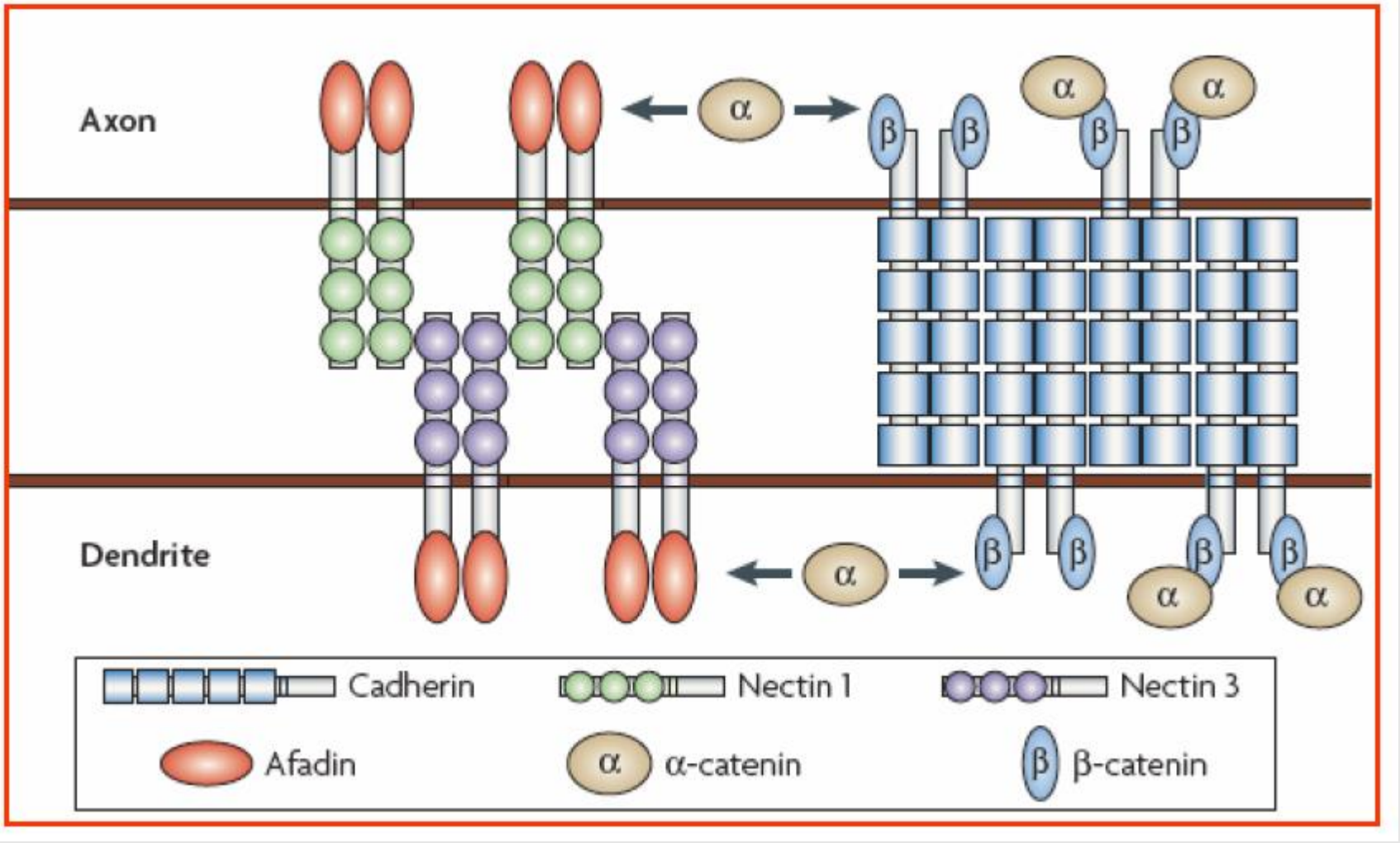



In maniera analoga al sistema caderine/catenine, nectine interagiscono con con il dominio intracitoplasmatico con le afidine

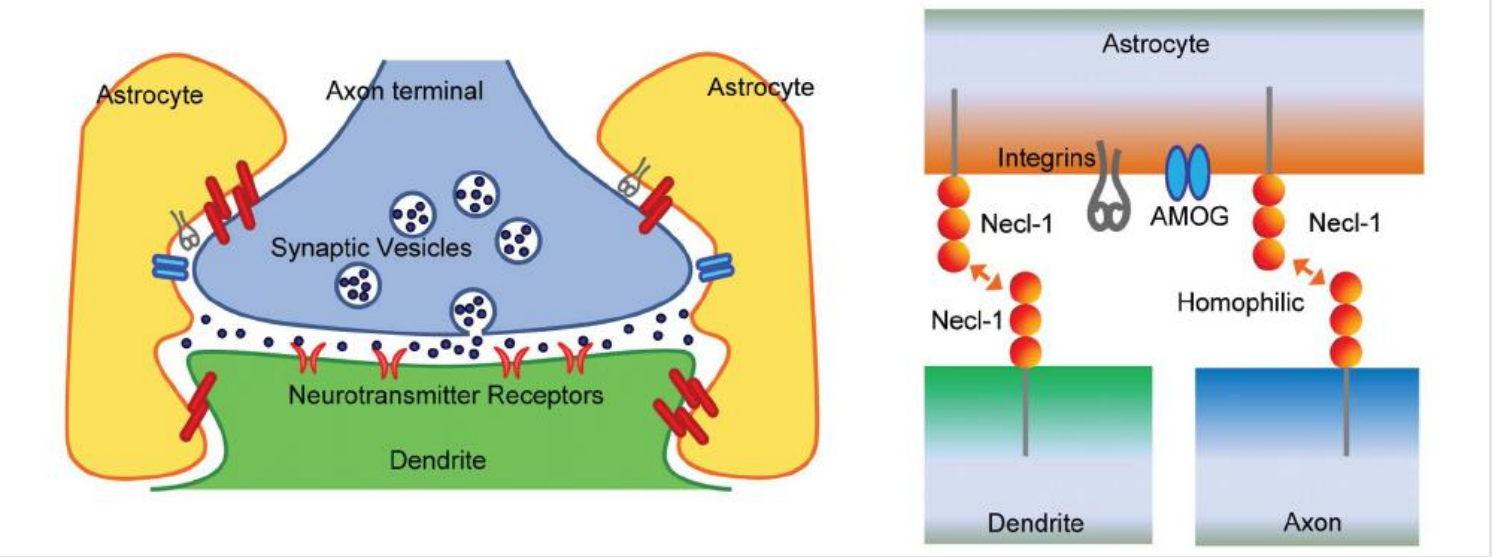
# COMPLESSI DI NECTINE E CADERINE SONO CONNESSI DA INTERAZIONI INTRACITOPLASMATICHE

COMPLESSI COMPOSTI DA NECTINE

COMPLESSI COMPOSTI DA CADERINE



# MOLECOLE DI ADESIONE SPECIFICHE FRA NEURONE E GLIA

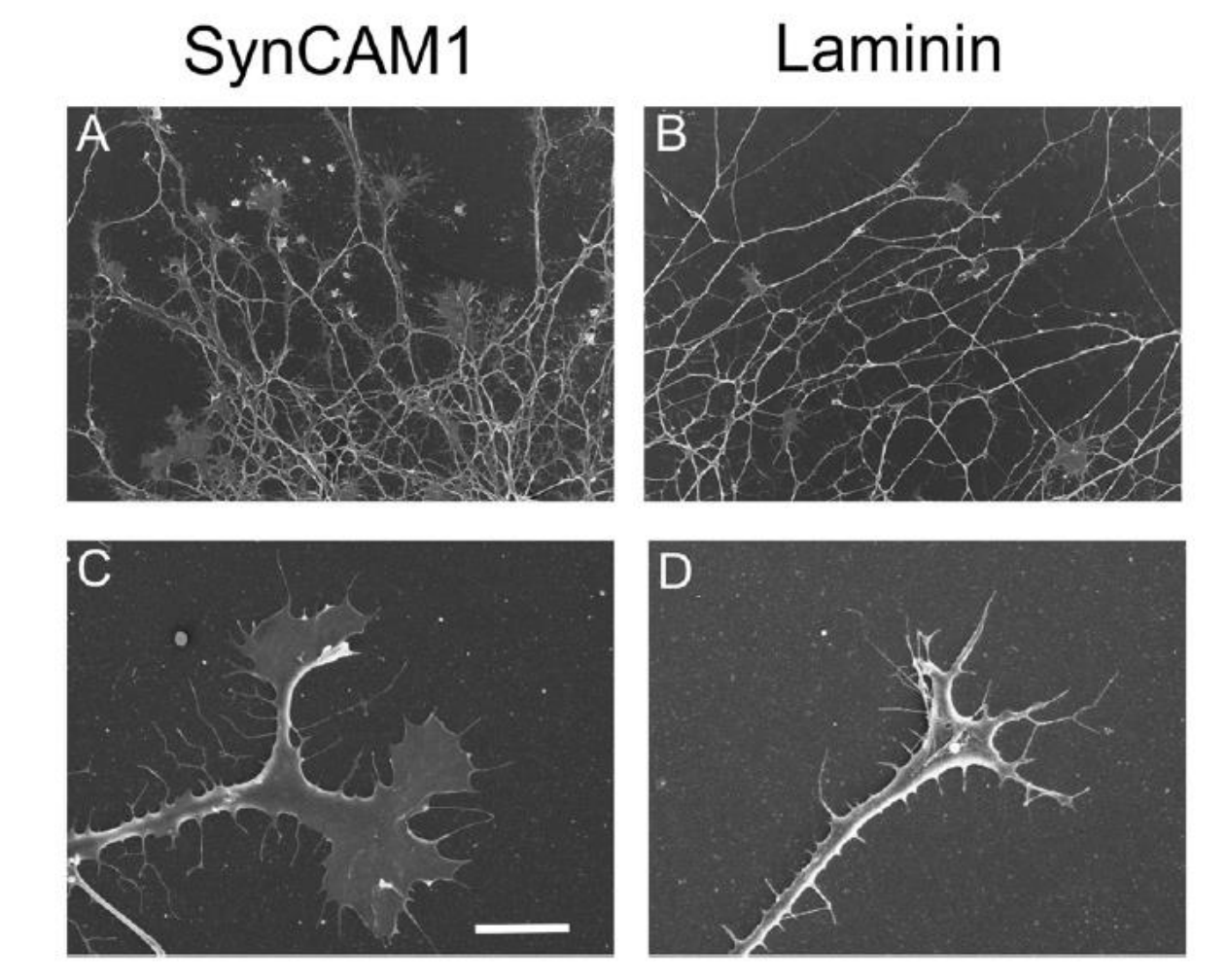



Le proteine Necls (Nectin like) sono molecole di adesione di tipo omofilico con struttura simile alle Nectine, non richiedono calcio per adesione e sono importanti per la interazione neurone-glia

Table 1 **Lists of the neuron-neuron and neuron-glia interactions in the nervous systems**

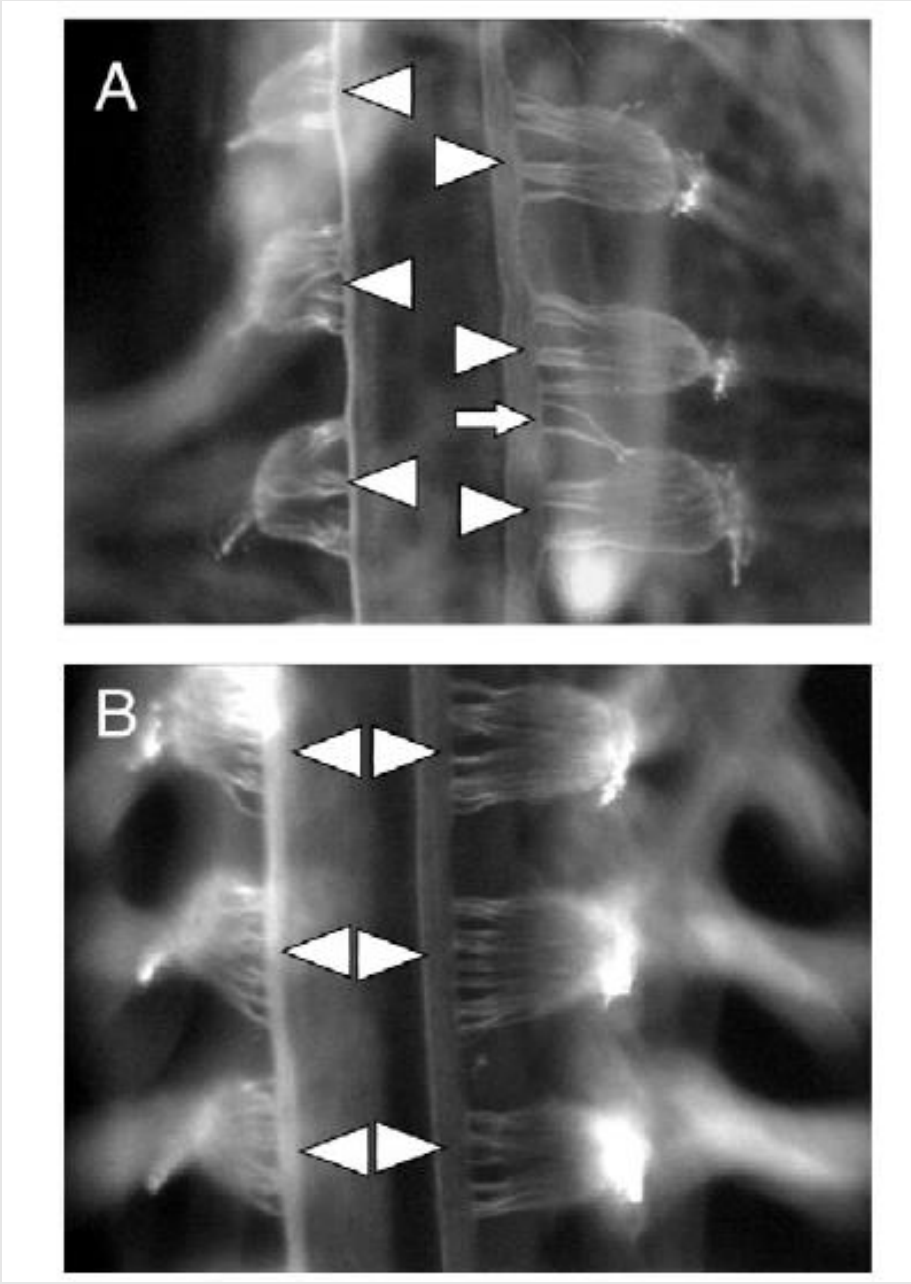
| Classification | Adhesion molecules | Localization |
|---|---|---|
| Cadherin Super Family | Classic cadherins | Synapse (PAJs), Neuron-Glia |
| | Proto-cadherins | Synapse (?) |
| Ig-like Molecules | Nectins | Synapse (PAJs), Neuron-Glia |
| | Nectin-like molecules (Necls) | Neuron-Glia |
| | NCAM | Synapse |
| | Syg-1, Syg-2 | Synapse |
| | Sidekicks | Synapse |
| | Integrins | Synapse, Neuron-Glia |
| Others | Neuroligins, neurexins | Synapse (SJs) |
| | Eph receptors, ephrins | Synapse (SJs) |

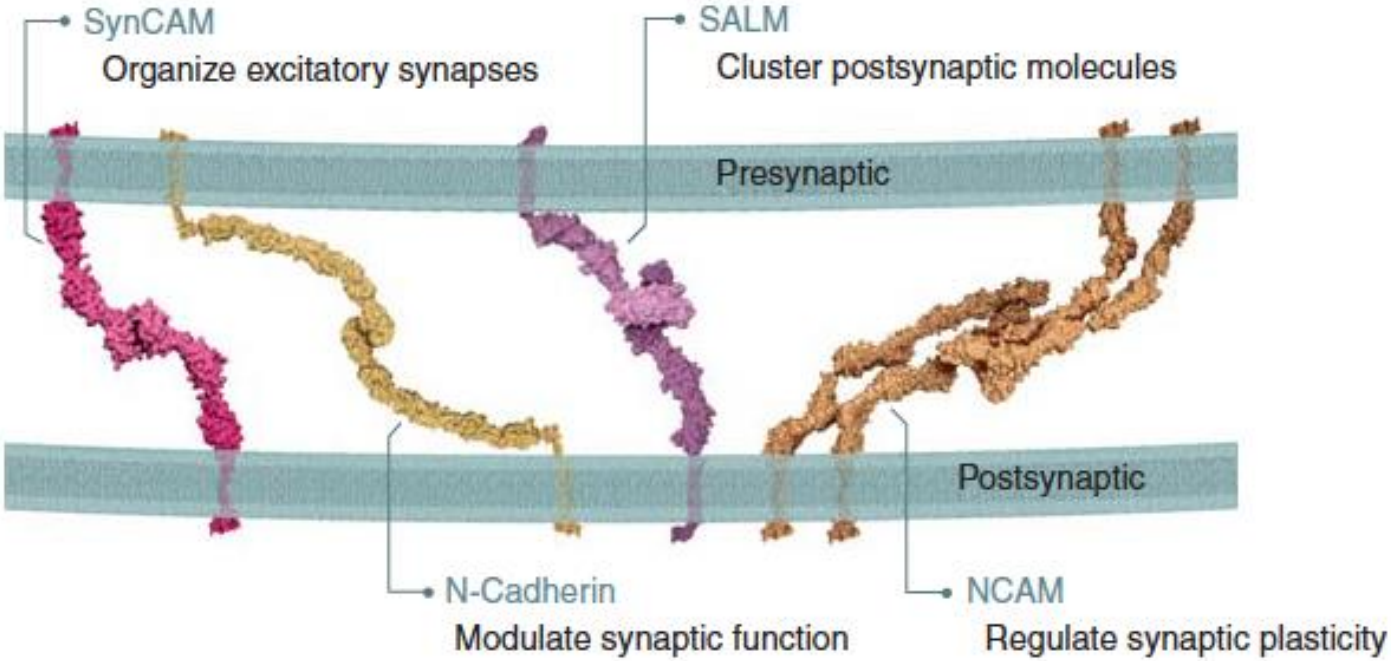
SynCAMs (synaptic cell adhesion molecules)
subfamily of the immunoglobulin superfamily of cell adhesion molecules.
They were first identified as cell adhesion molecules at the synapse which were sufficient to trigger synapse formation.
axon guidance,
synapse formation,
synaptic plasticity.

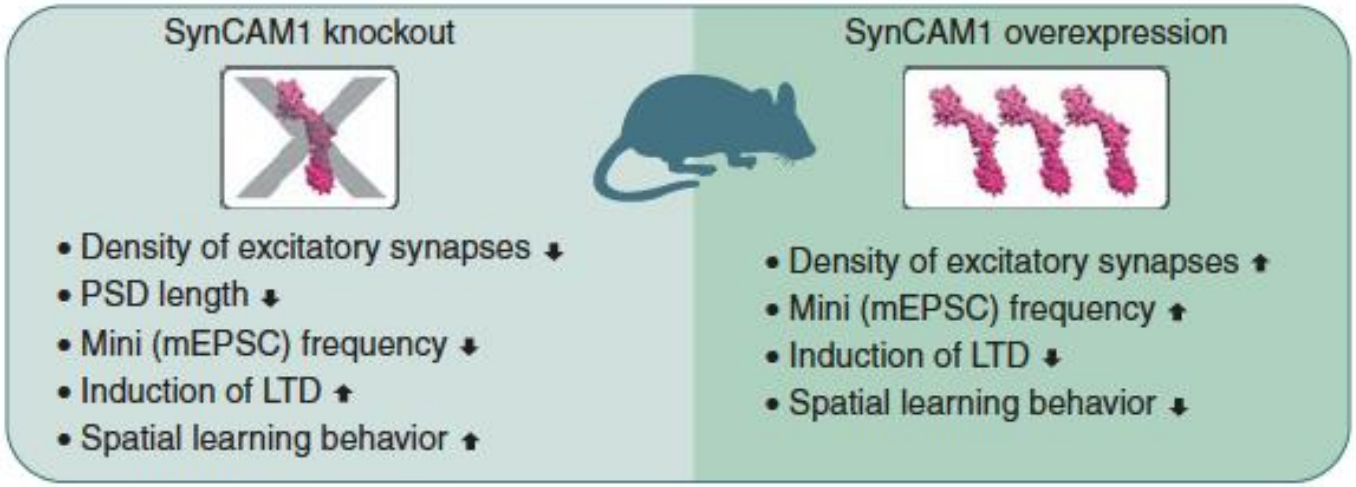



Sensory neurons grown on SynCAM1 substrate exhibit
a striking axonal flattening compared to Laminin.
The growth cones on SynCAM1 are three times larger than those on Laminin.

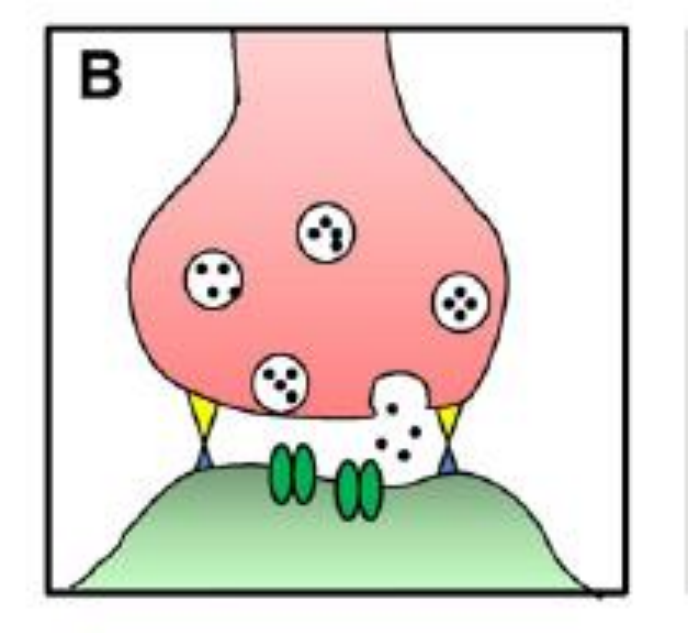
dorsal root ganglia and dorsal roots. Silencing SynCAM3 in neural crest cells by in ovo RNAi resulted in aberrant arrangement of dorsal root ganglia (compare position of arrowheads in the embryo lacking SynCAM3, shown in A, with a control-treated embryo shown in B)




SynCAM
Organize excitatory synapses
SALM
Cluster postsynaptic molecules
Presynaptic
Postsynaptic
N-Cadherin
Modulate synaptic function
NCAM
Regulate synaptic plasticity

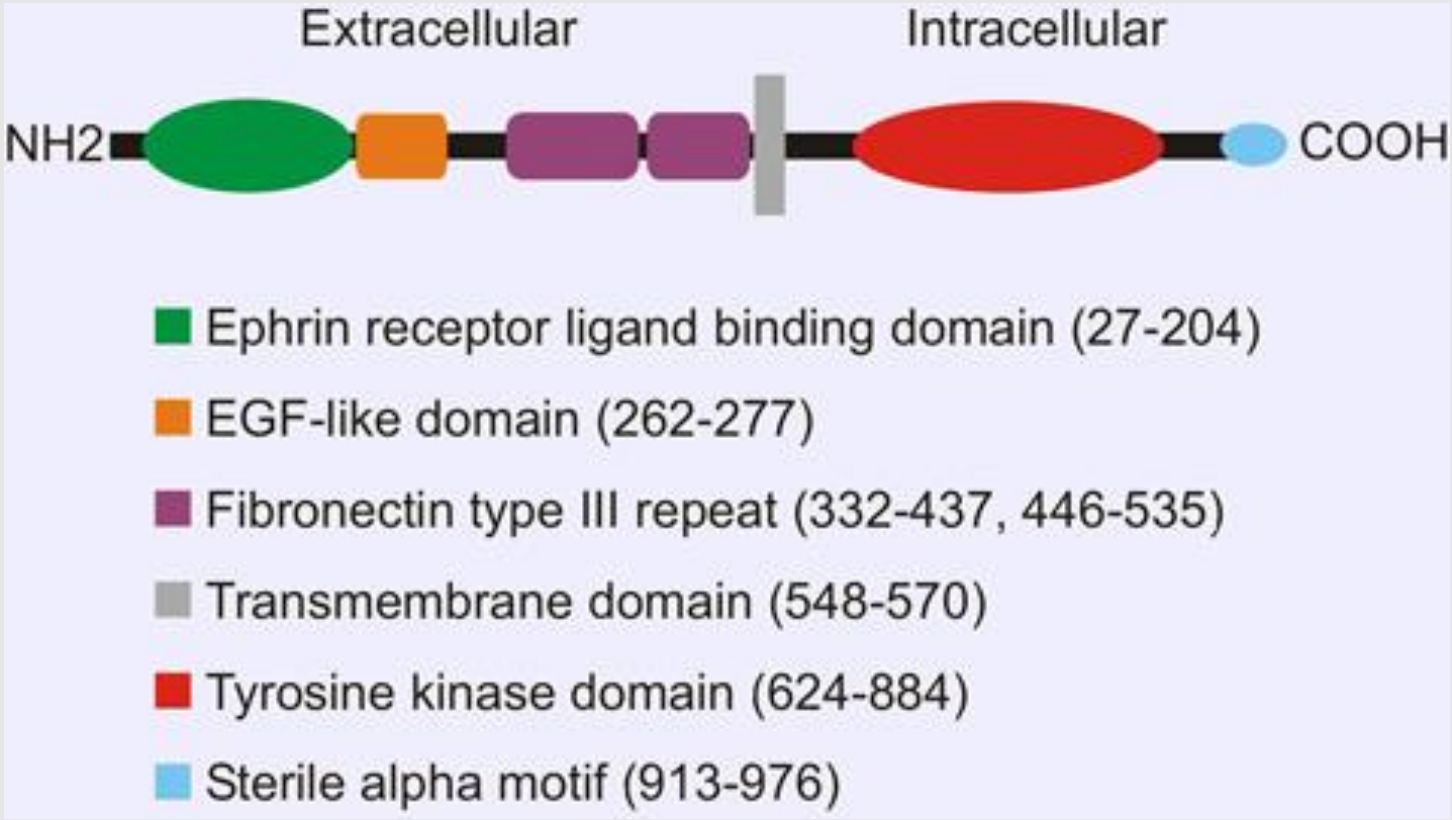

SynCAM1 knockout
• Density of excitatory synapses ↓
• PSD length ↓
• Mini (mEPSC) frequency ↓
• Induction of LTD ↑
• Spatial learning behavior ↑
SynCAM1 overexpression
• Density of excitatory synapses ↑
• Mini (mEPSC) frequency ↑
• Induction of LTD ↓
• Spatial learning behavior ↓

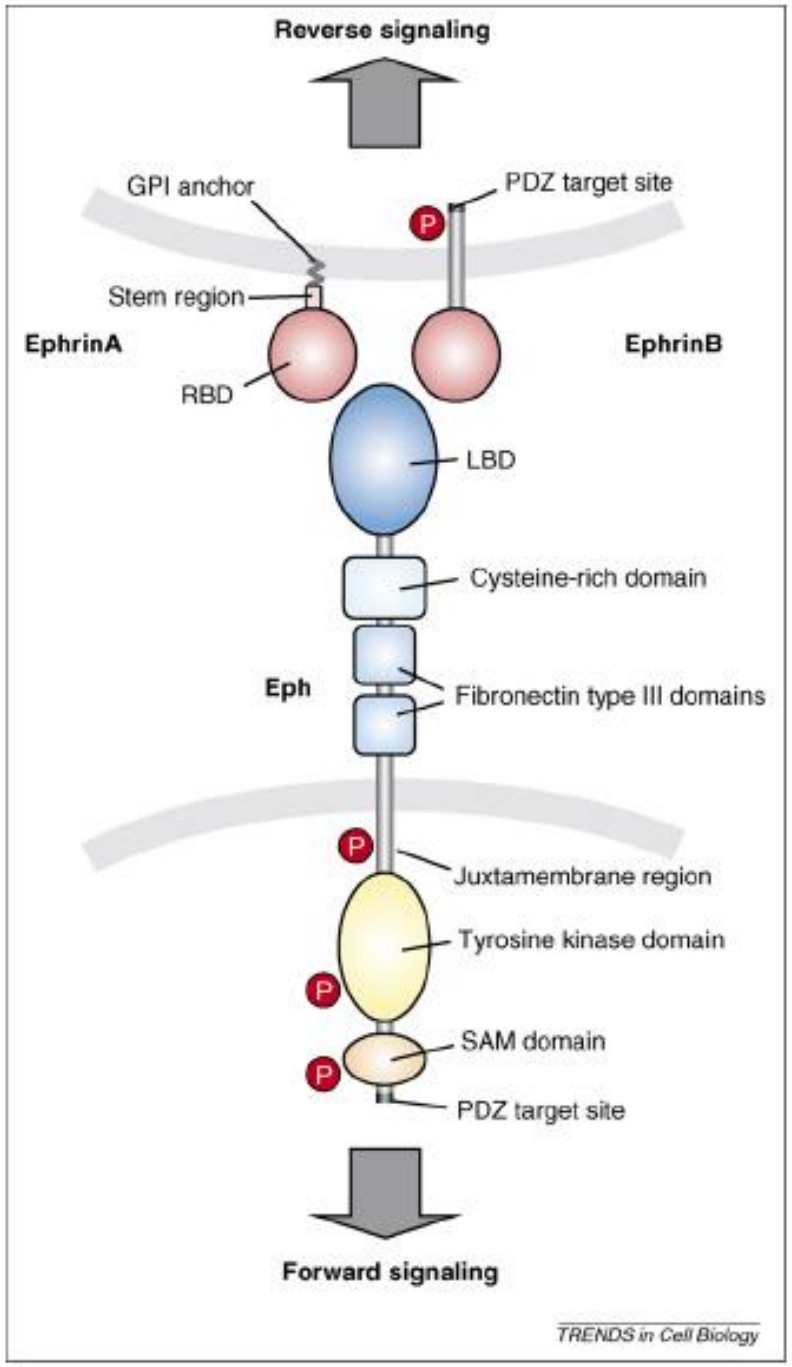
# LEGAME ETEROFILICO



# I RECETTORI EPH e LE EFRINE

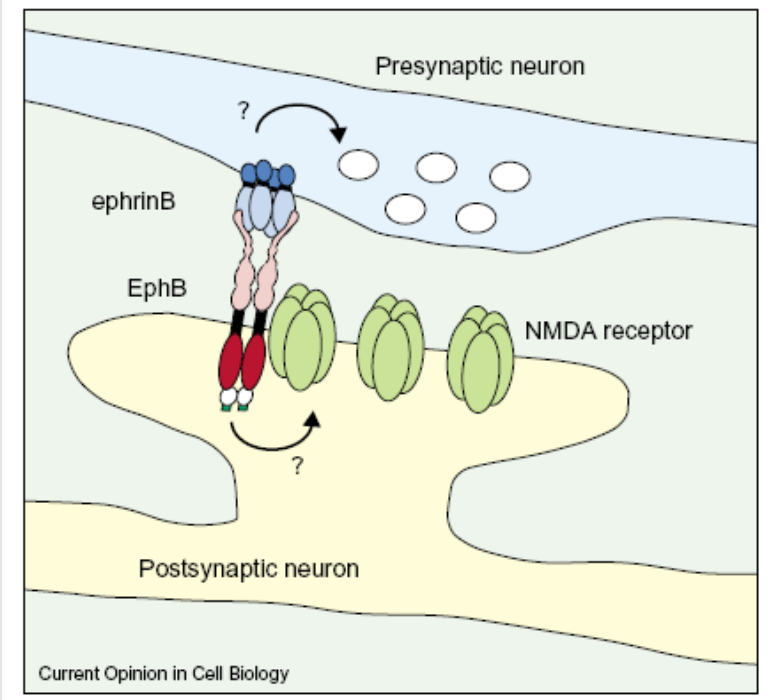
- Recettori tirosin chinasici (EphA, EphB)
- Legano proteine di membrana, le efrine (efrineA, efrineB)



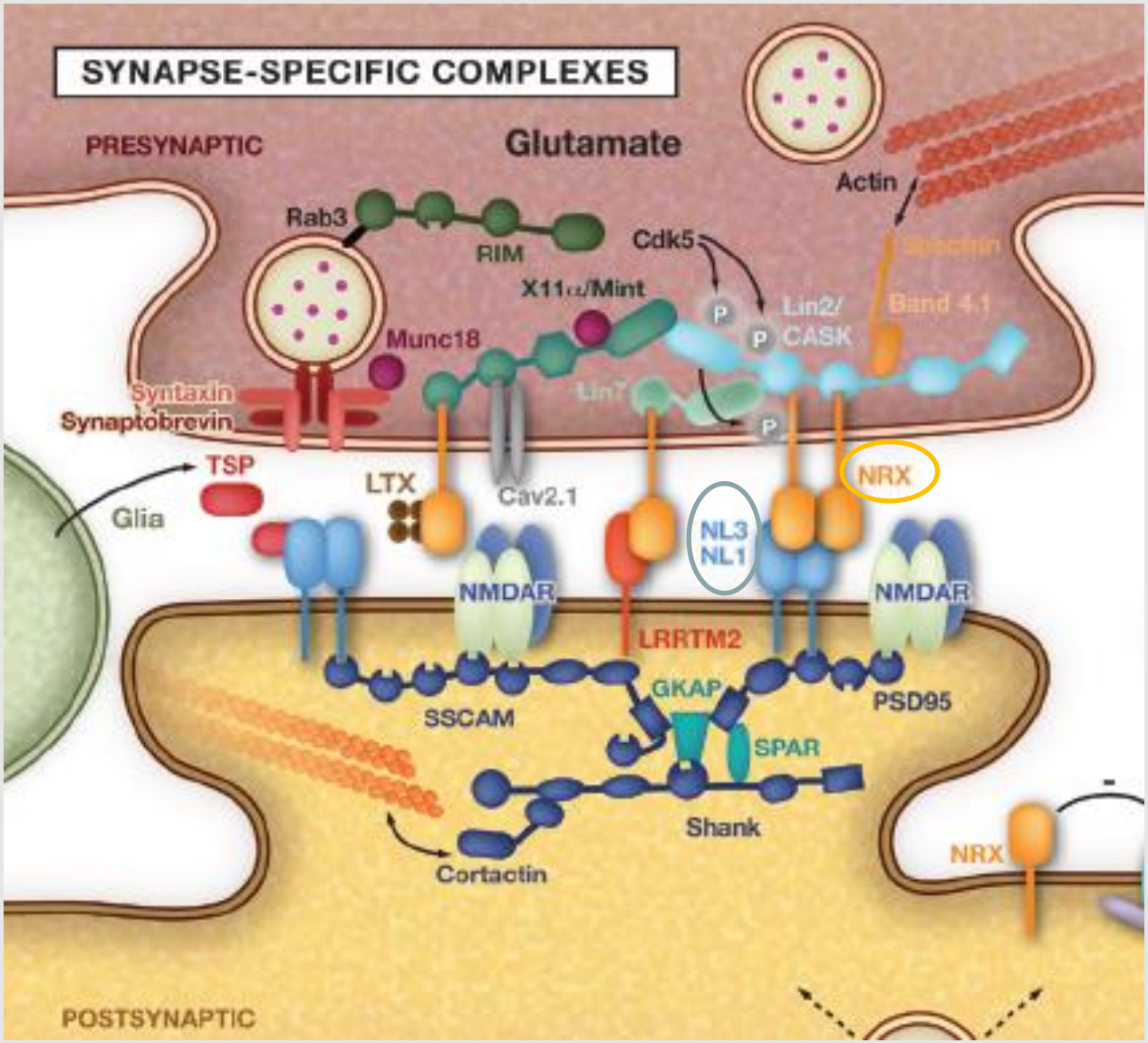



L'INTERAZIONE RECETTORE EPH-EFRINA NON E' UN ESEMPIO CLASSICO DI ADESIONE PERCHE' PUO' MEDIARE ANCHE REPULSIONE

I RECETTORI EphB SONO STATI LOCALIZZATI ALLA SINAPSI ECCITATORIE NELLE DENSITA' POST-SINAPTICHE DOVE LEGANO I RECETTORI DEL GLUTAMMATO DI TIPO NMDA



# Neurolighine e Neurexine




Baudouin and Scheiffele, 2010

# The Neuroligin Family

•4 genes: NL1, NL2, NL3, NL4 :
(NL3 e NL4 map on X chr in humans)

•Form non-covalent dimers

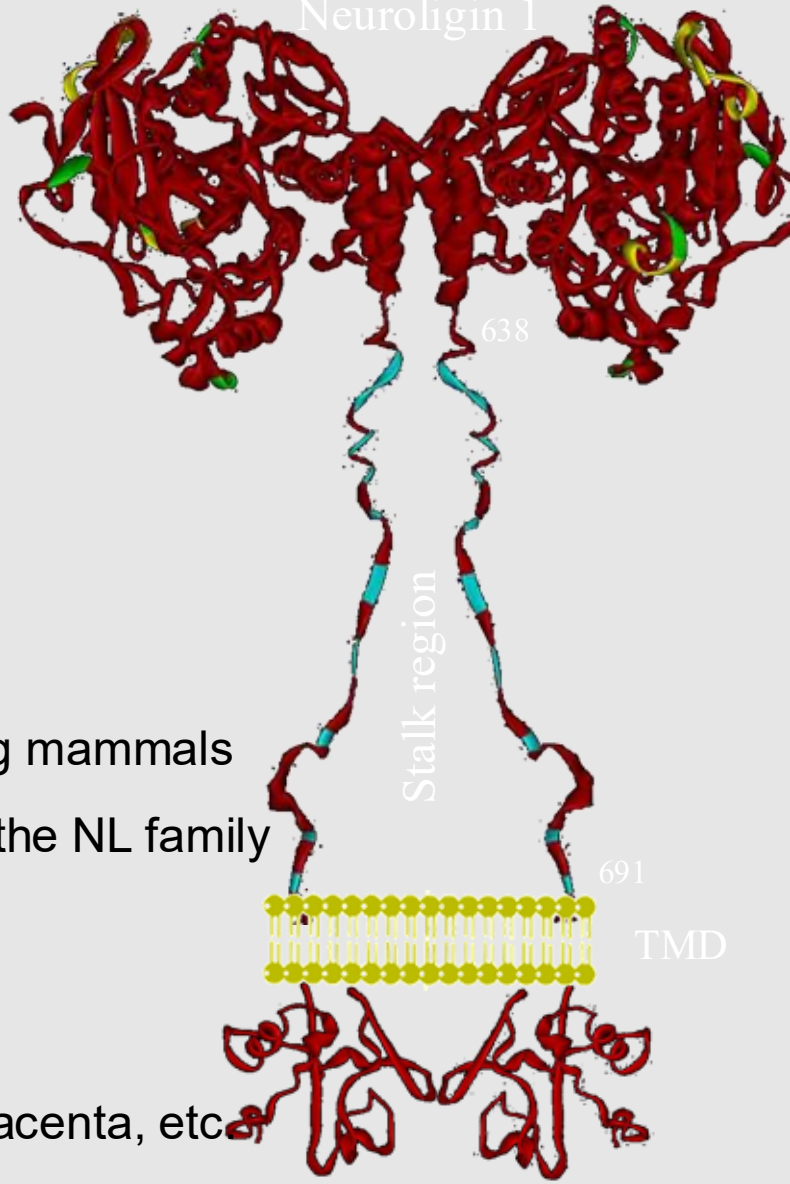
•Extra-cellular cholinesterase-like domain:
- 2 splice inserts
- An oligomerization domain
- Several N- and O-linked glycosylation sites
- Binds to the Neurexins

• High degree (~99% identity) of conservation among mammals

•High degree (~60% identity) of conservation within the NL family

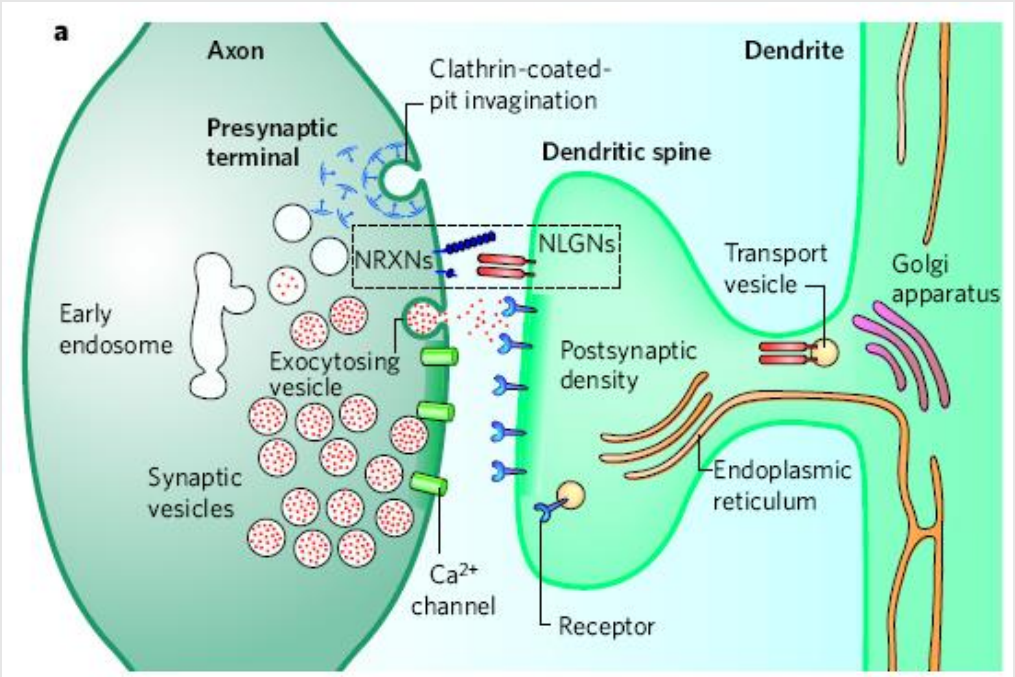
• NL1 to 4 mainly expressed in the CNS

•NL3 and 4 also in heart, lung, pancreas, muscle, placenta, etc.



AChE homology model

# NEUREXINE E NEUROLIGINE MEDIANO ADESIONE ETEROFILICA ALLA SINAPSI

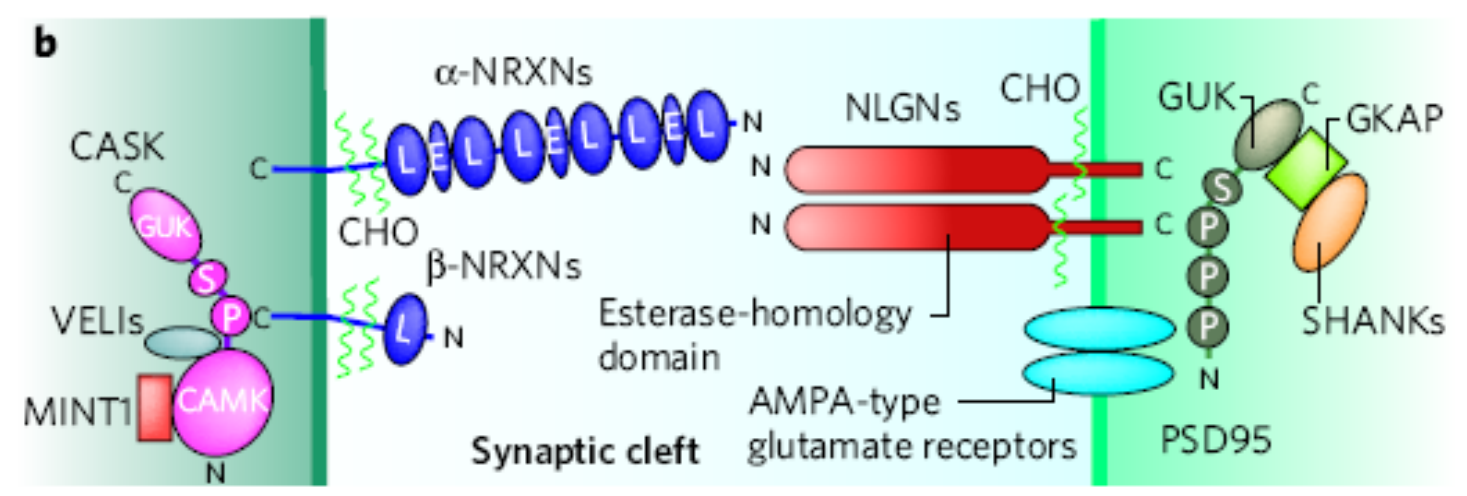



## NEUREXINE

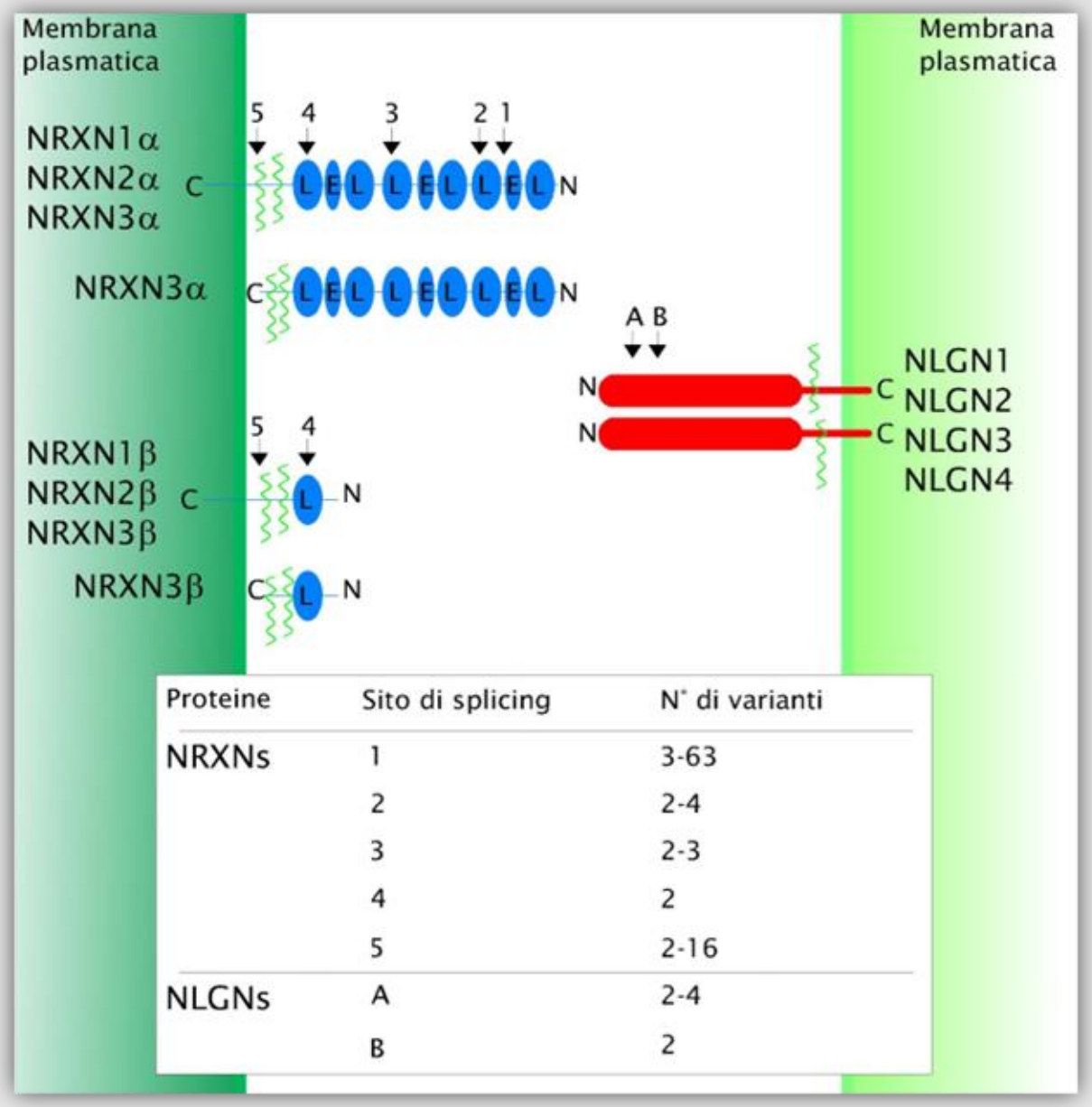
- Espresse nel SNC a livello presinaptico
- Esistono neurexine α e β
- Differiscono nel dominio extracellulare ma hanno identici dominii transmembrana e citoplasmatico.

## NEUROLIGINE

- Espresse nel SNC a livello postsinaptico
- Dominio extracellulare e' simile all' acetilcolinesterasi e lega le neurexine



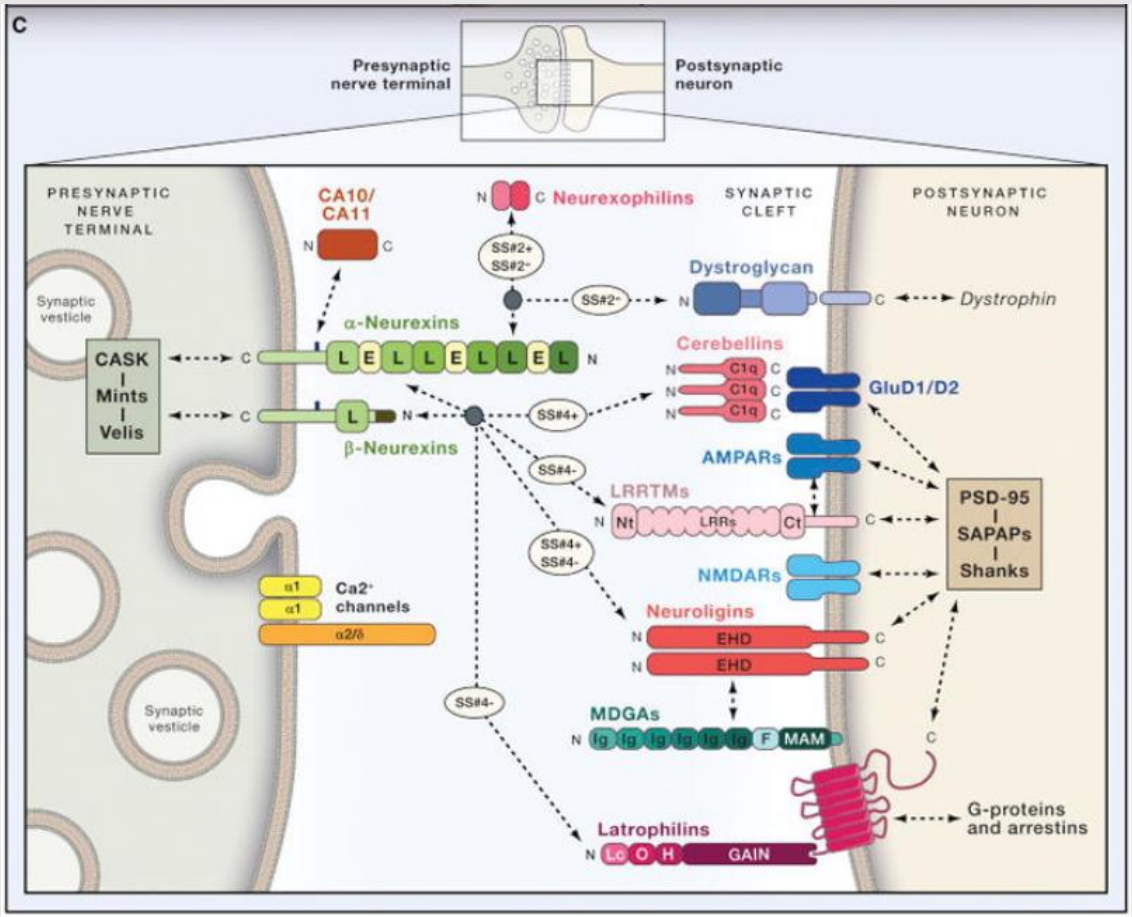
# Cosa regola il legame tra Neuroligine e Neurexine?




| Proteine | Sito di splicing | N° di varianti |
|---|---|---|
| NRXNs | 1 | 3-63 |
| | 2 | 2-4 |
| | 3 | 2-3 |
| | 4 | 2 |
| | 5 | 2-16 |
| NLGNs | A | 2-4 |
| | B | 2 |

- Il dominio extracellulare delle α-NRXNs contiene 5 siti di splicing e 2 nelle β-NRXNs
- Questo significa che esistono più di 3000 isoforme delle Neurexine!
- Le Neuroligine contengono solo 2 siti di splicing alternativo

# NLGN-NRXN




Neurexins (blue ovals) bind many protein partners tethered to the postsynaptic membrane including neuroligins, LRRTMs, $\alpha$-dystroglycan, calsyntenins (CLSTN), and the GABAA-receptor, as well as partners that are secreted such as neurexophilins. (

b) NLGN1 and LRRTM2 can both bind neurexins at an overlapping binding site generating two competing trans-interactions.

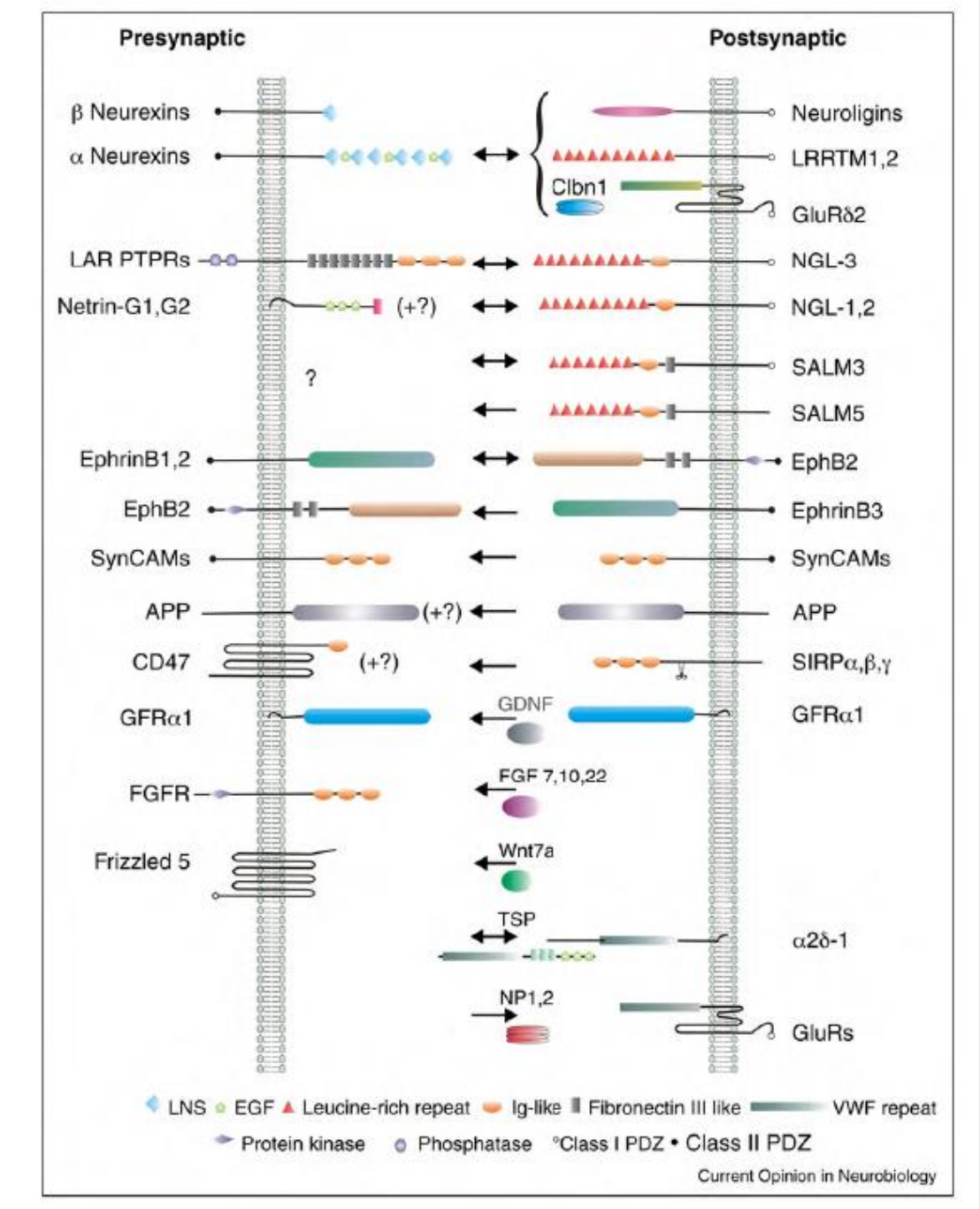
(c) Neurexins and MDGA1 can both bind NLGN2 at an overlapping binding site generating competing cis- and trans-interactions.

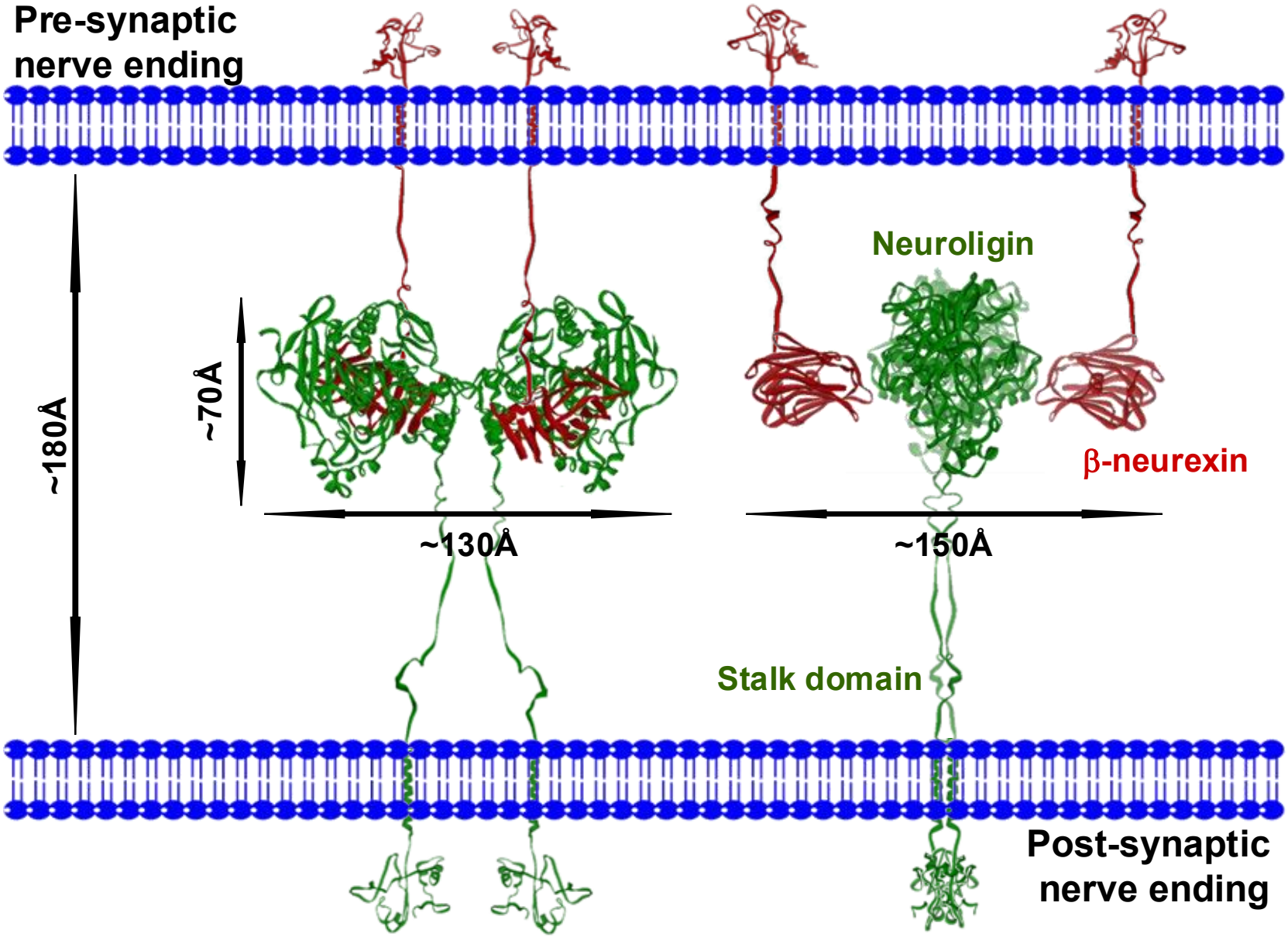
Südhof TCS, 2017

Le Neurexine non legano solo le Neurolighine

leucine-rich repeat (LRR) domains

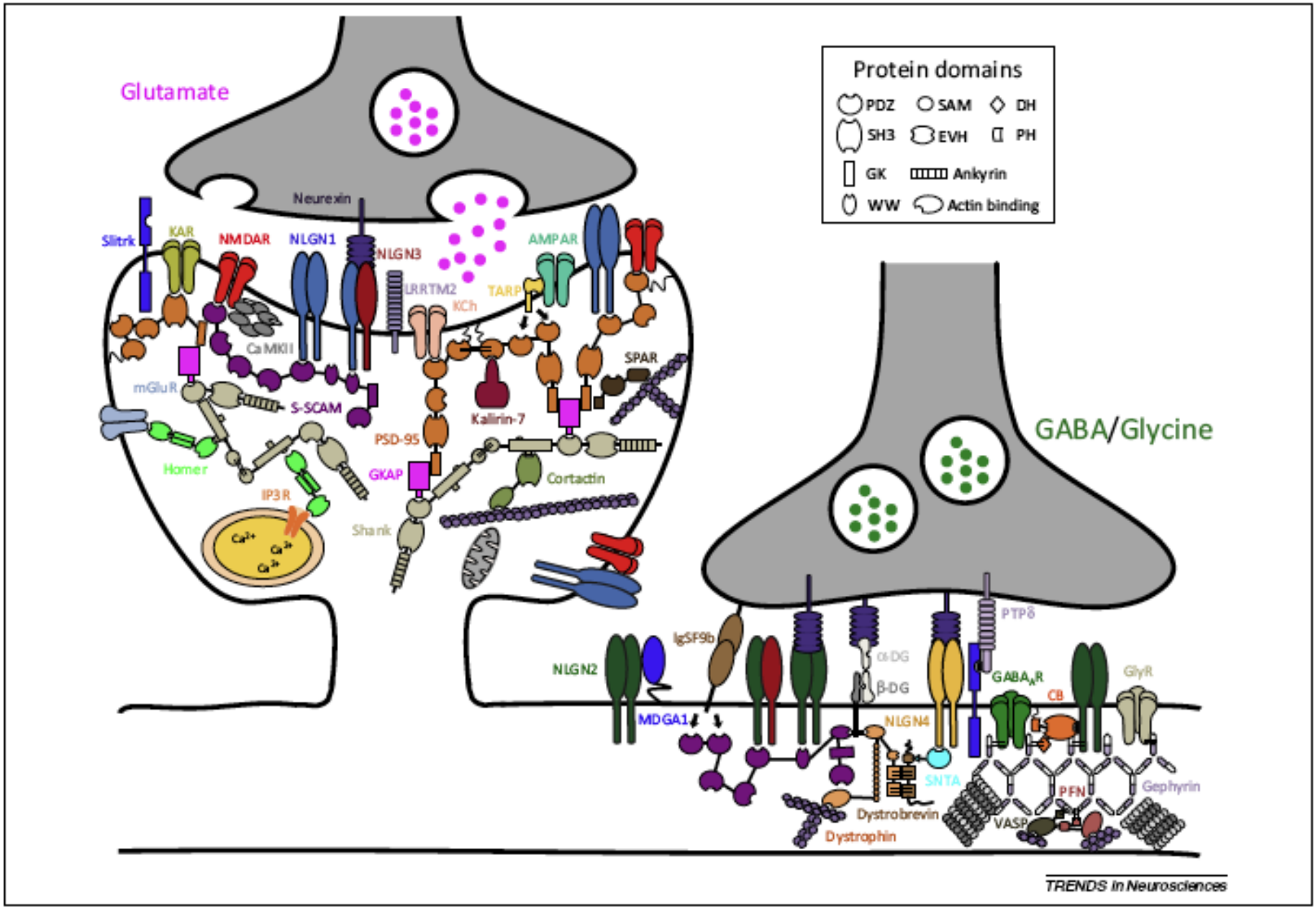
LRRTMs and Slitrks



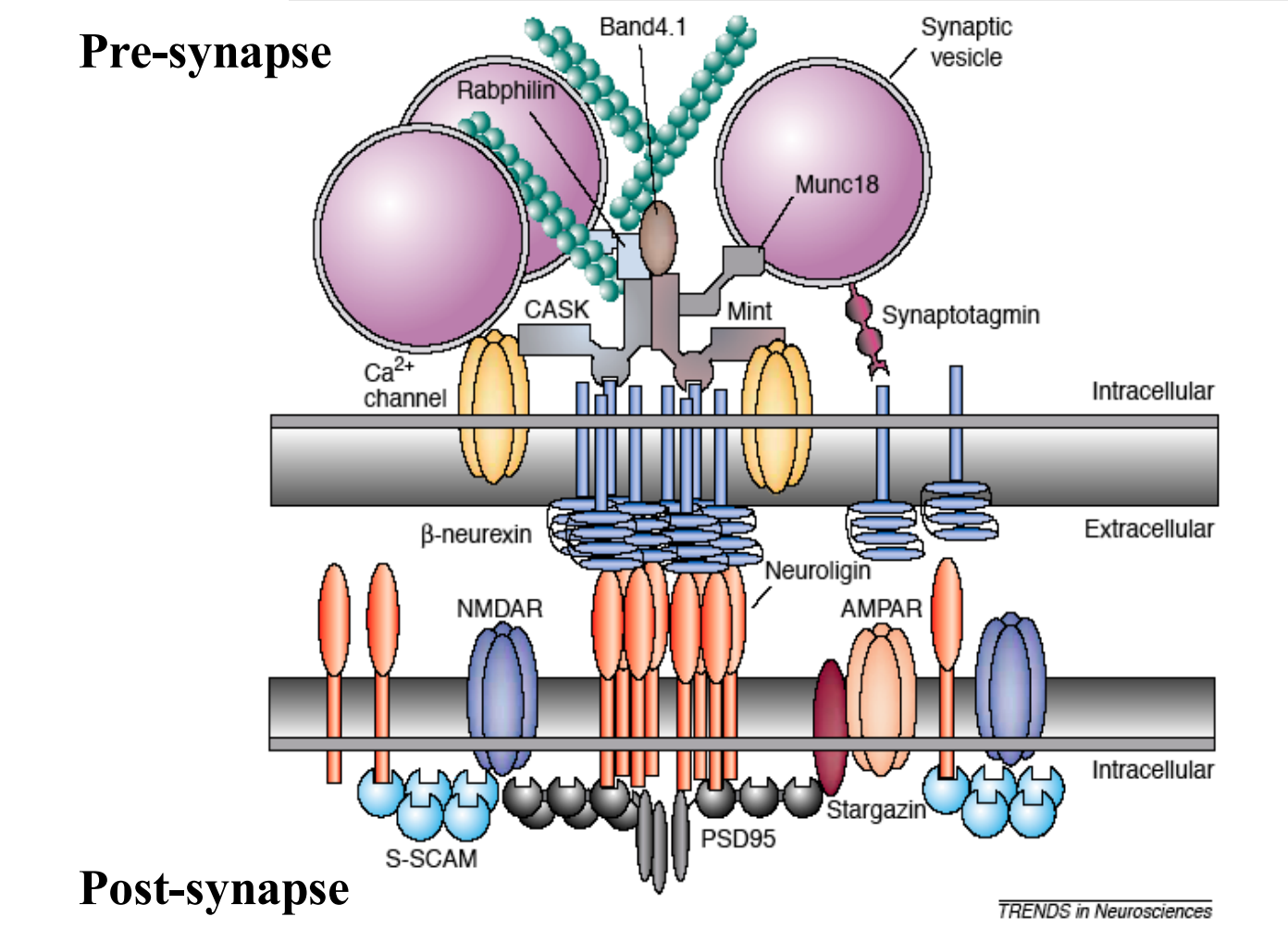



*Comoletti et al., 2007*

# Distribuzione di Neuroligina 1, 2, 3 e 4 alle sinapsi



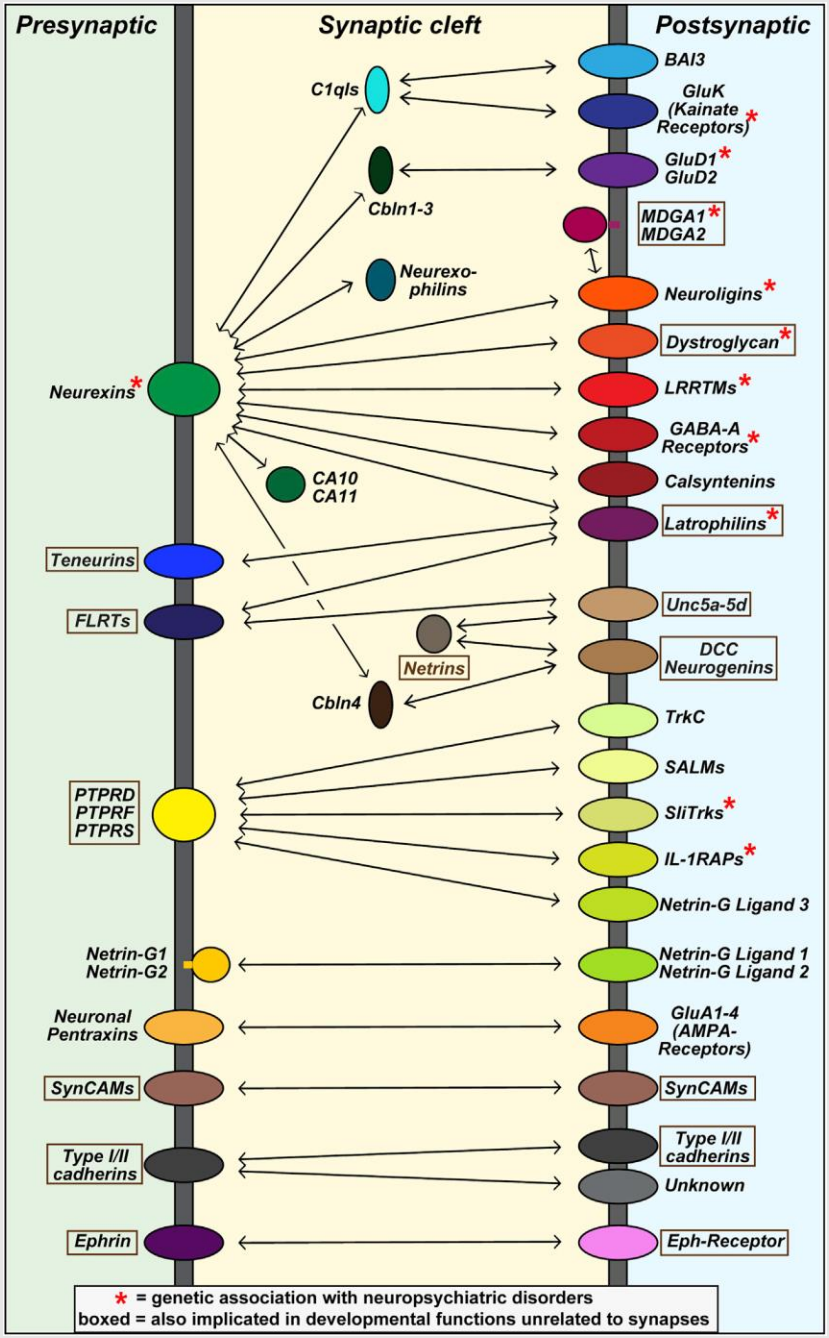
# Interazione *in trans:* Neurexina/Neuroligina




NEUROLIGINE E NEUREXINE LEGANO CON LA REGIONE CITOPLASMATICA PROTEINE CHE PRESENTANO DOMINII PDZ, IN PARTICOLARE CASK PER NEUREXINA E PSD95 PER NEUROLIGINA CHE HANNO IL RUOLO DI "ANCORARE" LE MOLECOLE DI ADESIONE ALLA RETE DI PROTEINE SOTTOSTANTI LA MEMBRANA

# ALCUNE MOLECOLE CAMs HANNO PIU' INTERATTORI

*GENETIC ASSOCIATION WITH NEUROPSYCHIATRIC DISORDERS



The LRR-containing proteins
mainly localized to postsynaptic sites and bind across the synap-tic cleft
to their presynaptic partners
-leucine-rich-repeat transmembrane neuronal proteins (LRRTMs) (Linhoff et al.,2009),
-netrin-G ligands (NGLs)
-SLIT and NTRK-likeprotein (Slitrks)
-synaptic adhesion like molecules (SALMs)
-fibronectin leucine richtransmembrane proteins (FLRTs)

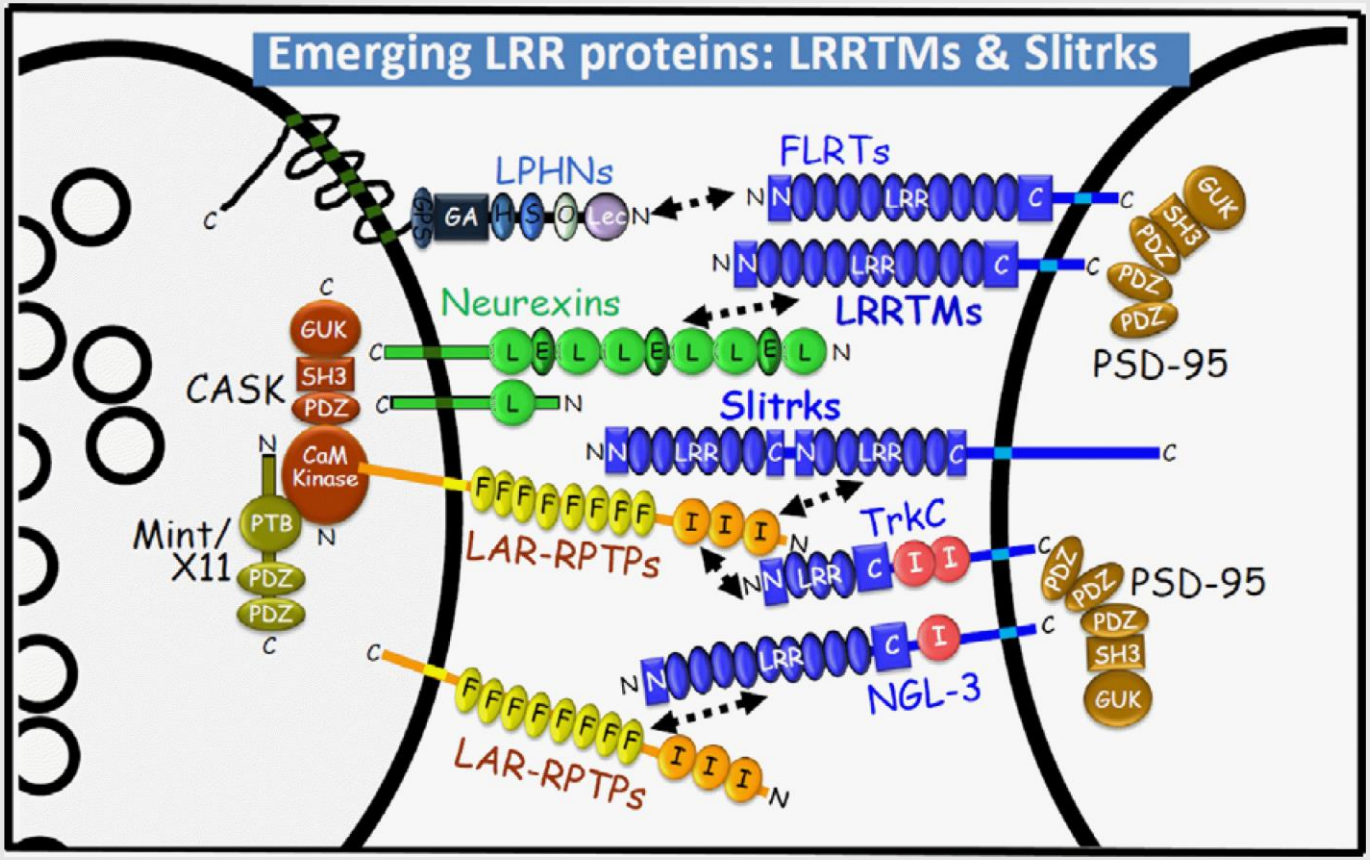


**Table 1**
Leucine-rich repeat containing synapse organizing proteins and their presynaptic binding partners.

| Proteins | Protein domains | Presynaptic partner |
|---|---|---|
| LRRTMs | | |
| LRRTM1 | LRRNT, LRR1–10, LRRCT, TM, C-terminal | Neurexin 1,2,3 α/β (−S4) |
| LRRTM2 | LRRNT, LRR1–10, LRRCT, TM, C-terminal | Neurexin 1,2,3 α/β (−S4) |
| LRRTM3 | LRRNT, LRR1–10, LRRCT, TM, C-terminal | Neurexin 1β (−S4) |
| LRRTM4 | LRRNT, LRR1–10, LRRCT, TM, C-terminal | Heparan sulphate proteoglycans |
| Slitrks | | |
| Slitrk1 | LRRNT1, LRR1–6, LRRCT1, LRRNT2, LRR7–12, LRRCT2, TM, C-terminal | PTPσ, PTPδ |
| Slitrk2 | LRRNT, LRR1–6, LRRCT1, LRRNT, LRR7–12, LRRCT2, TM, C-terminal | PTPσ, PTPδ |
| Slitrk3 | LRRNT, LRR1–6, LRRCT1, LRRNT, LRR7–12, LRRCT2, TM, C-terminal | PTPδ |
| Slitrk4 | LRRNT, LRR1–6, LRRCT1, LRRNT, LRR7–12, LRRCT2, TM, C-terminal | PTPσ |
| Slitrk5 | LRRNT, LRR1–6, LRRCT1, LRRNT, LRR7–12, LRRCT2, TM, C-terminal | PTPσ, PTPδ |
| Slitrk6 | LRRNT1, LRR1–5, LRRCT1, LRRNT2, LRR6–11, LRRCT2, TM, C-terminal | PTPσ |
| NGLs | | |
| NGL-1 | LRRNT, LRR1–9, LRRCT, Ig, TM, C-terminal | Netrin-G1, LAR |
| NGL-2 | LRRNT, LRR1–9, LRRCT, Ig, TM, C-terminal | Netrin-G2 |
| NGL-3 | LRRNT, LRR1–9, LRRCT, Ig, TM, C-terminal | LAR-RPTPs |
| SALMs | | |
| SALM1 | LRRNT, LRR1–7, LRRCT, Ig, FNIII, TM, C-terminal | |
| SALM2 | LRRNT, LRR1–7, LRRCT, Ig, FNIII, TM, C-terminal | |
| SALM3 | LRRNT, LRR1–7, LRRCT, Ig, FNIII, TM, C-terminal | LAR-PTPs |
| SALM4 | LRRNT, LRR1–6, LRRCT, Ig, FNIII | SALM4 |
| SALM5 | LRRNT, LRR1–7, LRRCT, Ig, FNIII | SALM5, LAR-RPTPs |
| Trks | | |
| TrkC | LRRNT, LRR1–3, LRRCT, Ig1, Ig2, TM, C-terminal | PTPσ |
| FLRT | | |
| FLRT1 | LRRNT, LRR1–7, LRRCT, Ig, FNIII, C-terminal | Not known |
| FLRT2 | LRRNT, LRR1–7, LRRCT, Ig, FNIII, C-terminal | LPHN3 |
| FLRT3 | LRRNT, LRR1–6, LRRCT, Ig, FNIII, C-terminal | LPHN3, Unc5 |

Tecniche di studio per le molecole di adesione sinaptica

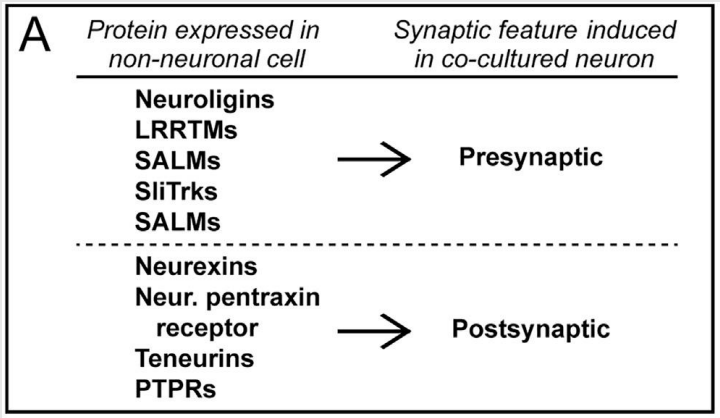
Tecniche di guadagno di funzione (gain of function)

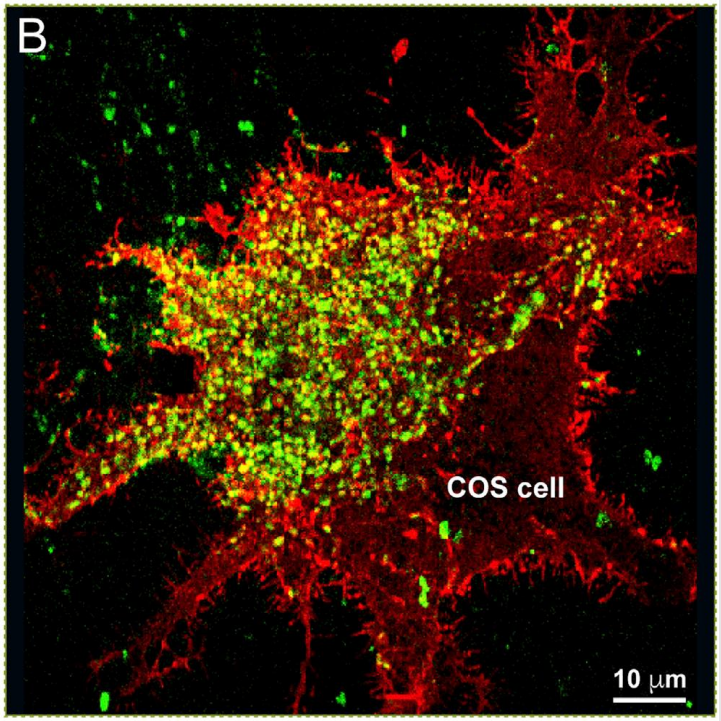
- Saggi di adesione cellulare (sopra-espressione della molecola in studio in cellule non-neuronali in coltura)
- Saggio di formazione di sinapsi artificiali (co-coltura di neuroni e cellule non-neuronali trasfettate)
- Saggio di trasfezione di neuroni

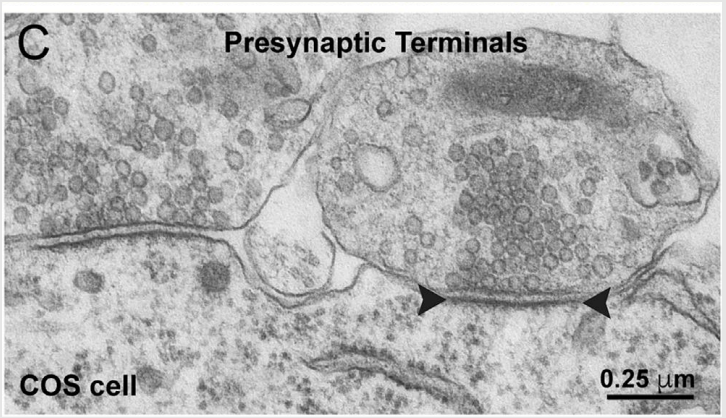
Tecniche di perdita di funzione (loss of function)

- RNA interference. Limitazione: knockdown inefficiente; redundancy; reazioni compensatorie
- Manipolazione genetica (animali transgenici), costitutiva o condizionale. Limitazioni per quella costitutiva: fenotipo di malformazione o morte embrionale
- Inibizione farmacologica. Limitazioni: mancanza d'inibitori per tutte le molecole sotto studio

# ESPERIMENTI DI STUDIO DI FUNZIONE PER CAMS

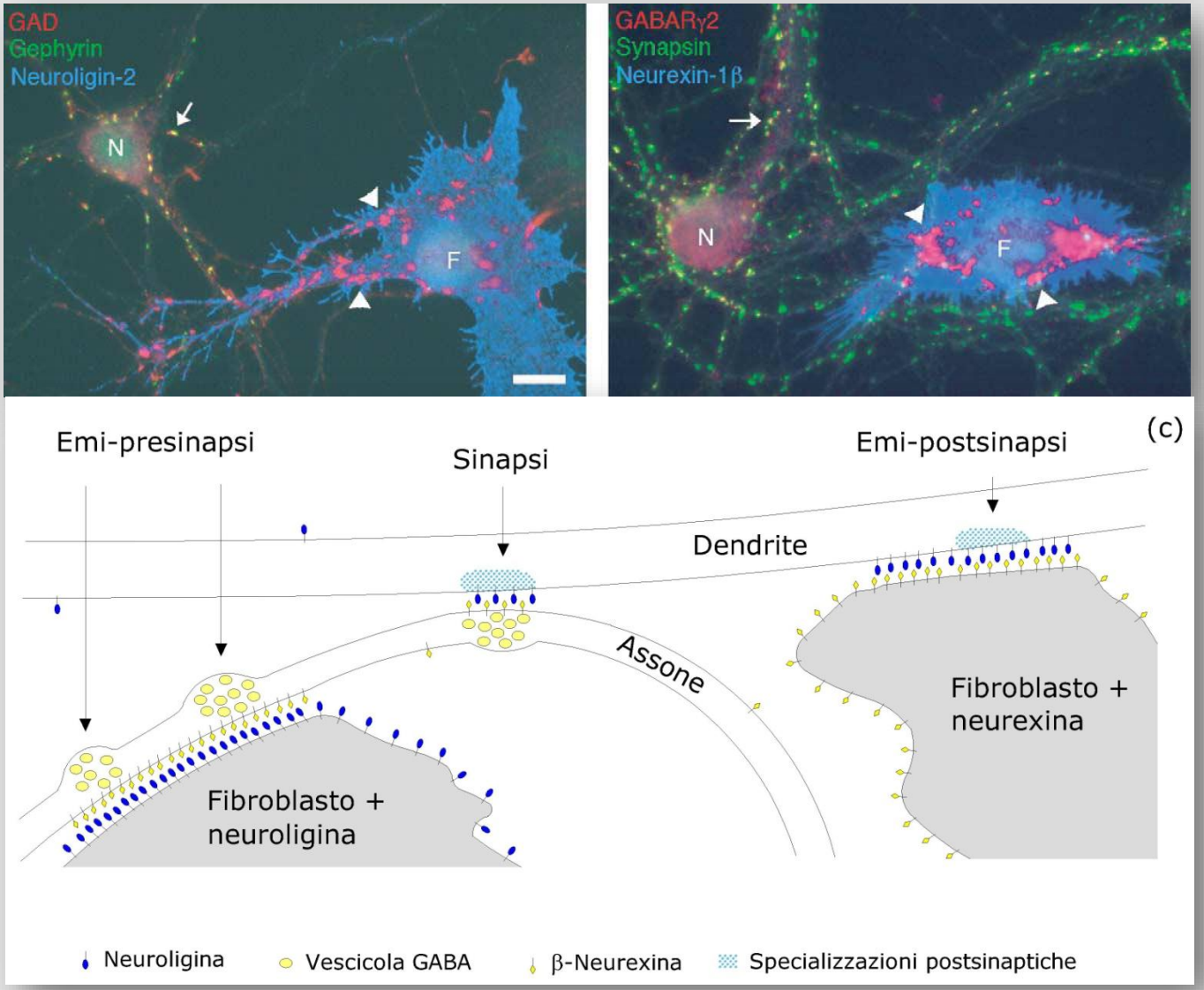


# ESPRESSIONE ESOGENA DI NLGNs E NRXNs

## "Saggio di formazione di sinapsi artificiali"



•Esperimenti di RNAi knockdown delle NLGNs correlano un decremento di NLGNs con un decremento nel numero di sinapsi (Chih et al., 2004).

•Overespressione di NLGN1 in neuroni in coltura aumenta il numero delle sinapsi eccitatorie mentre NLGN2 aumenta il numero delle sinapsi inibitorie (Chih et al., 2005).

•Overespressione di NLGN1 in neuroni in coltura aumenta il numero delle sinapsi eccitatorie e queste nuove sinapsi sono funzionali (capaci di evocare neurotrasmissione). NLGN1 e' selettiva per le sinapsi di tipo eccitatorio. Mentre NLGN2 incrementa il numero delle sinapsi inibitorie (Chubykin et al., 2007).

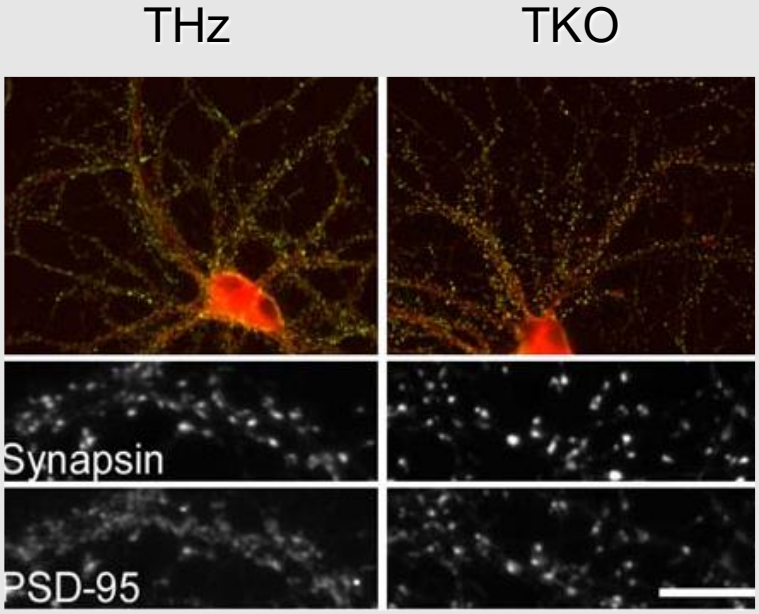
# MOLECOLE DI ADESIONE E SINAPTOGENESI

Table 1 | ***In vitro* evidence for trans-synaptic signalling in synaptogenesis**

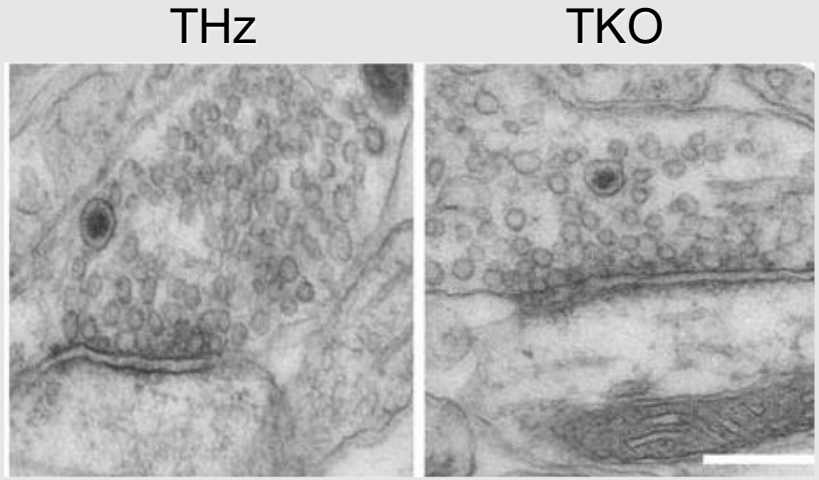
| SAM | Excitatory postsynaptic differentiation | Inhibitory postsynaptic differentiation | Presynaptic differentiation | Dendritic spine formation |
|---|---|---|---|---|
| Neurexins/ neuroligins | Direct association with PSD-95 via PDZ binding domain interactions[14]; NMDARs recruited by neuroligin[17,20], but mechanism unclear; AMPARs recruited in co-culture only in the presence of glutamate[22] | Recruitment of GABAR and gephyrin in co-culture[20]; fewer inhibitory synapses with neuroligin knockdown[17] | Induced in co-culture[21]; neurexin associates with CASK/MINT[15,16,25] | Induced with overexpression of neuroligins[17]; mechanism unknown |
| EphBs/ ephrin-Bs | Direct extracellular domain interaction of EphB2 with NMDARs[43]; EphB2 also associates with AMPARs via PDZ binding domain interactions[46,50] | ND | Induced in co-culture and requires ephrin binding domain[50]; mechanism unknown | Induced by EphBs/ ephrin-Bs via multiple signalling pathways (Rho GEFs, syndecan, FAK)[44,49,51,53,54]. Fewer spines form with expression of EphB2 kinase inactive mutant[51] |
| SynCAM | Direct association with PSD-95 via PDZ binding domain interactions[57] | ND | Induced in co-culture[57]; SynCAM binds CASK/ MINT[57] | ND |
| SALM2 | Direct association with PSD-95 via PDZ binding domain interactions[59]; AMPARs recruited in co-culture, but NMDARs less so[59] | ND | No induction in co-culture[59]; presynaptic ligand is unknown | Induced with overexpression[59]; mechanism unknown |
| NGL2 | Direct association with PSD-95 via PDZ binding domain interactions[60]; NMDARs recruited in co-culture, but not AMPARs[60] | ND | Induced in co-culture and requires NGL2 extracellular domain[60]; mechanism unknown | Induced with overexpression of NGL2 via PSD-95-dependent and -independent mechanisms[60] |
| N-cadherin | Fewer clusters of PSD-95 with inhibition of cadherin signalling[76]; evidence for interaction with AMPARs[78] | ND | No induction in co-culture[80] (but see *in vivo* evidence in TABLE 2) | Fewer spines with N-cadherin dominant-negative[76]; α-catenin stabilizes spines[82]; p120 required for spine formation and maturation[81] |

# FUNZIONE: PROVE *IN VIVO*

THz: neuroni di controllo (tripli eterozigoti)
TKO: neuroni con triplo knockout per le NLGNs 1-3



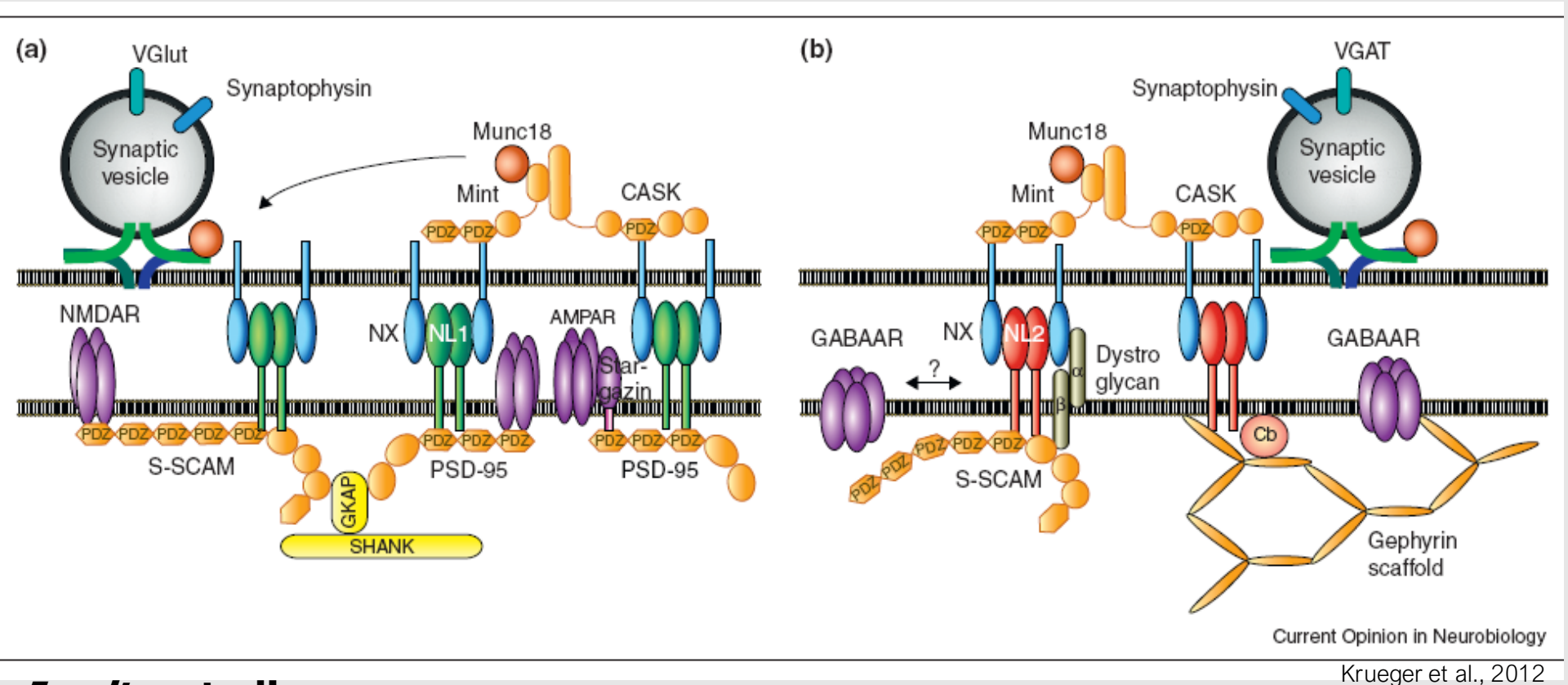

Sinapsina: marker presinaptico
PSD-95: marker postsinaptico




Espianti corticali
dopo 2 settimane in coltura

Topi KO per Neuroligine 1-3 muoiono per insufficienza respiratoria, ma il numero delle sinapsi e' normale ed anche la ultra struttura al microscopio elettronico

# Proposed roles for the Neuroligins proteins




Krueger et al., 2012

***In vitro* studies:**

**Neuroligins and Neurexins may induce synapse formation**

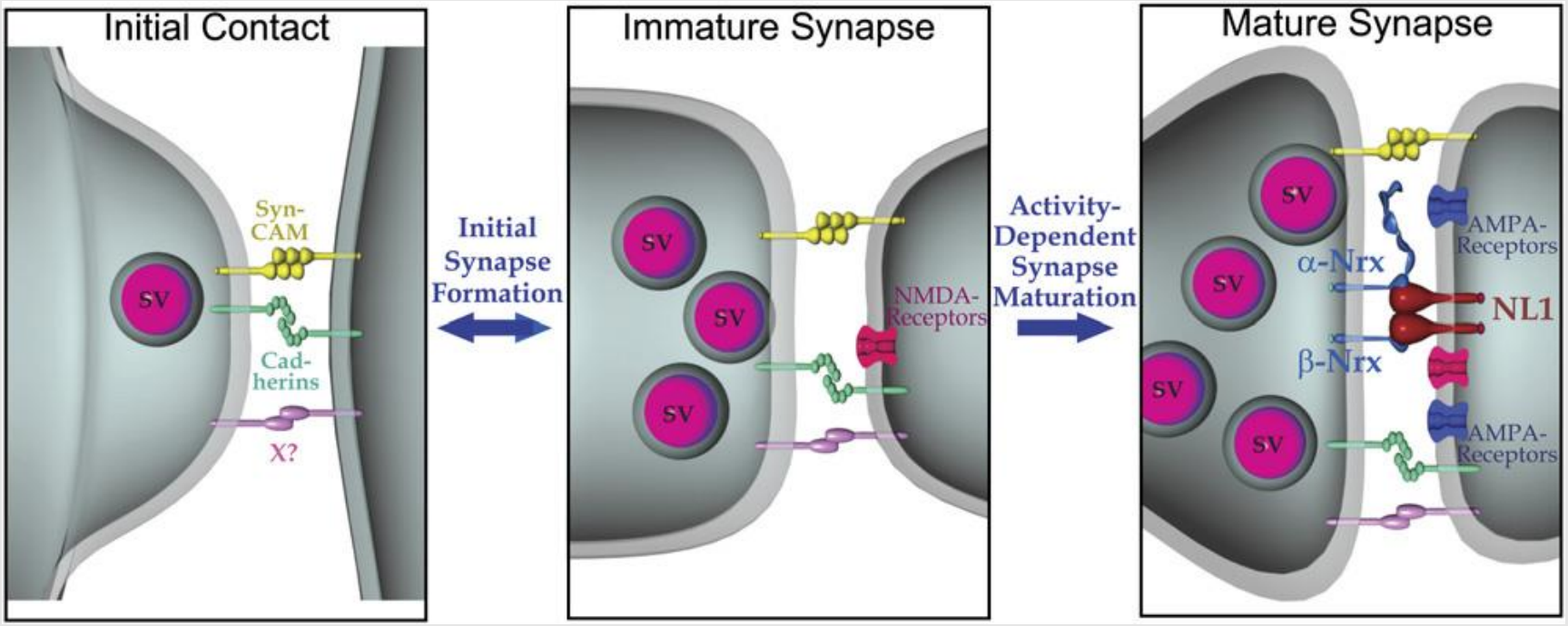
*(Artificial synapse formation assay, Neuronal transfection assay )*

**Gene Knock out studies:**

Neuroligins and Neurexins are essential for **synapse function**

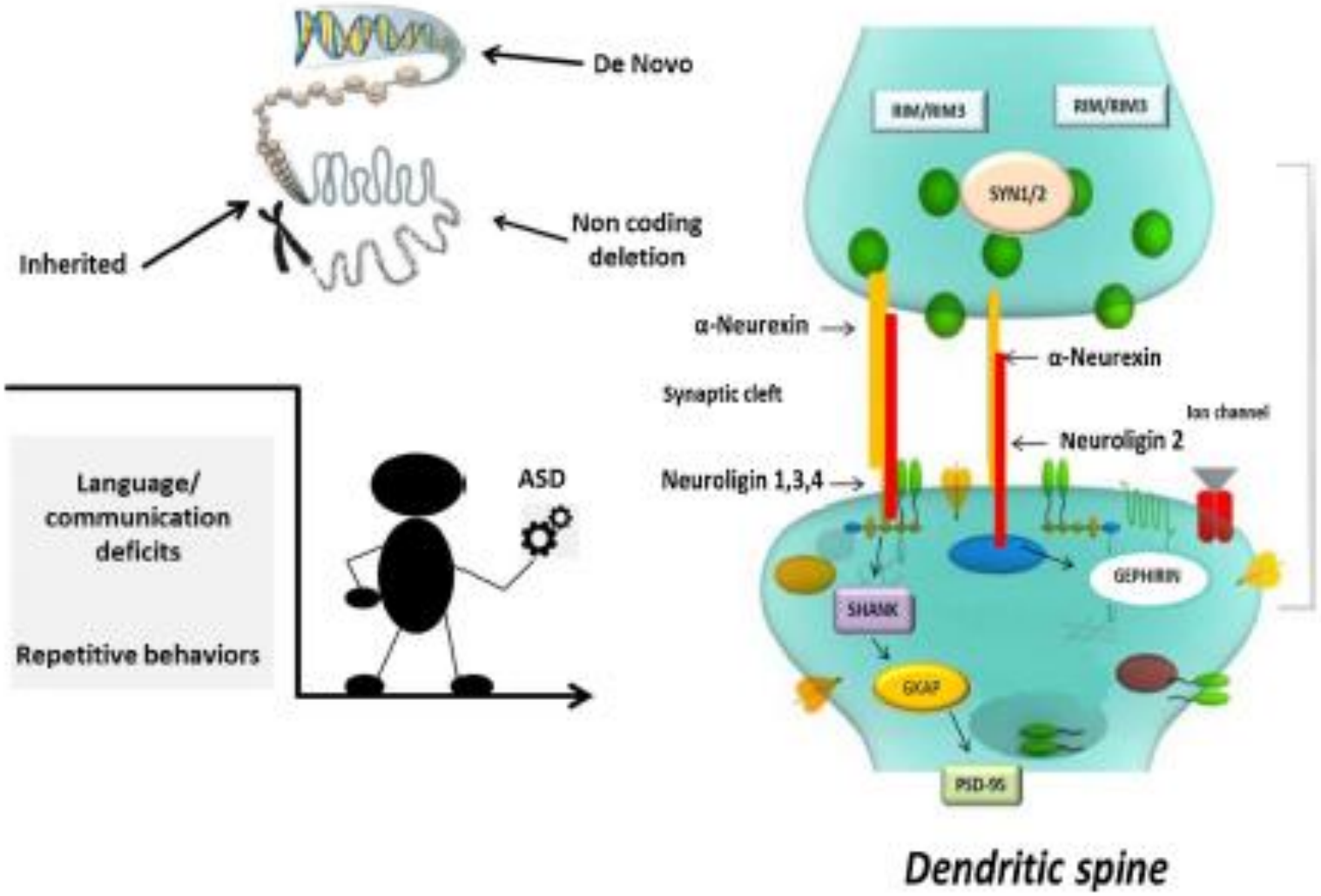
**Role in the specification and identity of synaptic connections**

# LA TEORIA CORRENTE



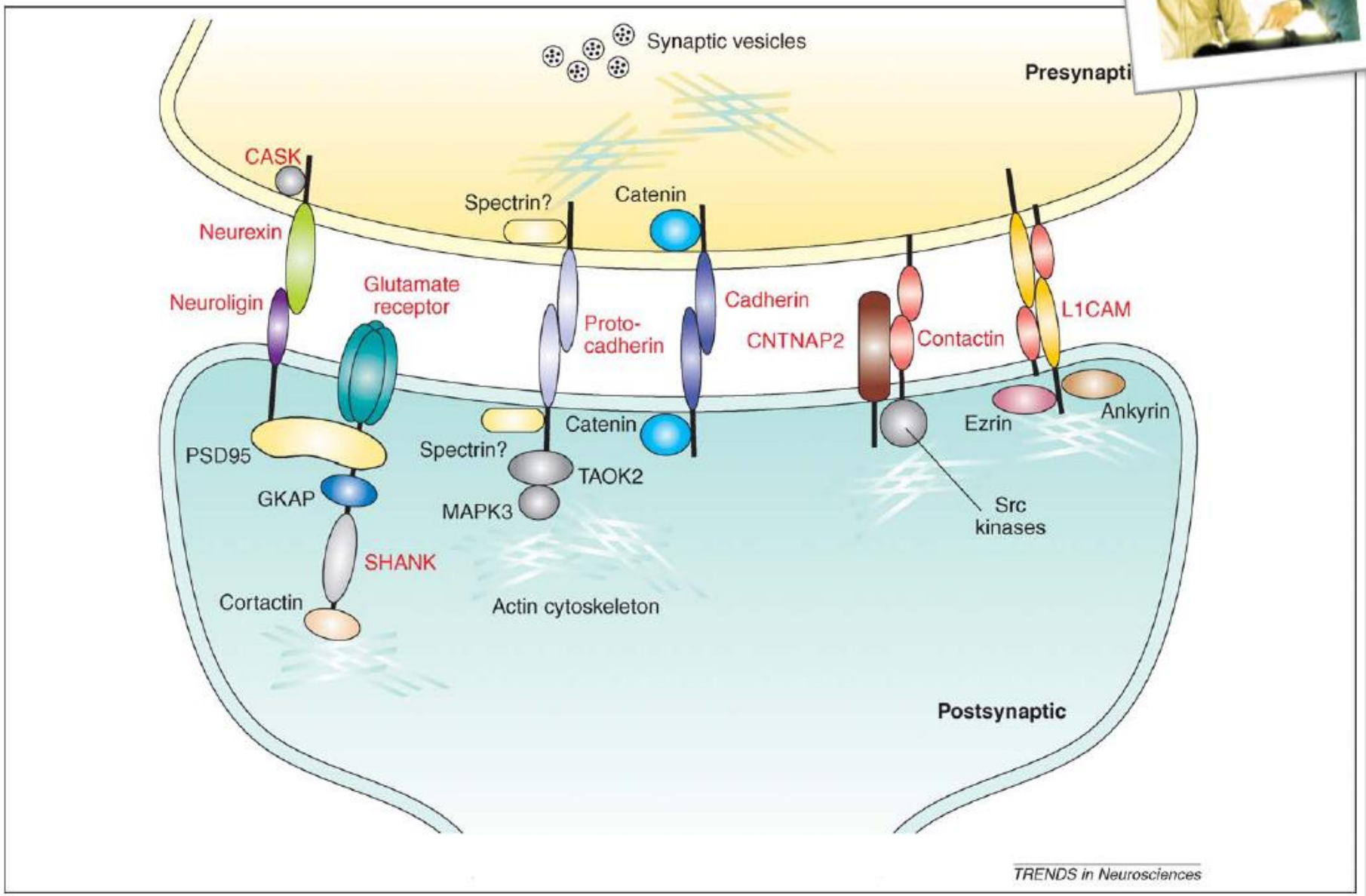

Contatti sinaptici iniziali coivolgono diverse molecole di adesione che conferiscono specificità. La sinapsi immatura è funzionale ma ha bisogno di acquisire proprietà specifiche attraverso processi dipendenti dall'attività. Le neuroligine possono mediare la stabilizzazione dipendente dall'attività dei contatti sinaptici transienti. Neuroligine e neurexine non sono fondamentali per la formazione della sinapsi ma sono importanti per formazione di complessi sinaptici funzionali.

# From the genetics to the synapse



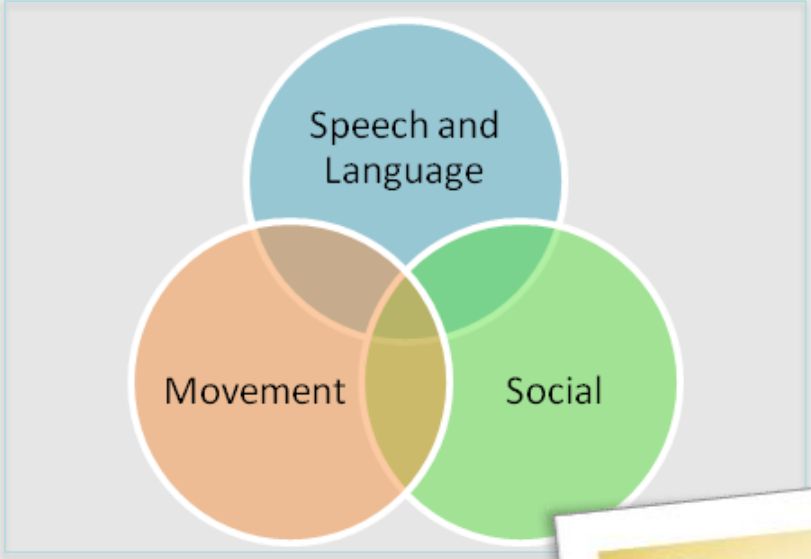
# MOLECOLE SINAPTICHE E DISTURBI DELLO SPETTRO AUTISTICO





# Autism Spectrum Disorders

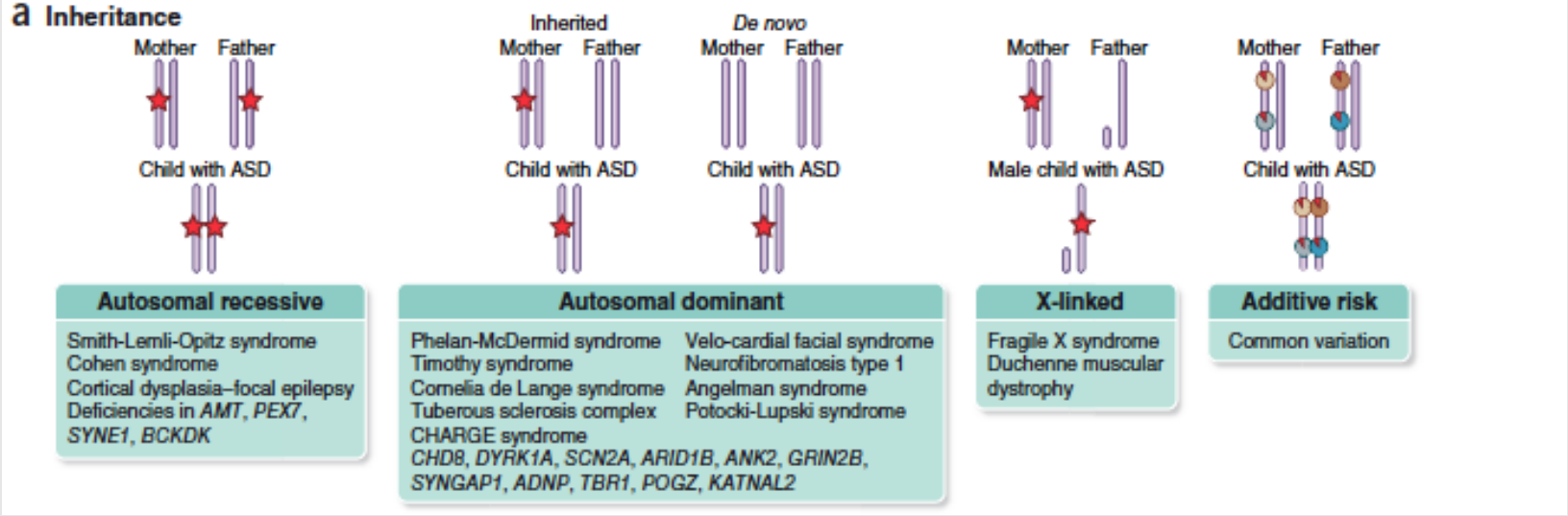


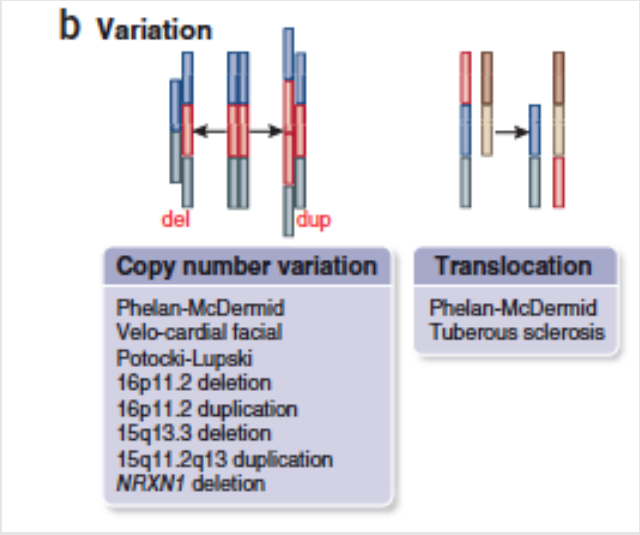



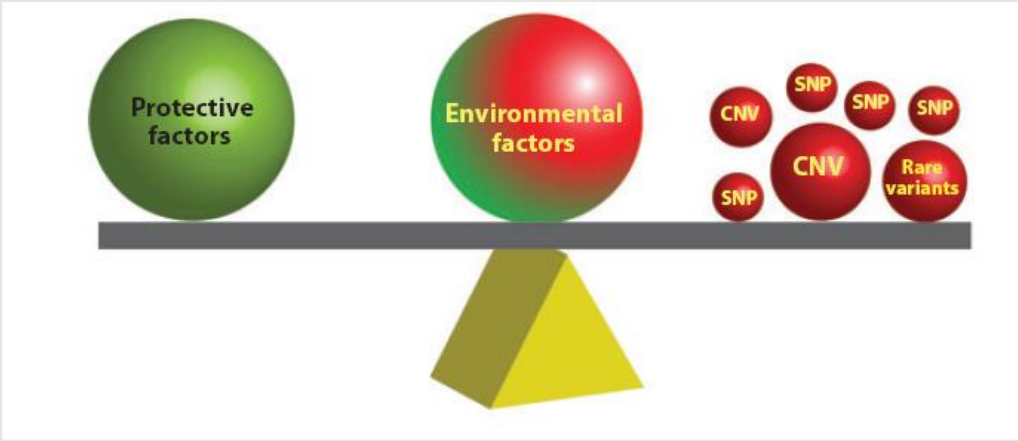



- Onset by the age of 3
- Male to female ratio: 4 to 1
- Prevalence: once 1/1,000; recently up to 1/68 (USA)
- Diagnosis is based on behavioral criteria
- Strong genetic background interlinked with unknown environmental influences

# The genetics of autism spectrum disorders



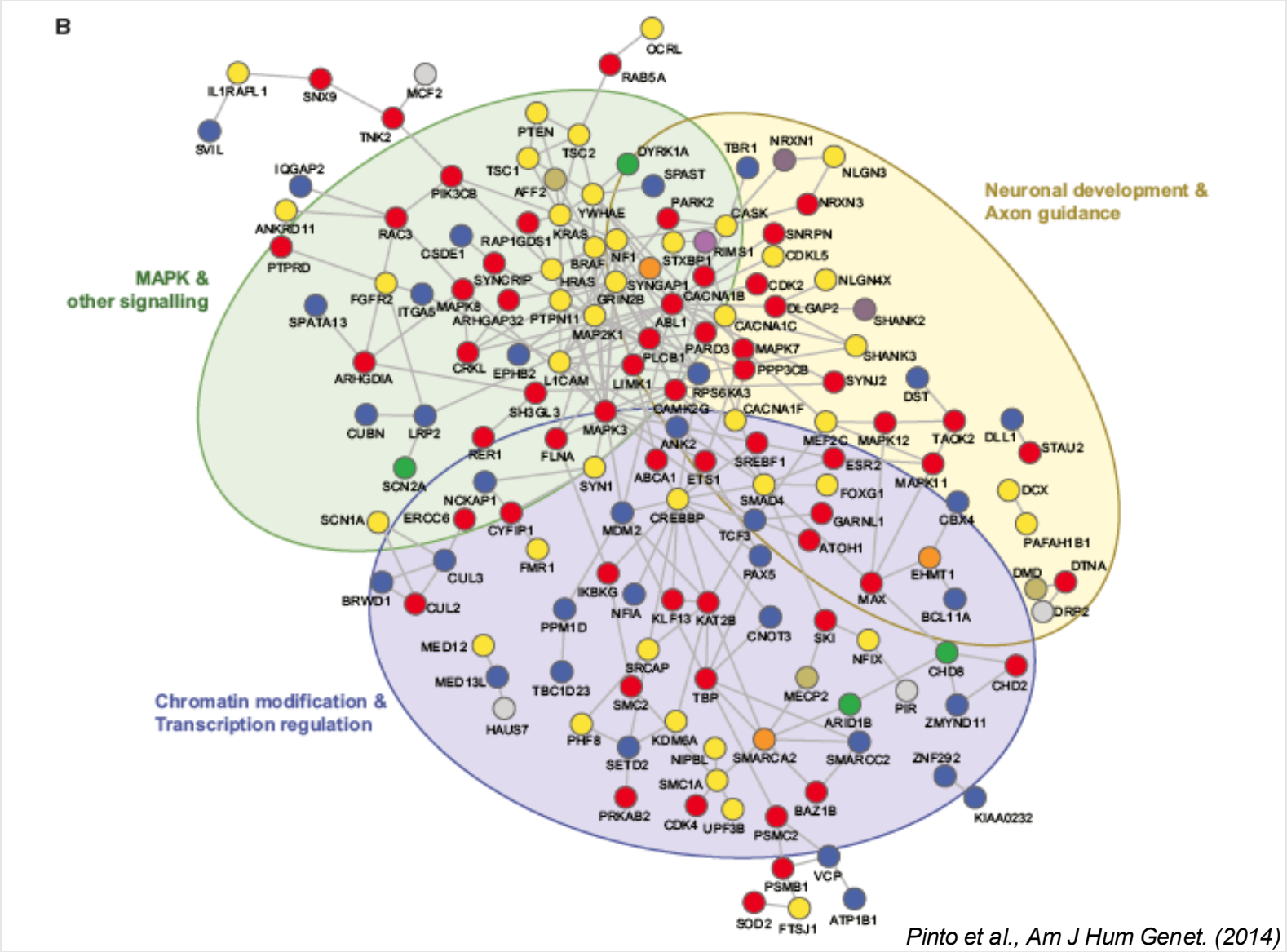




*De la Torre-Ubieta et al., 2016*

unique and recurrent CNV as significant contributors to ASD.

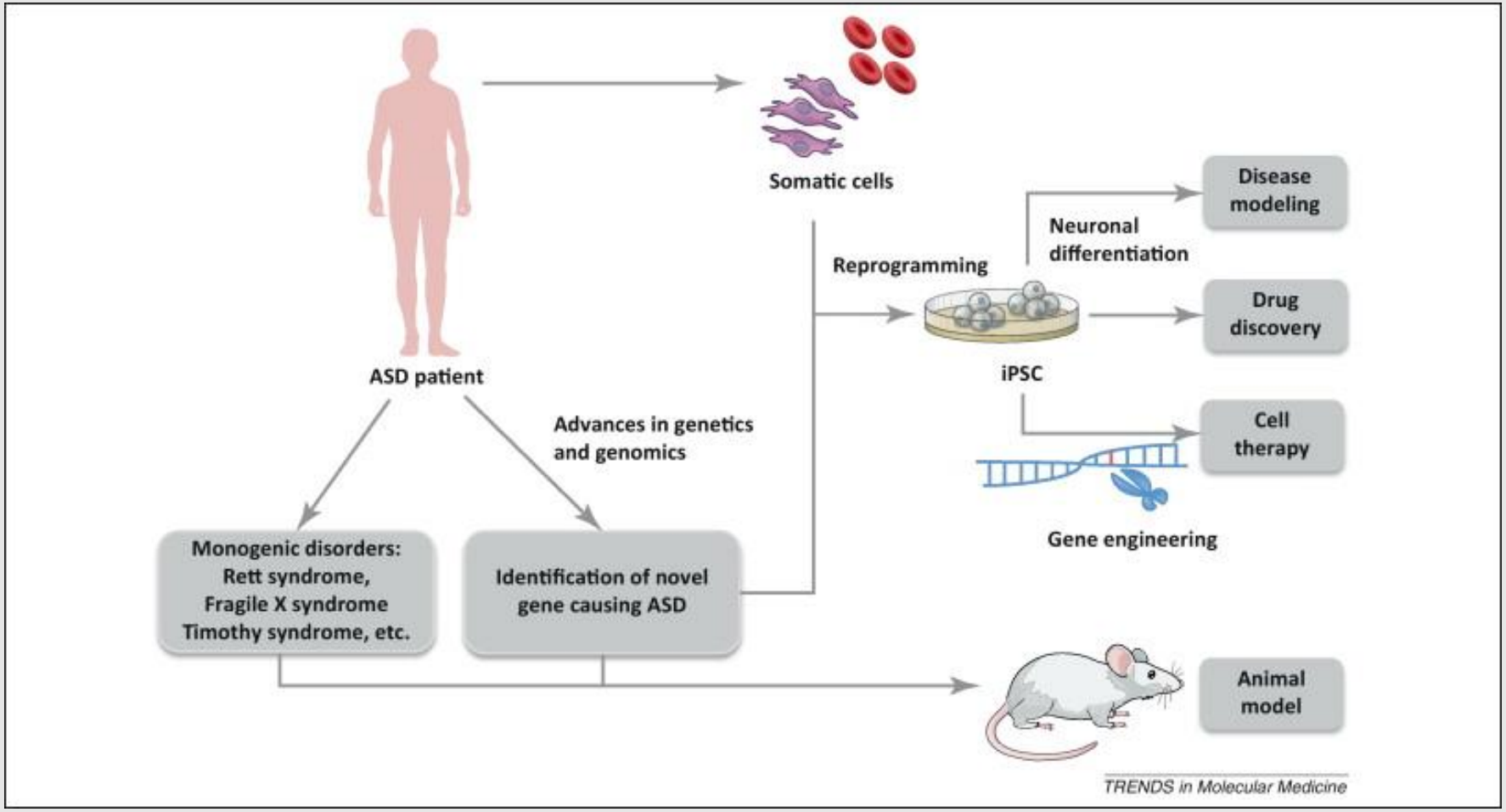
# Genes and cellular pathways dysregulated in ASD




*Pinto et al., Am J Hum Genet. (2014)*

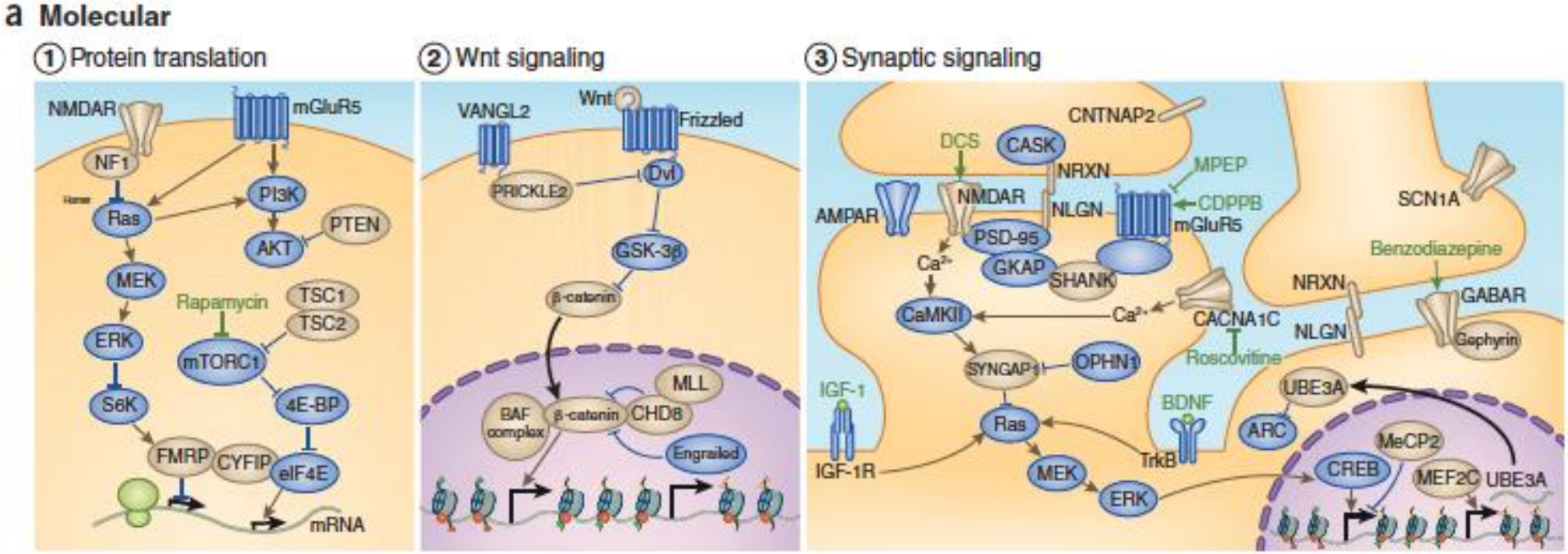
No single genetic mutation accounts for more than 1% of ASD

# Current in vitro and in vivo model systems




Mouse models have been valuable in traslating genetic findings and have provided Evidence for shared molecular pathways in ASD

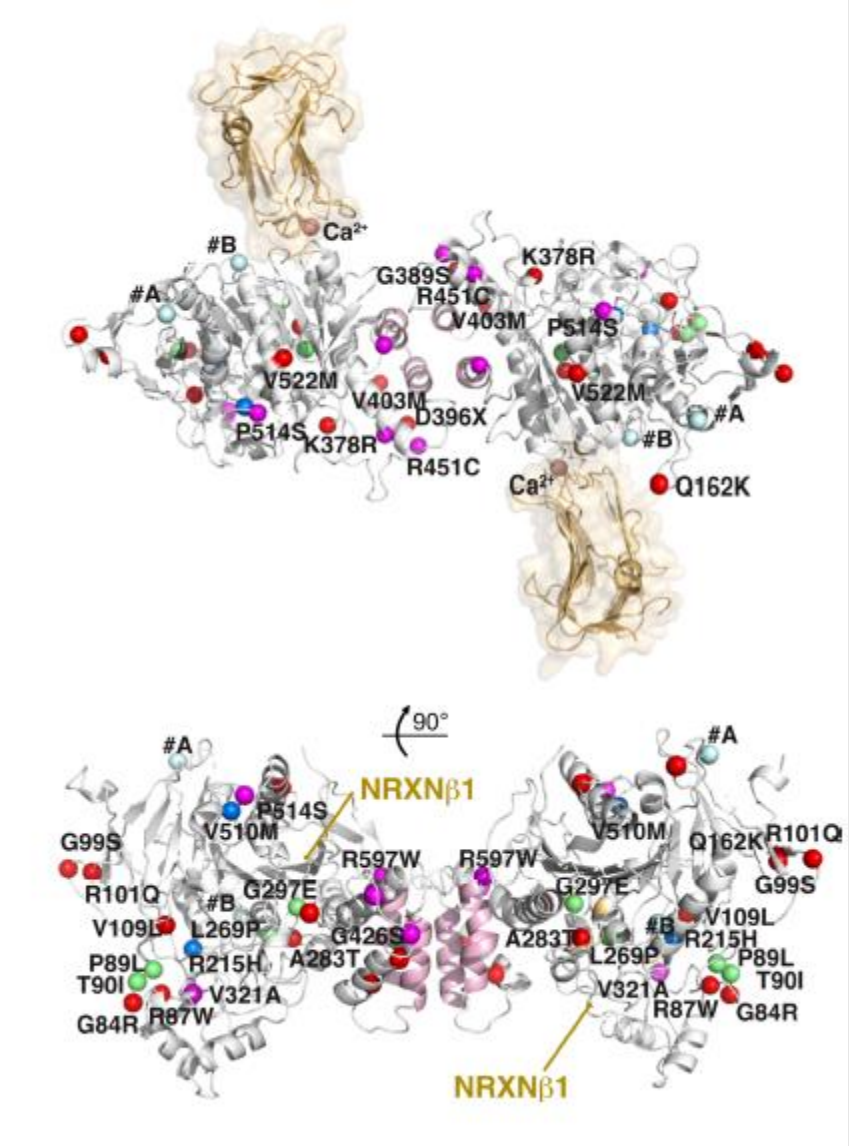
# Molecular Signaling involved in ASDs



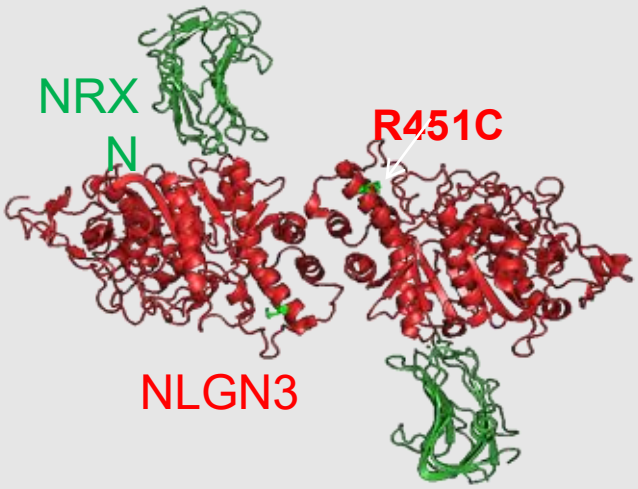

1. Activity dependent transcriptional and traslational regulators are associated with ASDs.
2. Wnt signaling is known to regulate neurogenesis
3. Dysregulation in synaptogenesis and synaptic trasmission.

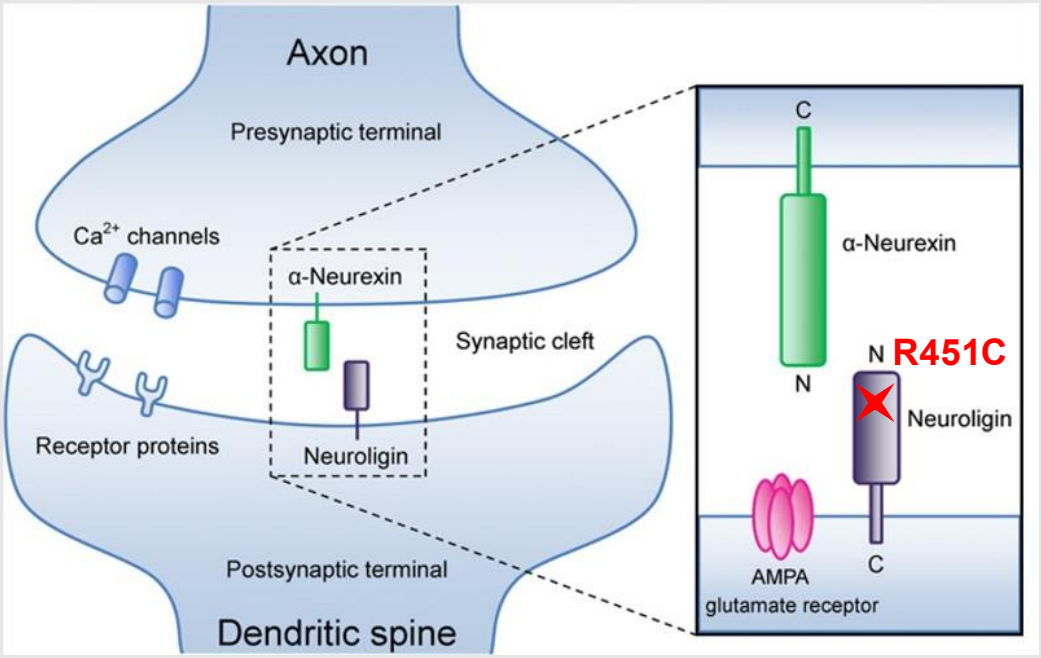
*De la Torre-Ubieta et al., 2016*

# MUTAZIONI RISCONTRATE NEL DOMINIO EXTRACELLULARE DELLE PROTEINE DELLA FAMIGLIA DELLE NEUROLIGHINE



# The R451C mutation maps in the extracellular domain of Neuroligin3

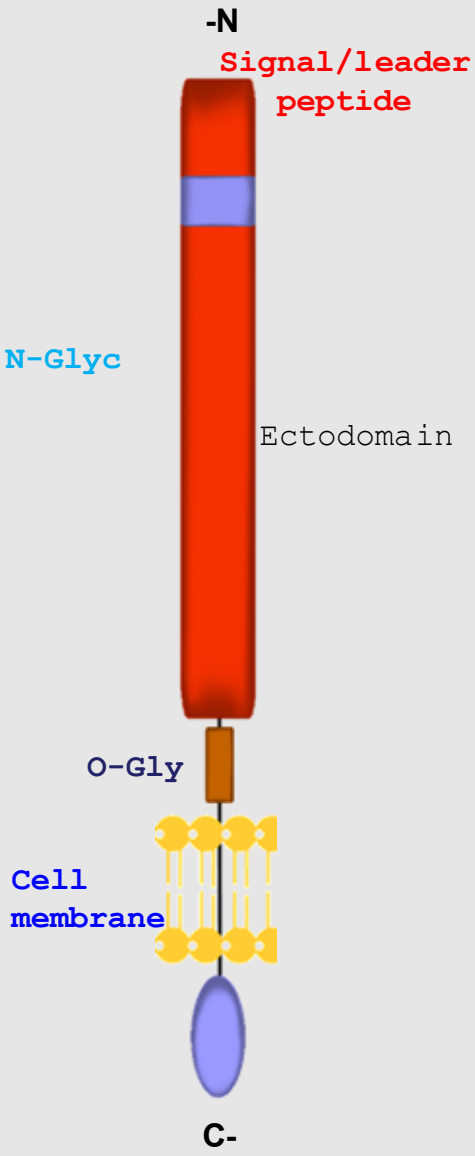



More than 30 genetic alterations have been associated with autism including a Neuroligin3 point mutation and a deletion

*Jamain et al., 2003*

# Basic architecture of cell surface molecules




MAWLGVVIFALVIALFPSAPVLVTVRHLKANSAVVSWDVLEDEVVIGFAISQQKKDVRMLRF
IQEVNTTTRSCALWDLEEDTEYIVHVQAISIQGQSPASEPVLFKTPREAEKMASKNKDEVTM
KEMGRNQQLRTGEVLIIVVVLFMWAGVIALFCRQYDIIKDNEPNNNKEKTKSASETSTPEHQ
GGGLLRSKI

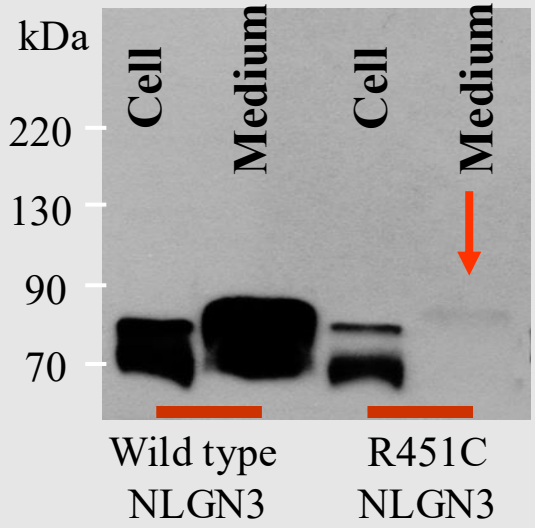
**MAWLGVVIFALVIALFPSAPVLVTV**RHLKANSAVVSWDVLEDEVVIGFAISQQKKDVRMLRF
IQEVNTTTRSCALWDLEEDTEYIVHVQAISIQGQSPASEPVLFKTPREAEKMASKNKDEVTM
KEMGRNQQLRTGEVLIIVVVLFMWAGVIALFCRQYDIIKDNEPNNNKEKTKSASETSTPEHQ
GGGLLRSKI

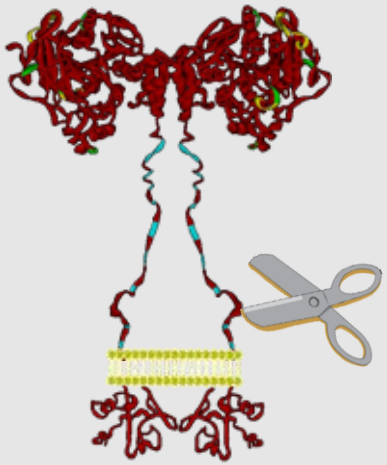
**MAWLGVVIFALVIALFPSAPVLVTV**RHLKANSAVVSWDVLEDEVVIGFAISQQKKDVRMLRF
IQEVNTTTRSCALWDLEEDTEYIVHVQAISIQGQSPASEPVLFKTPREAEKMASKNKDEPTM
STTPRNSSLRT**GEVLIIVVVLFMWAGVIALF**C**R**Q**K**D**R**I**K**DNEPNNNKEKTKSASETSTPEHQ
GGGLLRSKI

**MAWLGVVIFALVIALFPSAPVLVTV**RHLKANSAVVSWDVLEDEVVIGFAISQQKKDVRMLRF
IQEVNTTTRSCALWDLEEDTEYIVHVQAISIQGQSPASEPVLFKTPREAEKMASKNKDE**PTM**
**STTPRNSSLRTGEVLIIVVVLFMWAGVIALF**C**R**Q**K**D**R**I**K**DNEPNNNKEKTKSASETSTPEHQ
GGGLLRSKI

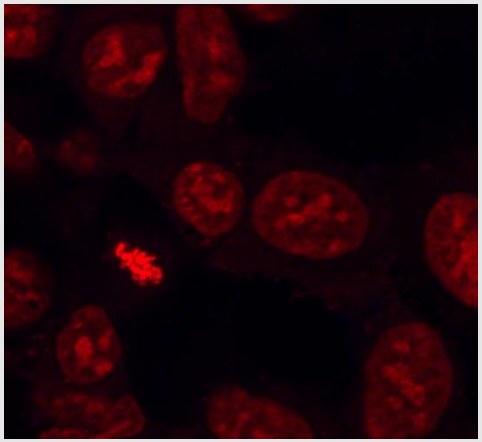
**MAWLGVVIFALVIALFPSAPVLVTV**RHLKANSAVVSWDV**C**LKF**C**SGRGQ**C**TRHG**C**K**C**DPGFS
GPA**C**IQEV**NAS**TTRSCALWDLEEDTEYIVHVQAI**NDT**GQSPASEPVLFKTPREAEKMASKNK
DE**PTMSTTPTNSSLRTGEVLIIVVVLFMWAGVIALF**C**R**Q**K**D**R**I**K**DNEPNNNKEKTKSASETS
TPEHQGGGLLRSKI

# Intracellular retention of R451C mutant soluble Neuroligin3

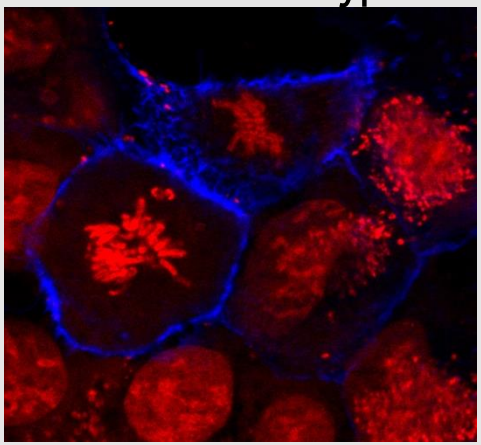


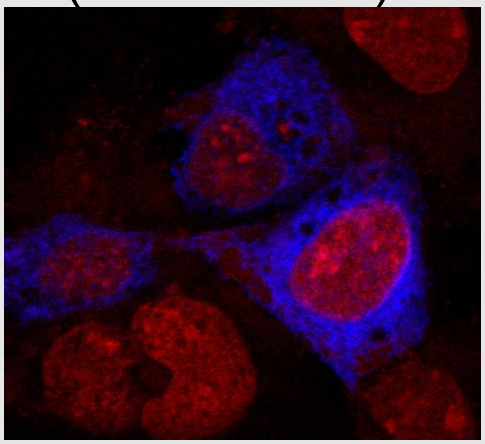
HEK-293 mock

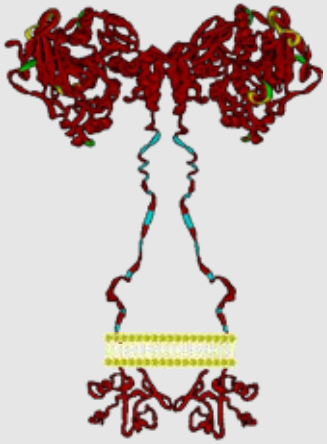


NLGN3-wild type



NLGN3-R451C (Autism mutant)





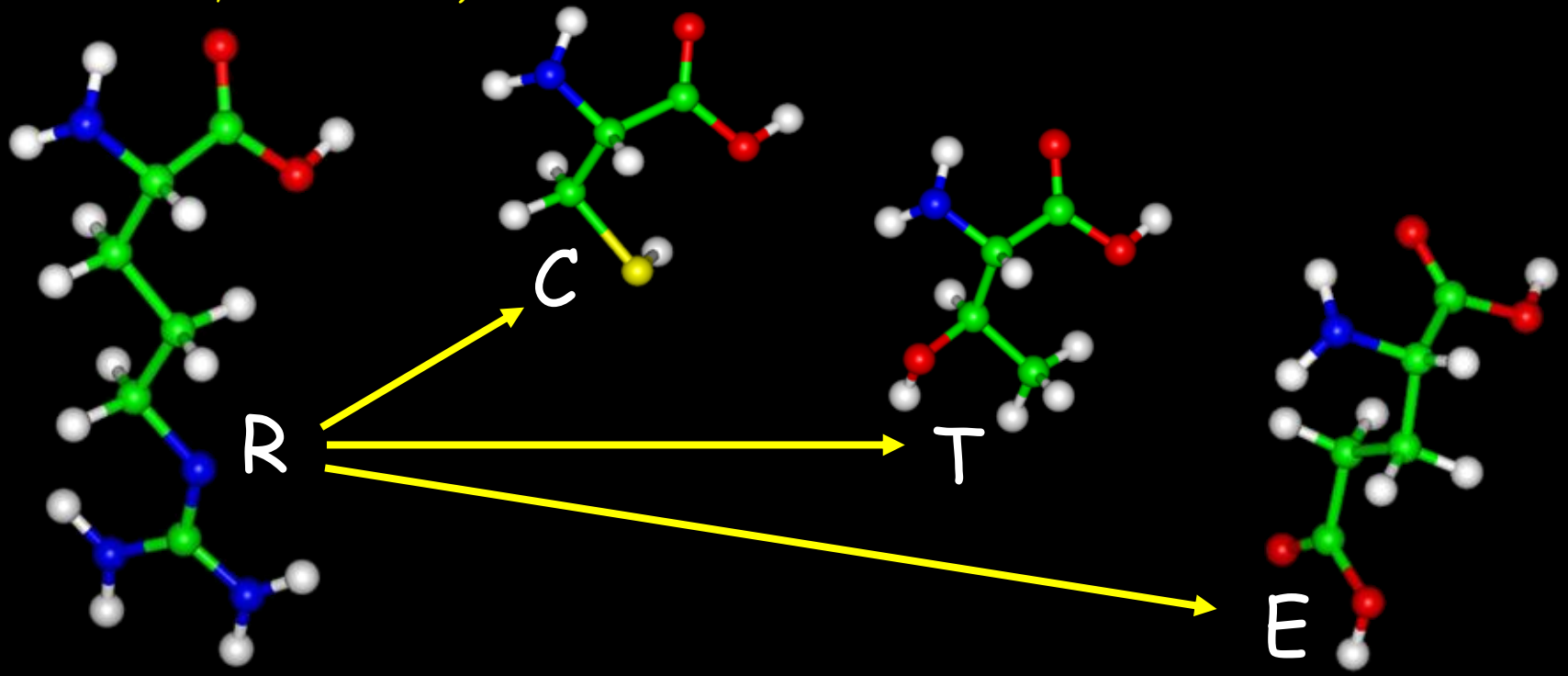
# NL3 R451C mutant

| neuroligins | | 451 | | |
|---|---|---|---|---|
| affected | YTDWADRD-NPETR | C | KTLVALFTD |
| control | YTDWADRD-NPETR | R | KTLVALFTD |
| rat | YTDWADRD-NPETR | R | KTLVALFTD |
| mouse | YTDWADRD-NPETR | R | KTLVALFTD |
| fruit fly NL | YTDWEKAIRNPLSS | R | DATLQFLSD |
| fruit fly GL | YTFWPDPN-NNTII | R | DQYINMLSD |
| | | | |
| ACHE | | | |
| human | YTDWLHPE-DPARL | R | EALSDVVGD |
| mouse | YTDWLHPE-DPTHL | R | DAMSAVVGD |
| chicken | YTDWLDAD-NPVKN | R | EALDDIVGD |
| banded krait | YTDWQDQD-NREKN | R | EALDDIVGD |
| electric eel | YTDWMDED-NPIKN | R | EAMDDIVGD |
| zebra fish | YTDWMDEN-NGQKN | R | DAMDDIVGD |

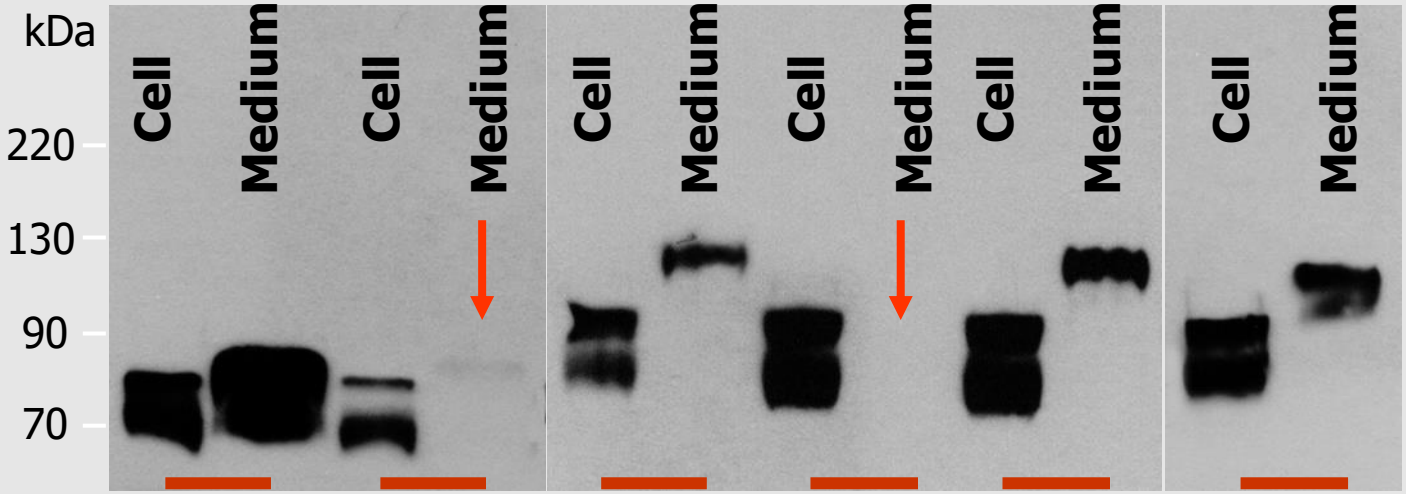
(Jamain et al. 2003, *Nature Genetics*)

Is it the removal of Arg?

Is it the presence of Cys?



# Cellular secretion of wild-type and mutant NLGN1 and NLGN3

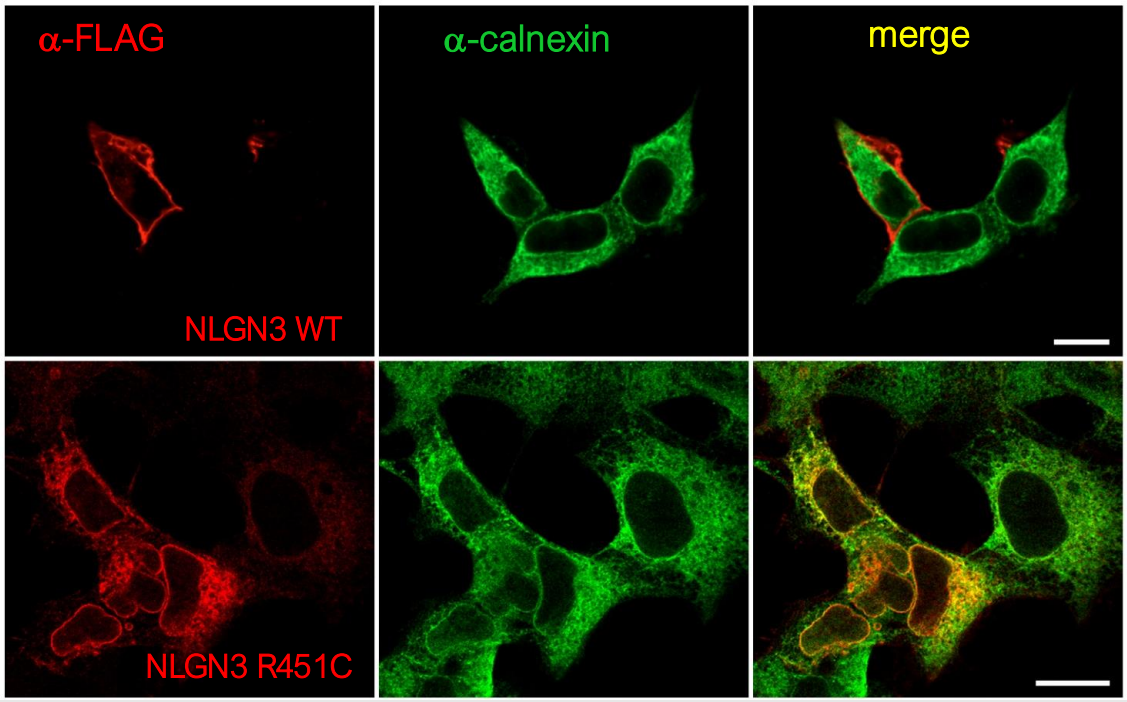


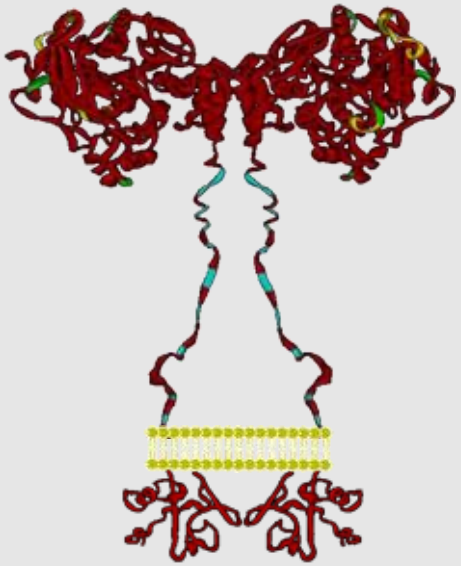


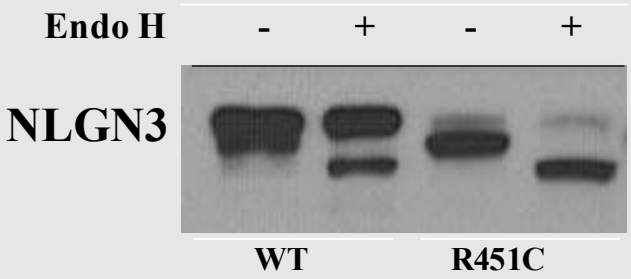

•Arg451 to Cys mutation in NL3 and Arg453 in NL1 compromises the export of NL1 and NL3. When Arg451 is mutated to Thr or Glu the protein is normally expressed.

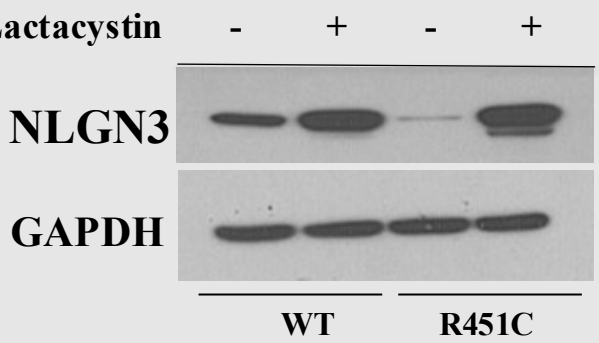
*Comoletti et al. J.Neurosci., 2004*

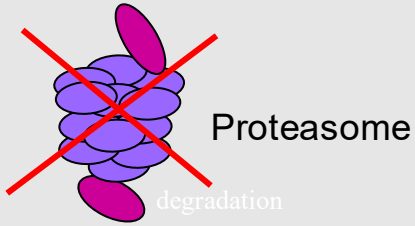
# Co-localization staining of Neuroligin3 and calnexin





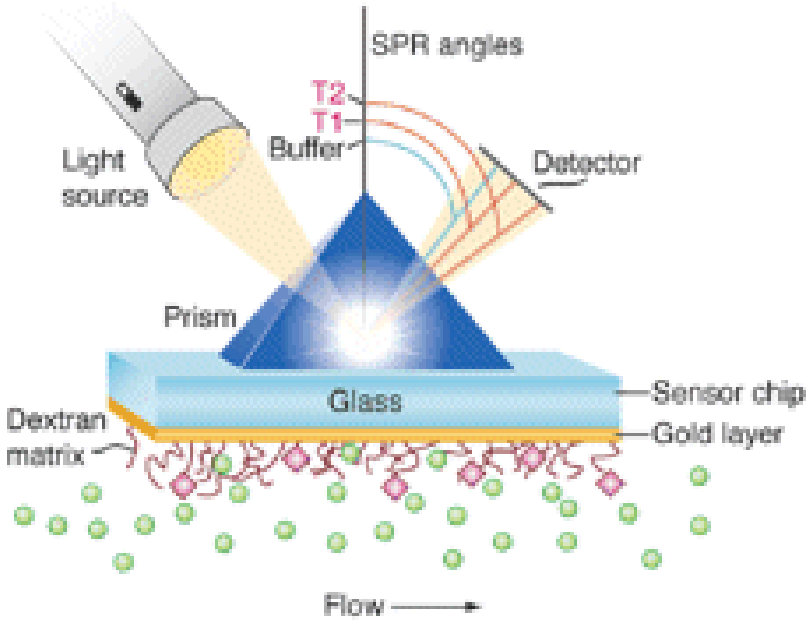



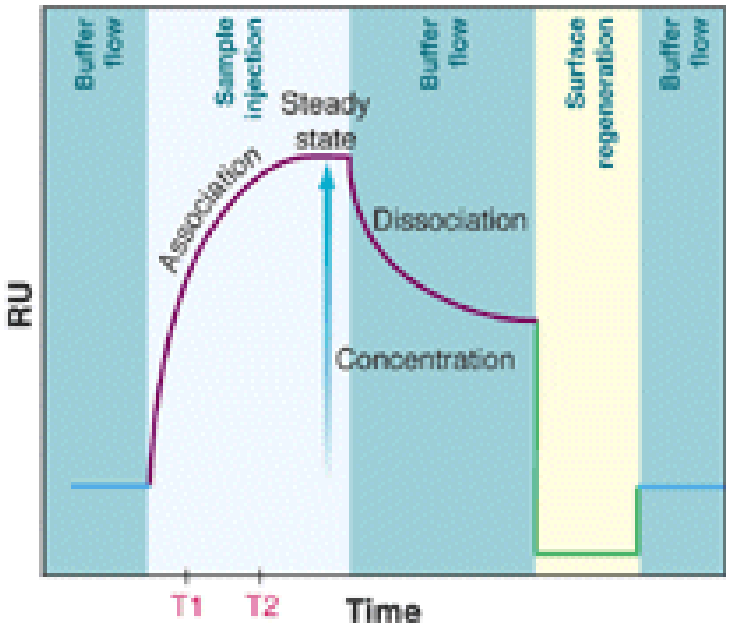




Neuroligin3 R451C is in the Endoplasmic Reticulum

*De Jaco et al., 2006*

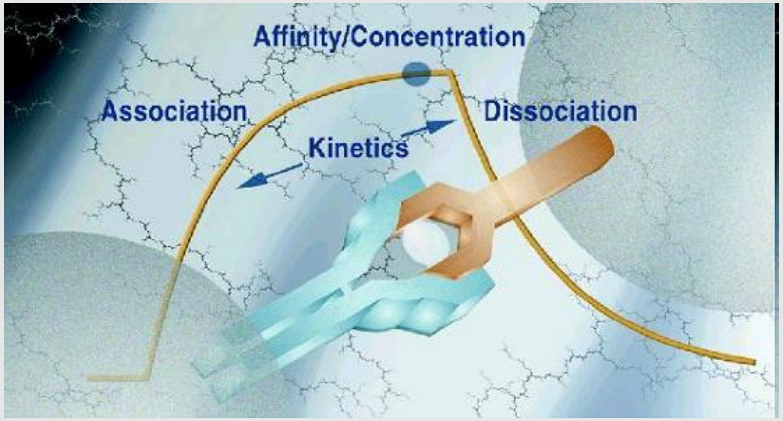
# SURFACE PLASMON RESONANCE

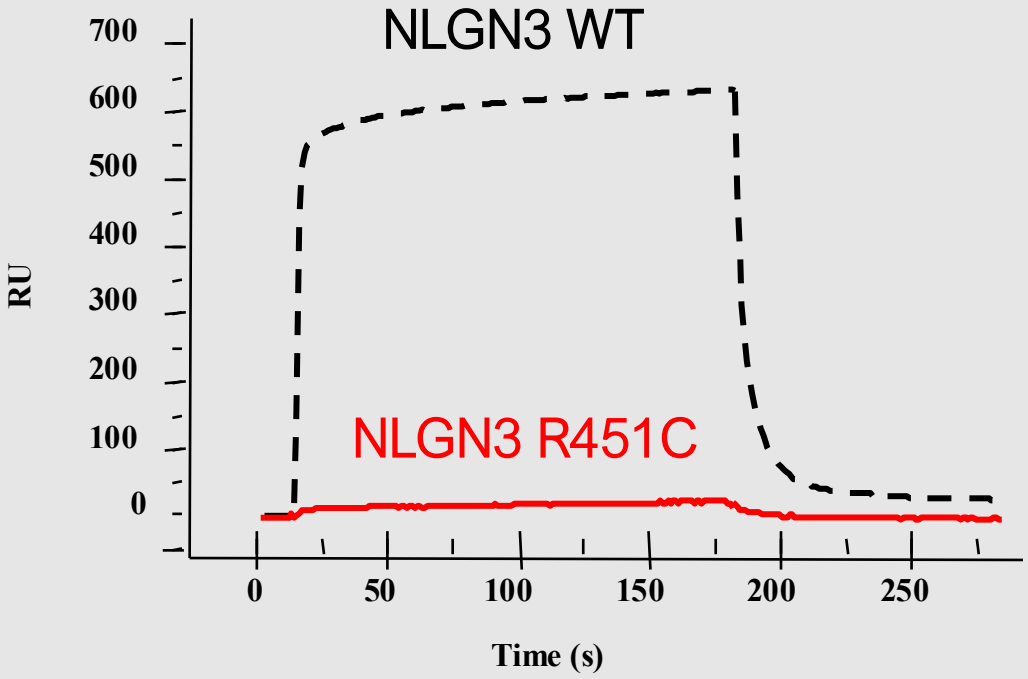



La variazione dell'angolo di risonanza e' direttamente proporzionale alla quantita' di Ligando legato alla macromolecola immobilizzata

# The mutation is affecting Neuroligin3 function

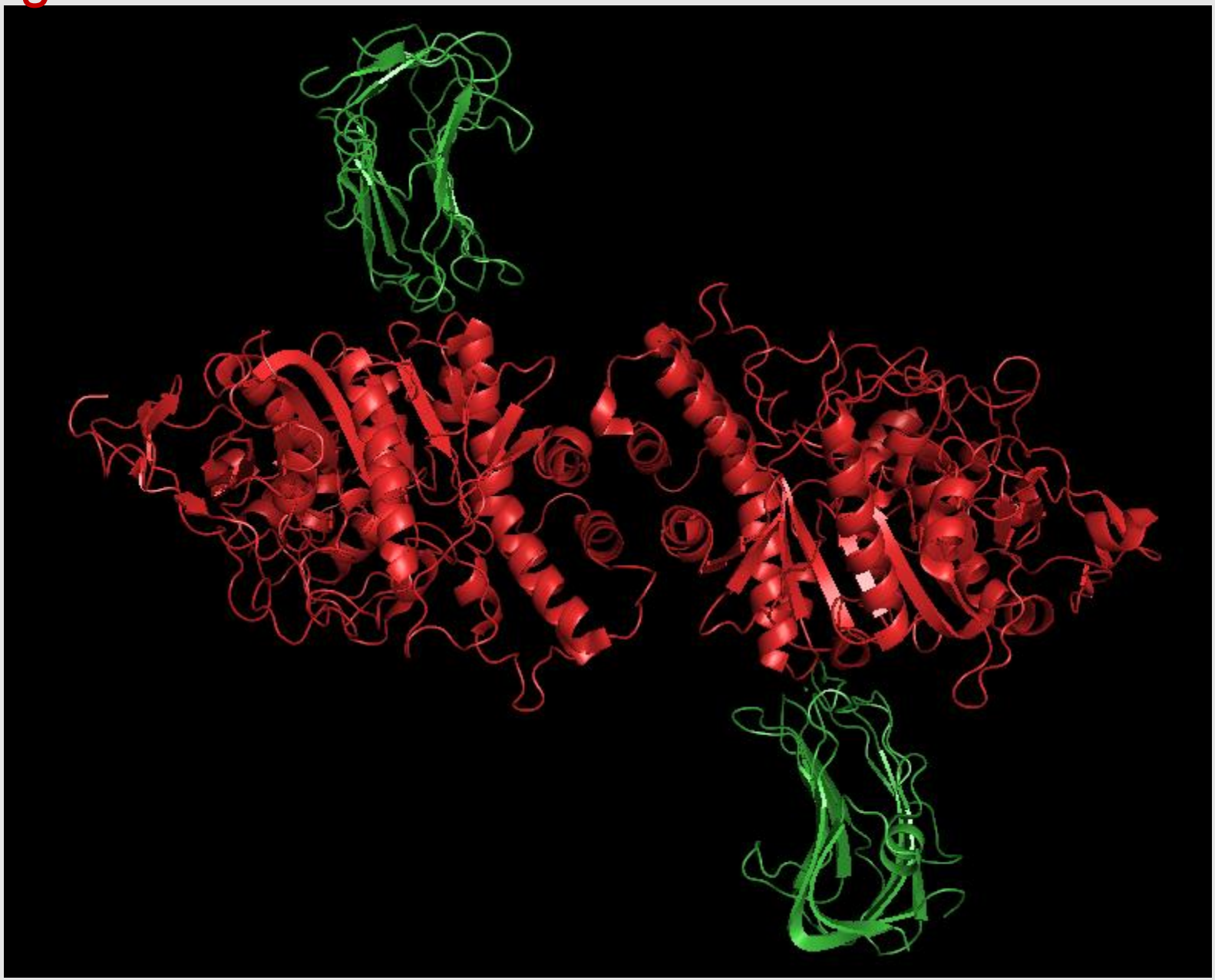


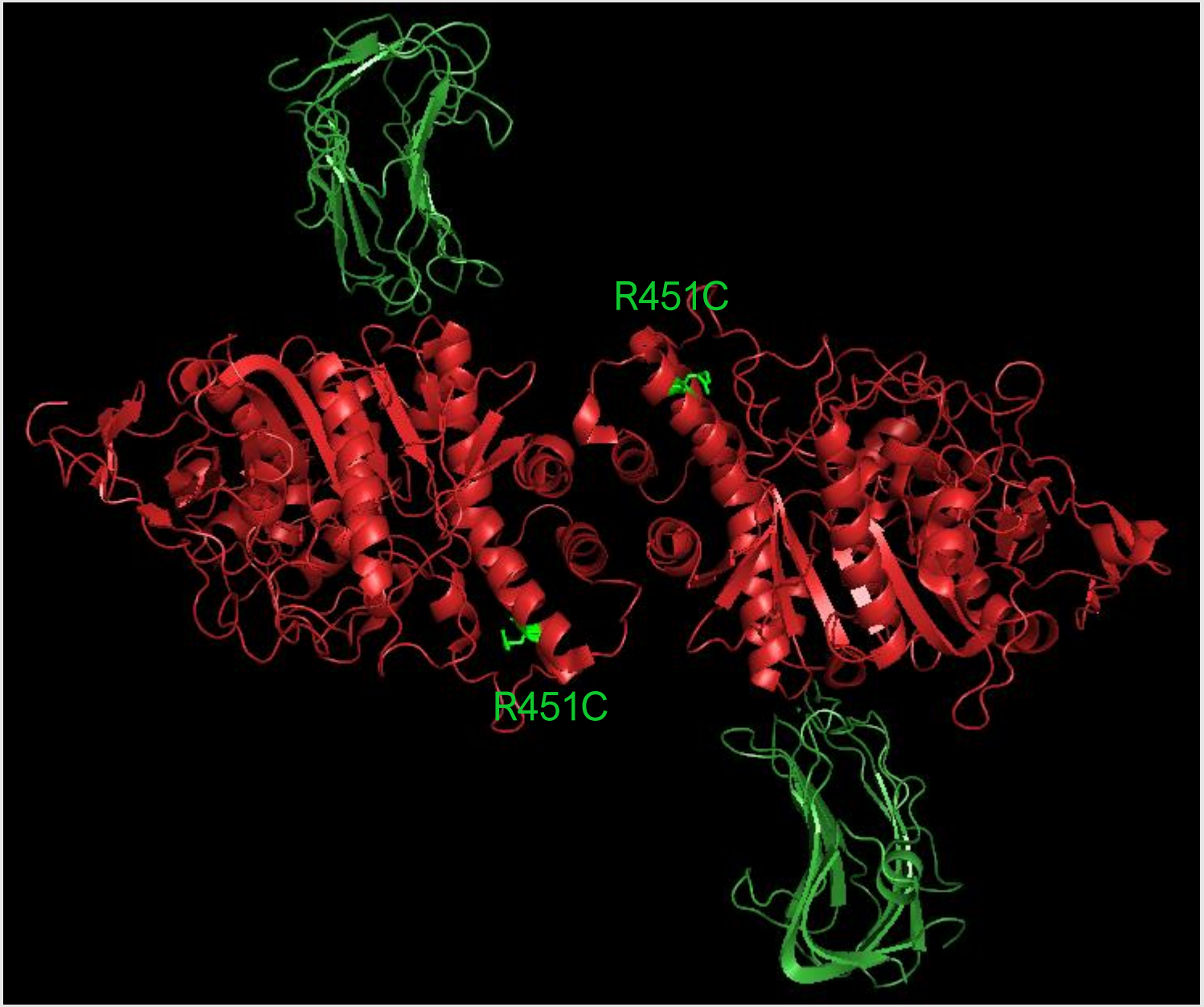

Reduced binding activity
of purified R451C Neuroligin3
in comparison to WT protein

*Comoletti et al., 2004*

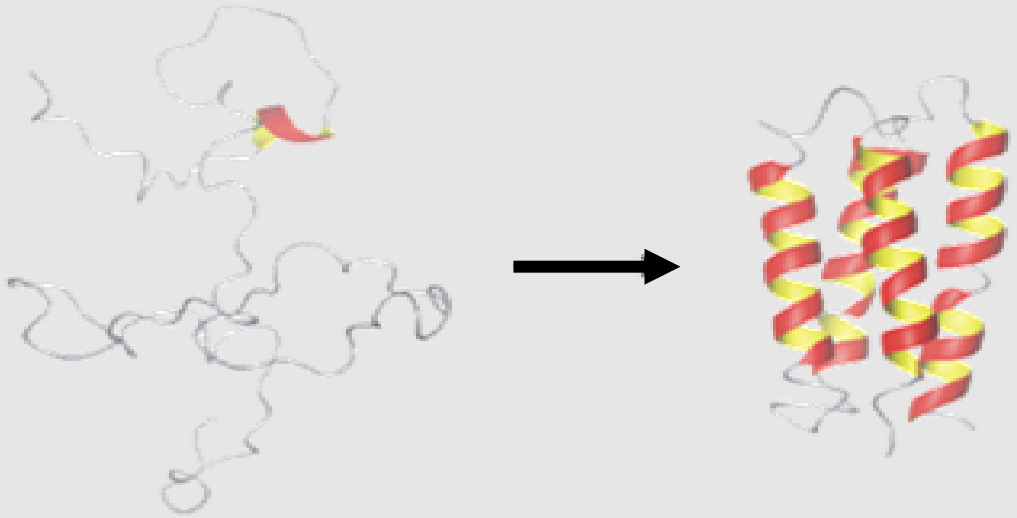
# Neuroligin-Neurexin



*Fabrichny et al., 2007*

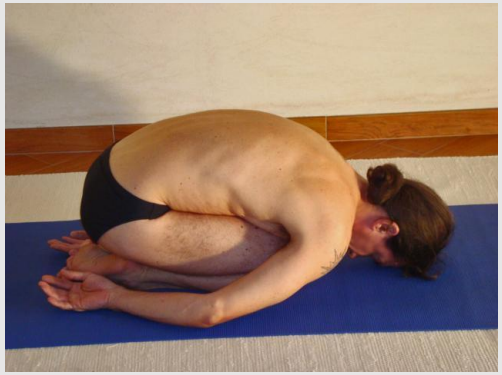

R451C
R451C

# PROTEIN FOLDING

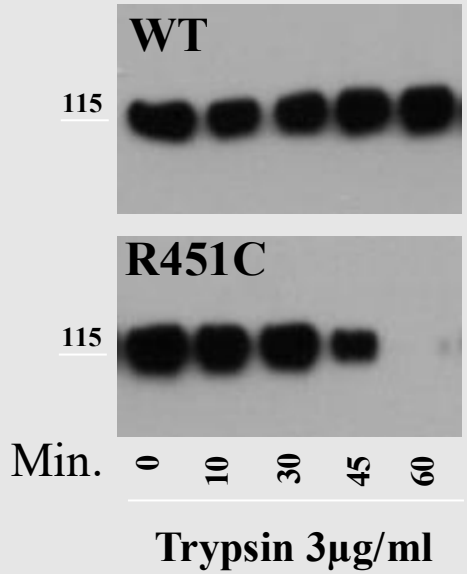


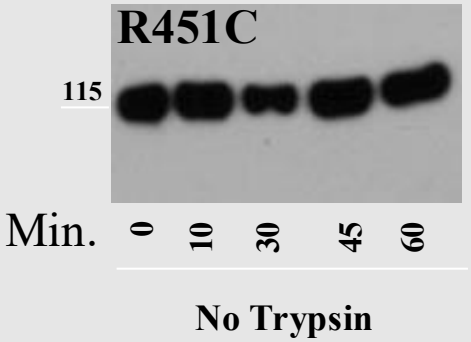
**unfolded** **folded**





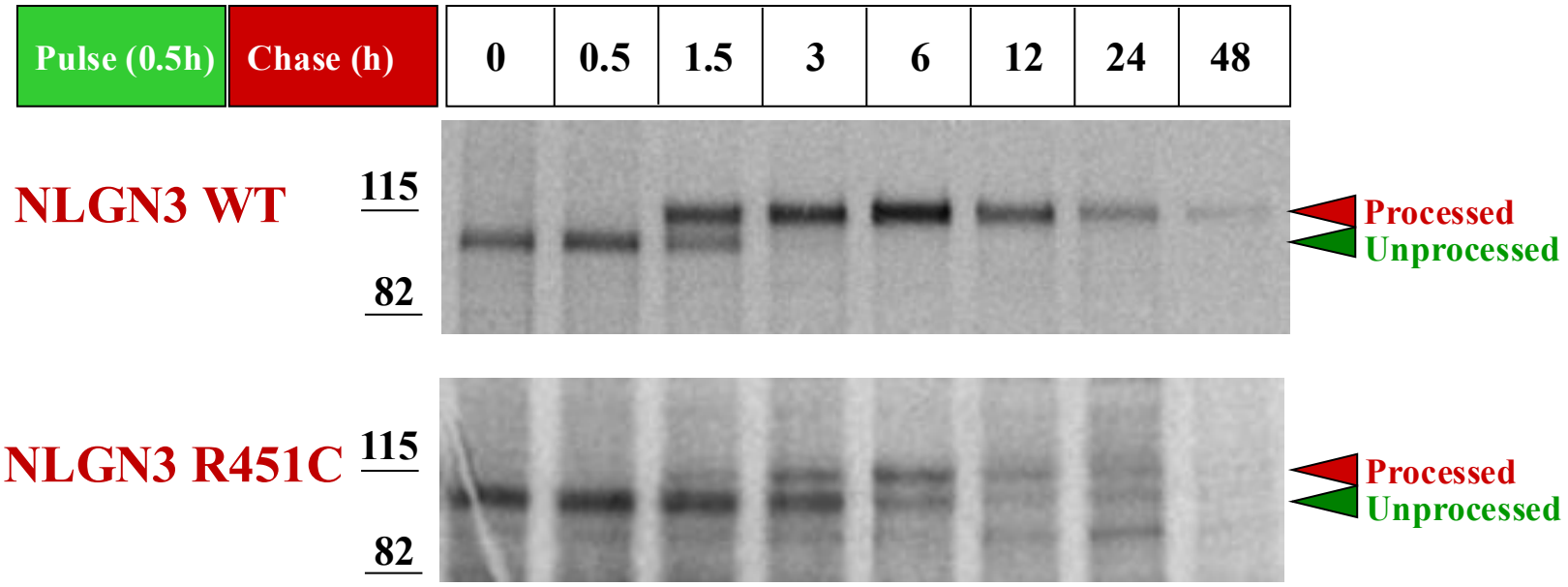
# Misfolding of NLGN3 R451C mutant protein: protease sensitivity





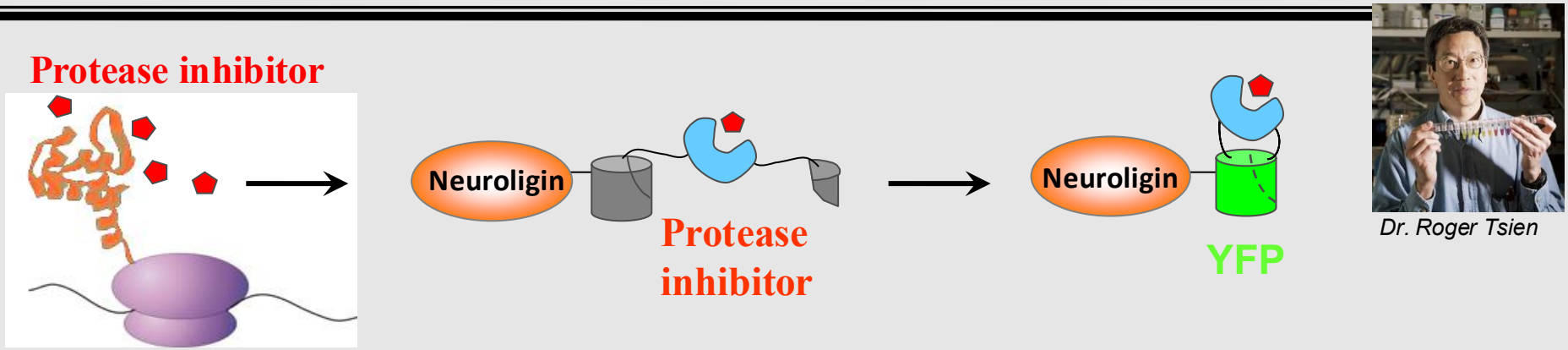

# Processing of NLGN3 in HEK-293 cells




R451C Mutation in NLGN3 affects glycosilation processing of the protein in the ER

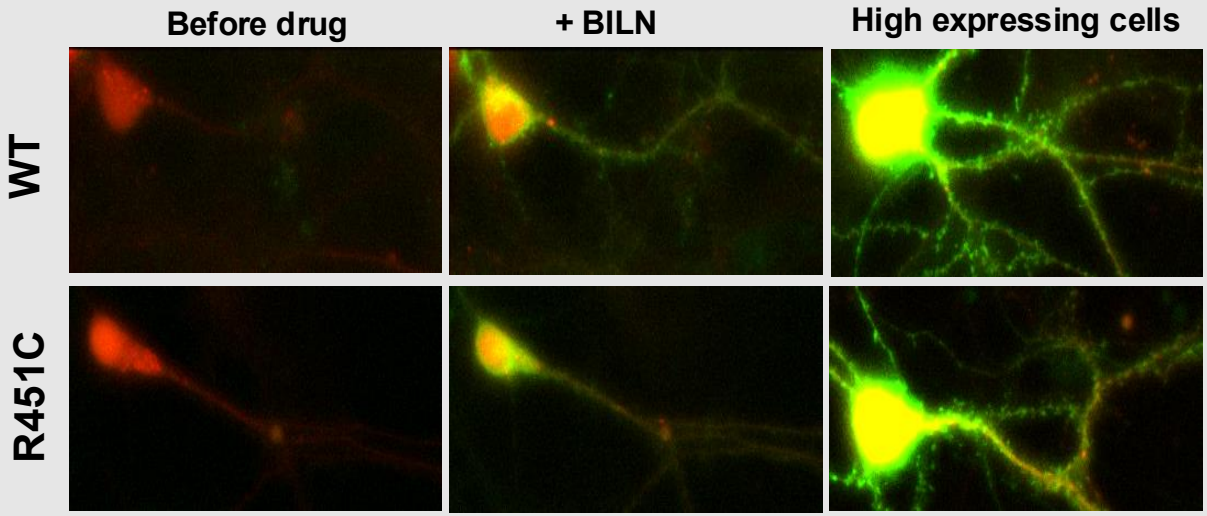
*De Jaco et al., 2010*

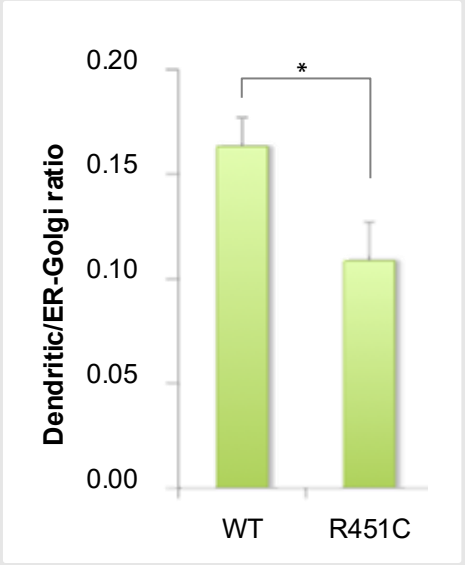
# Trafficking of fluorescent Neuroligin3 in hippocampal neurons






*Dr. Roger Tsien*




- Neuroligin3 protein is represented by the green dots.
- Protein translocation from the soma to dendrites was quantified by the ratio of the measured fluorescent signal!

*De Jaco et al., 2010*

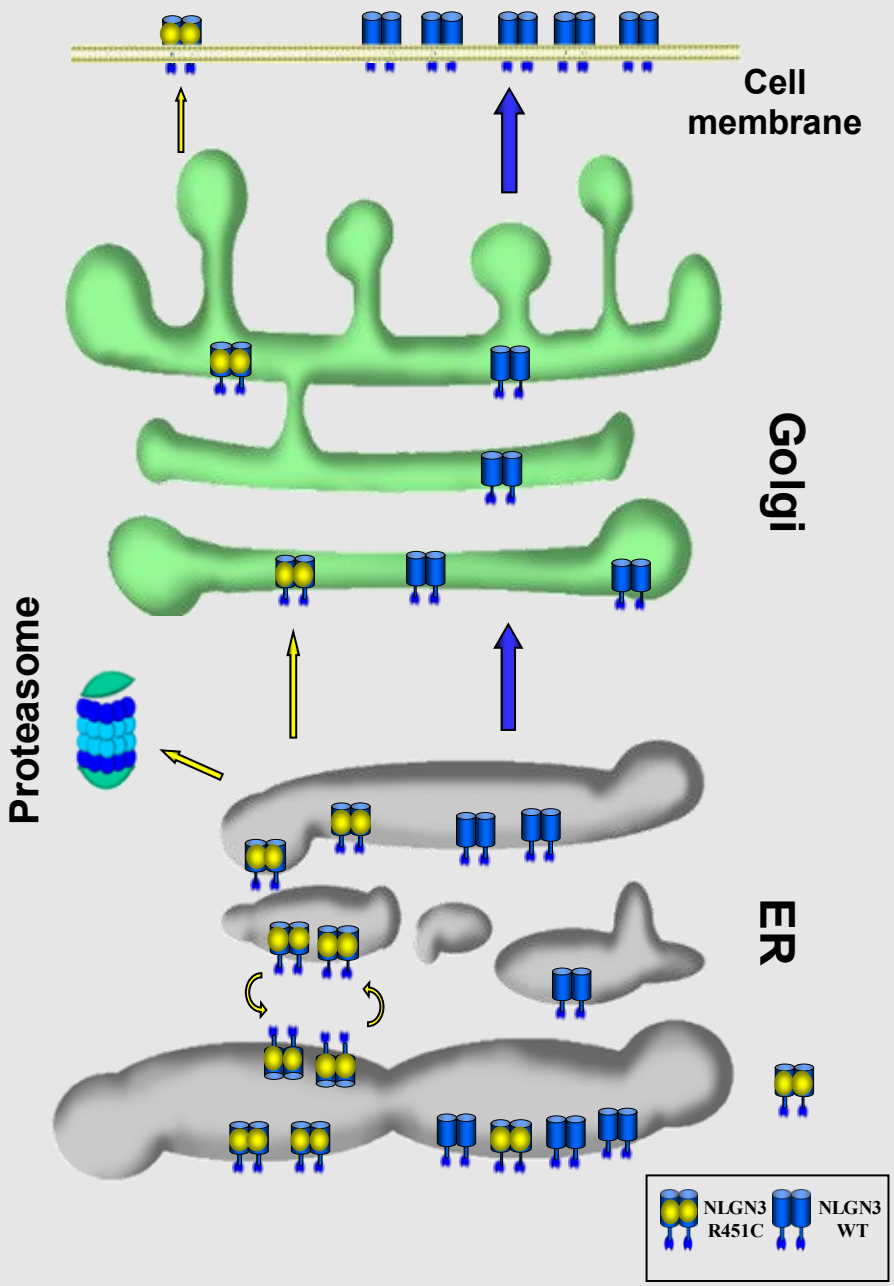
# R451C mutation in Neuroligin3 causes misfolding and ER retention

R451C NLGN3 is mainly retained in the Endoplasmic Reticulum (ER).

R451C mutation causes a local misfolding of NLGN3.

The majority of the protein is degraded by the proteasome

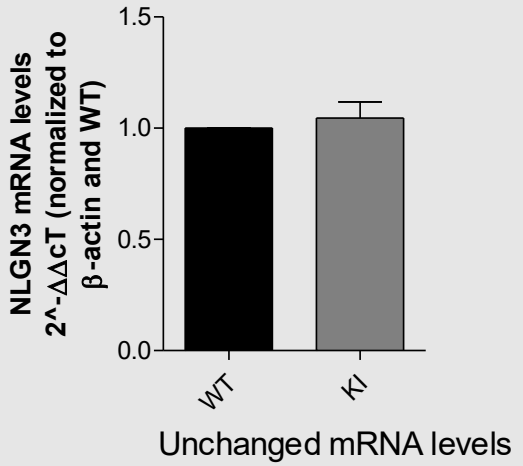
A small protein fraction reaches the cell surface to incorporate into synapses.



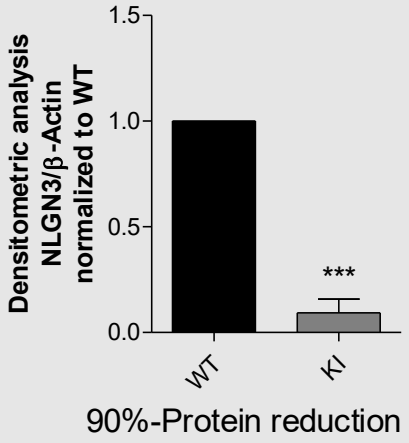

*Comoletti et al., 2004, De Jaco et al., 2010*

# Neuroligin3 R451C *Knock In* mouse model of autism




Unchanged mRNA levels




90%-Protein reduction

Behavioural and electrophysiological alterations of R451C Neuroligin3 KI mouse:

- 90% decrease in Neuroligin3 protein levels (adult)
- Autism-like behaviors (impaired sociality, enhanced spatial learning)
- Imbalance excitatory/inhibitory neurotransmission (hippocampus, cerebral cortex)
- "Gain of function" phenotype compared to Neuroligin3 *Knock Out* mice

Molecular mechanisms by which NLGN3 R451C may give rise to ASDs are not completely known

*Tabuchi et al., 2007; Etherton et al., 2011; Pizzarelli and Cherubini, 2013; Favaloro et al. In preparation*

**Table 1 Mouse models of ASD**

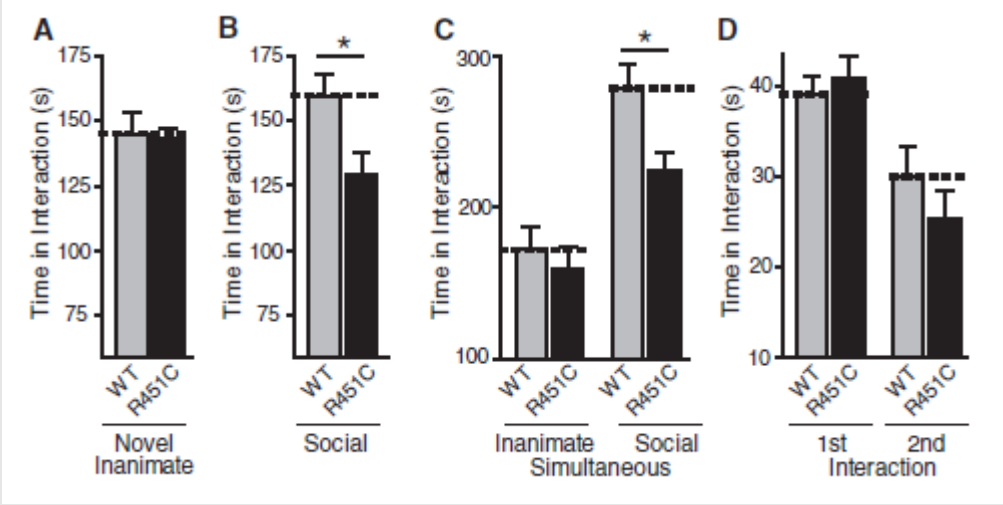
| Molecular function | Mouse model | Social interaction | Social communication | Repetitive behavior | Other symptoms | Molecular, cellular and circuit phenotypes | Treatment |
|---|---|---|---|---|---|---|---|
| Multiple | dup 15q11-q13 (refs. 267,268) | Impaired | ↓Calls | Behavioral inflexibility | NA | Altered serotonergic signaling, ↑spine dynamics | NA |
| Transcriptional regulator | *Tbr1* HT | Impaired | Impaired STFP | Behavioral inflexibility | CTA defects, learning deficits | Axonal projection defects in amygdala<br>↓NMDAR function | DCS (adult)[170]<br>clioquinol (A)[269] |
| Translational regulator | *Fmr1* KO[255] | Impaired | ↓Calls | Hand flapping | **PPI of startle, audiogenic seizure, learning deficits** | **↑mGluR function, immature protrusion** | MPEP (A)[233,237] |
| | | | | | **Learning impairment** | PI3K signaling, **↑spine density, impaired AMPAR-mediated synaptic plasticity** | 5-HT and DA compound (A)[270,271] |
| | | | | | **Audiogenic seizure** | Hypersensitivity to ERK1/2 pathway activation, **↑protein synthesis** | SL327 (A)[272] |
| | | | **↓Calls** | | NA | **↑Fetal or early postnatal GABA and $Cl^-$, abnormal EEG** | Bumetanide, oxytocin (P13-15)[251] |
| | *Tsc1* HT, *Tsc1*Cb KO | **Impaired** | ↑Calls | Grooming, **behavioral inflexibility** | Ataxia | **Cerebellar deficits** | Rapamycin (P7)[183] |
| | *Tsc2* HT | **Impaired** | ↑Calls | Increased marble burying | **Lethality, learning deficits** | **Brain enlargement**, hyperactive mTOR signaling, autophagy deficiency | Rapamycin (A)[144,240] |
| | *Pten* cKO | **Impaired** | NA | NA | **Learning deficits, seizure, anxiety** | **Macrocephaly, cellular hypertrophy**, PI3K pathway hyperactivation | Rapamycin (4–6 weeks)[114,184] |
| Neuron-glia interaction, $K^+$ channel clustering | *Cntnap2* KO | *Impaired* | ↓Calls | **Grooming** | Seizure, **hyperactivity** | ↓Interneuron number, abnormal neuronal migration | Risperidone (A)[109] |
| | | **Impaired** | | *Grooming* | *Hyperactivity* | **↓Oxytocin neurons** | Oxytocin (P7–P21; A)[249] |
| $Na^+$ channel | *Scn1a* KO | **Impaired** | NA | Grooming | Seizure, **learning impairment** | **↓GABAergic interneuron firing** | Clonazepam (A)[239] |
| Synaptic adhesion molecule | *Nrxn1a* KO[273,274] | Impaired, aggression in males | NA | Grooming | Anxiety, sensory-gating deficits, motor learning | ↓Glutamatergic trans. and synaptic density | NA |
| | *Nlgn3* R451C KI[275,276] | Impaired | ↑Calls | NA | Enhanced learning | Context-dependent impaired glutamatergic and GABAergic trans | NA |
| | *Nlgn3* KO[277] | Impaired | ↓Calls | Normal behavioral flexibility, **Stereotyped motor routine** | Hyperactivity | ↓Brain volume, cerebellar deficit | NA |
| | | | | | | ↓GABAergic trans. in D1-MSN in NAc | NLGN3 expression in D1-MSN[211] |
| | *Nlgn4* KO[278] | ↓Impaired, aggression | ↓Calls | Normal | NA | ↓Brain volume | NA |
| Synaptic scaffolding molecule | *Shank2* exon7 KO[279] | Impaired | ↓Calls, pattern change | Grooming | Hyperactivity, anxiety | ↑NMDAR function, ↑LTP | NA |
| | *Shank2* exons 6–7 KO | **Impaired** | ↓Calls | *Jumping* | *Hyperactivity, anxiety* | **↓NMDAR function, ↓LTP and LTD** | CDPPB, DCS (A)[232]<br>clioquinol (A)[269] |
| | *Shank3B* KO[210] | Impaired | NA | Grooming | Anxiety | Striatal dysfunction | NA |
| | *Shank3* exons 4–9 KO[280] | Impaired | Pattern change | Grooming | Learning deficits | ↓Activity-dependent AMPAR distribution and LTP | NA |
| | *Shank3* HT[281] | Impaired | ↓Calls | NA | **Motor coordination** | **↓Glutamatergic trans. by presynaptic mechanism**, ↓LTP | IGF1 (P13–P28)[177] |
| | *Shank3*$^{+/\Delta C}$ (ref. 282) | **Impaired** | NA | **Grooming** | NA | **↓NMDAR function, Rac1, PAK, cofilin signaling defects,** F-actin dysregulation in PFC | TAT–p-cofilin peptide (A) |
| | | **Impaired** | | Grooming | | | CA-Rac1 (A) |

# Neuroligin 3 R451C Knock In mice

**Animal model to study a monogenic form of ASD**

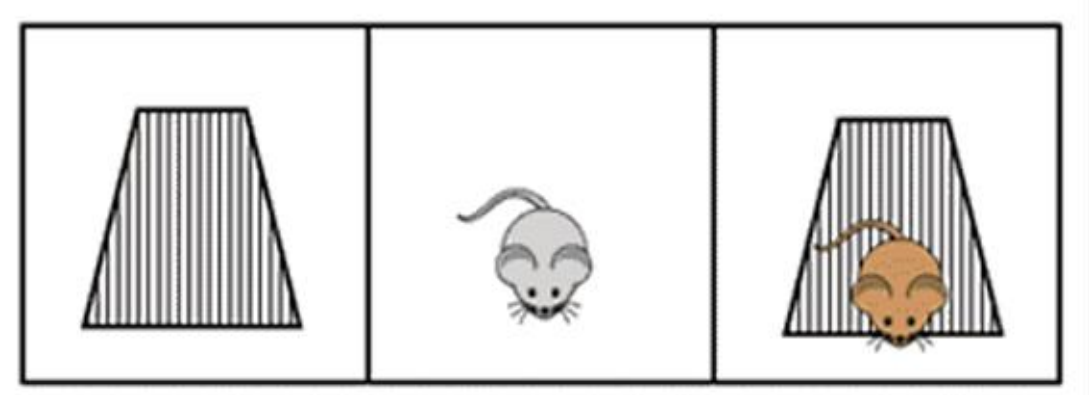
- Decrease of Neuroligin3 protein levels
- Autism-like behaviors (Impared social interactions)
- Imbalance of excitatory - inhibitory neurotransmission
- Gain of function phenotype compared to KO



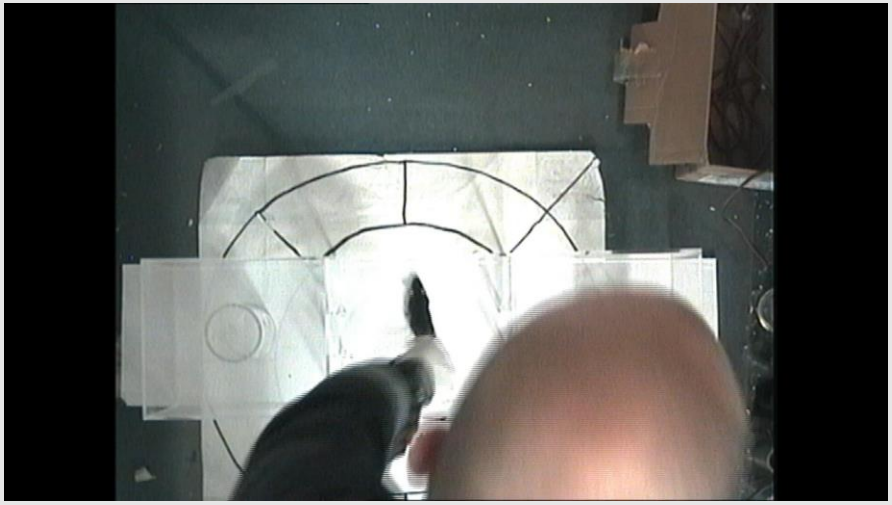

Tabuchi et al., 2007

# Il test delle TRE CAMERE per studiare il comportamento sociale



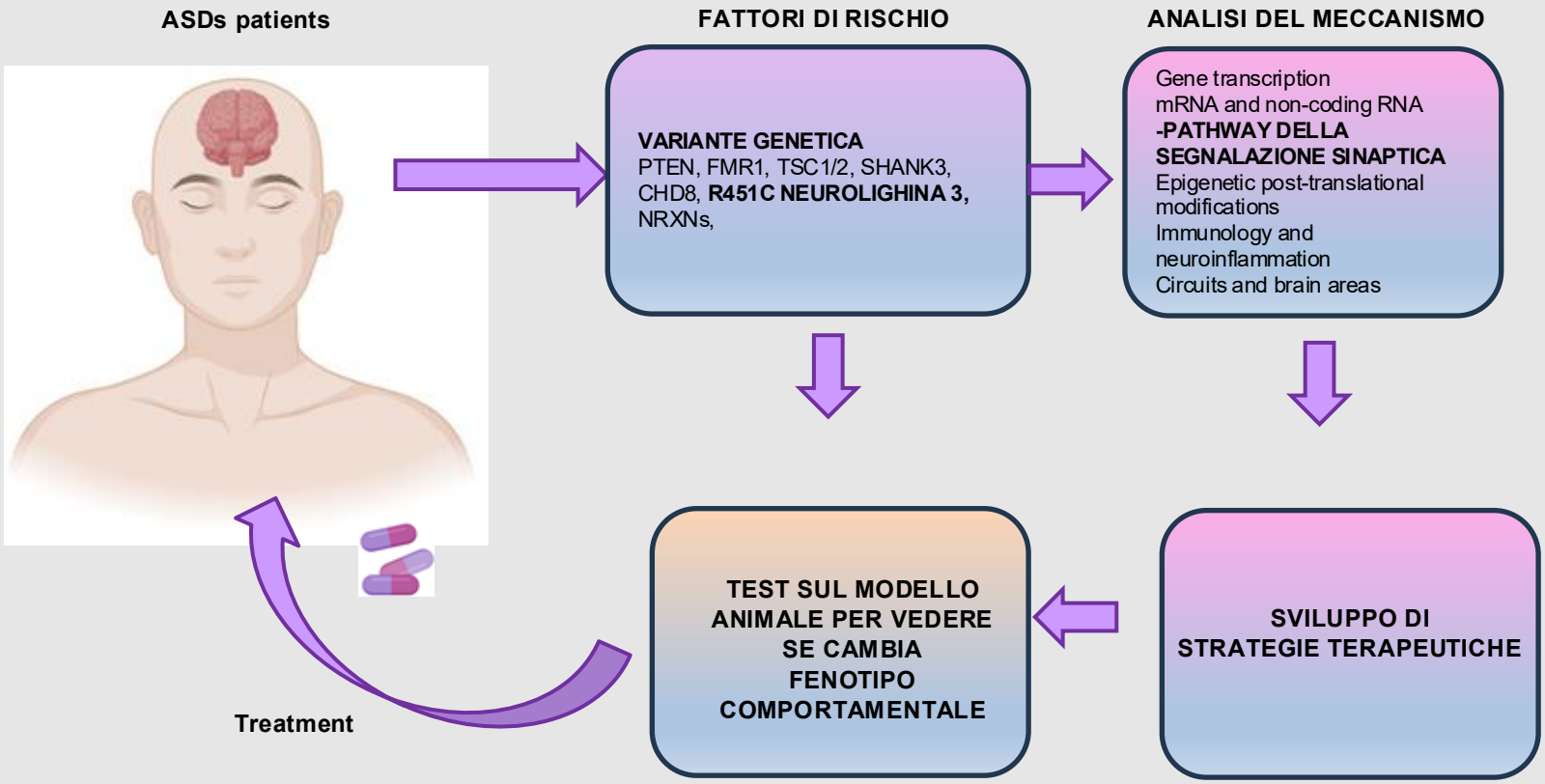
# Cosa fa di diverso il topolino autistico?

mostrare video

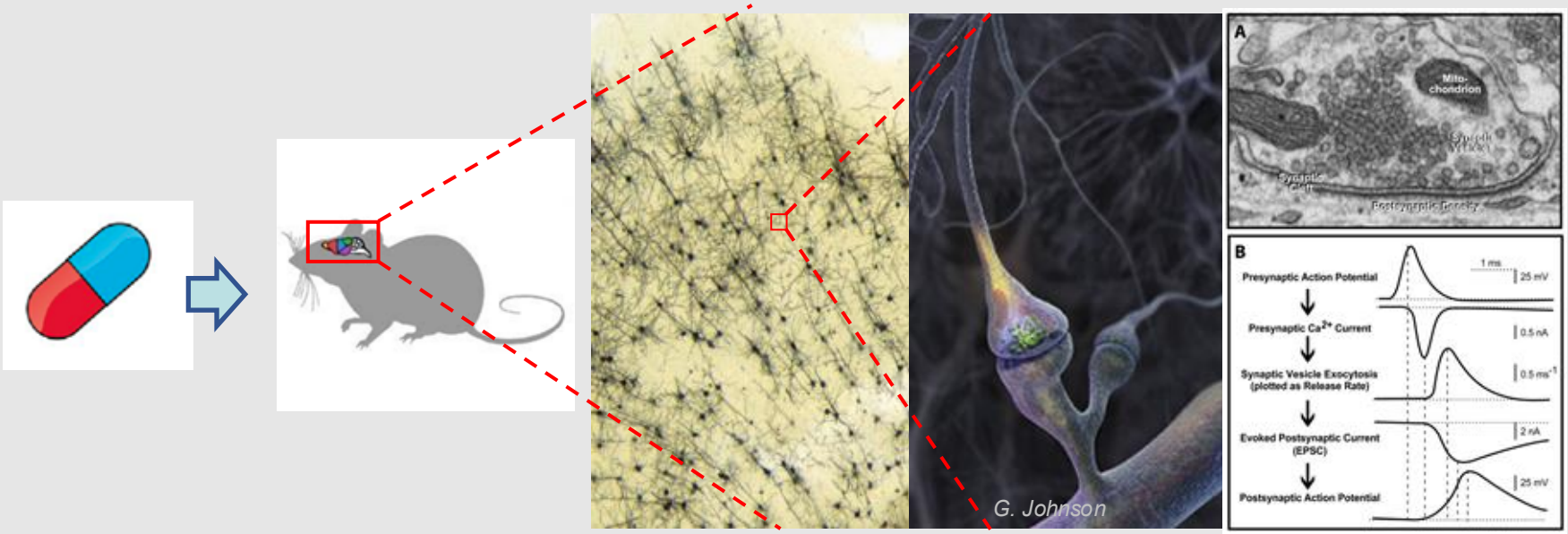


L548#0

# Dalle mutazioni nei geni alle terapie

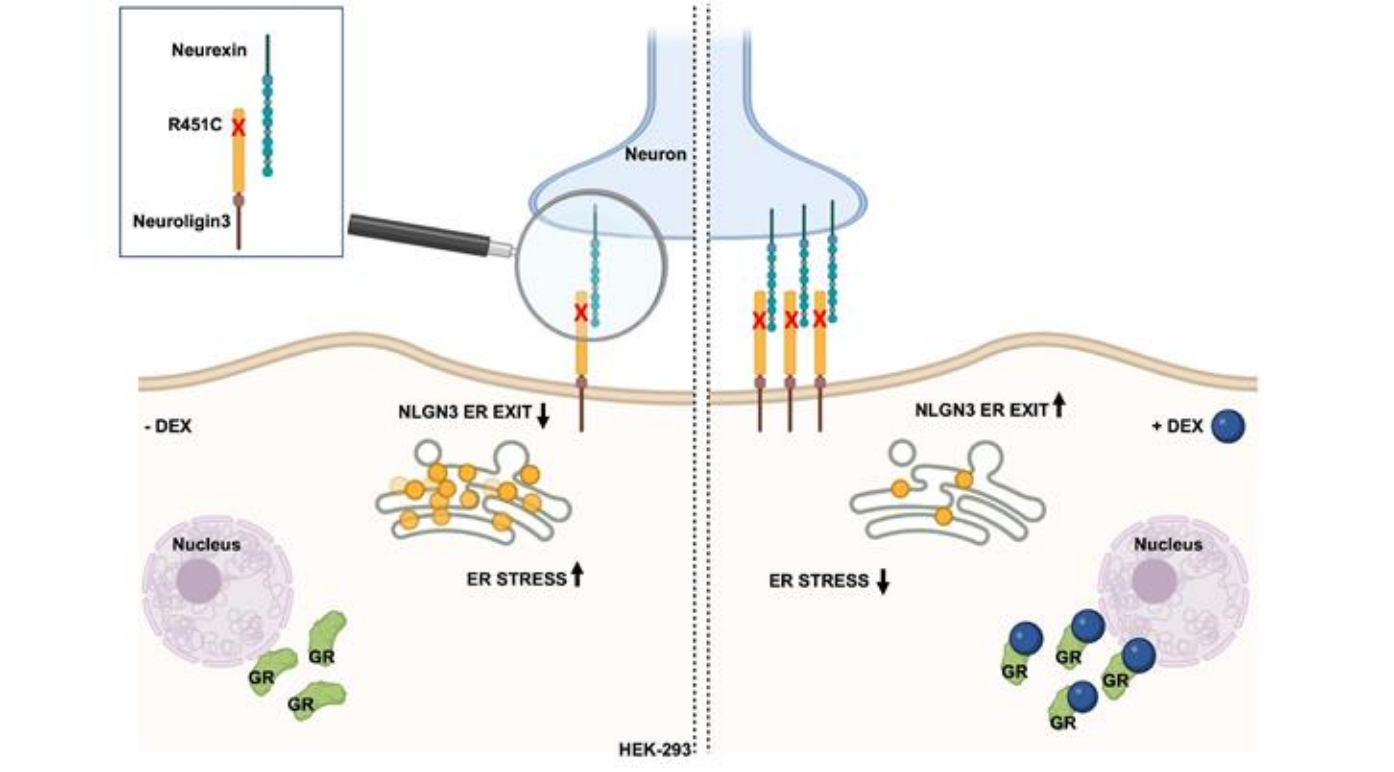


# Il topo ci permette anche di studiare come funziona la comunicazione tra neuroni, la neurotrasmissione




(Adapted from Südhof TC., 2008 Nature)

Trattamenti in vitro: per il recupero della proteina sulla superficie cellulare




Trattamenti in vivo:
-comportamento sociale
-neurotrasmissione

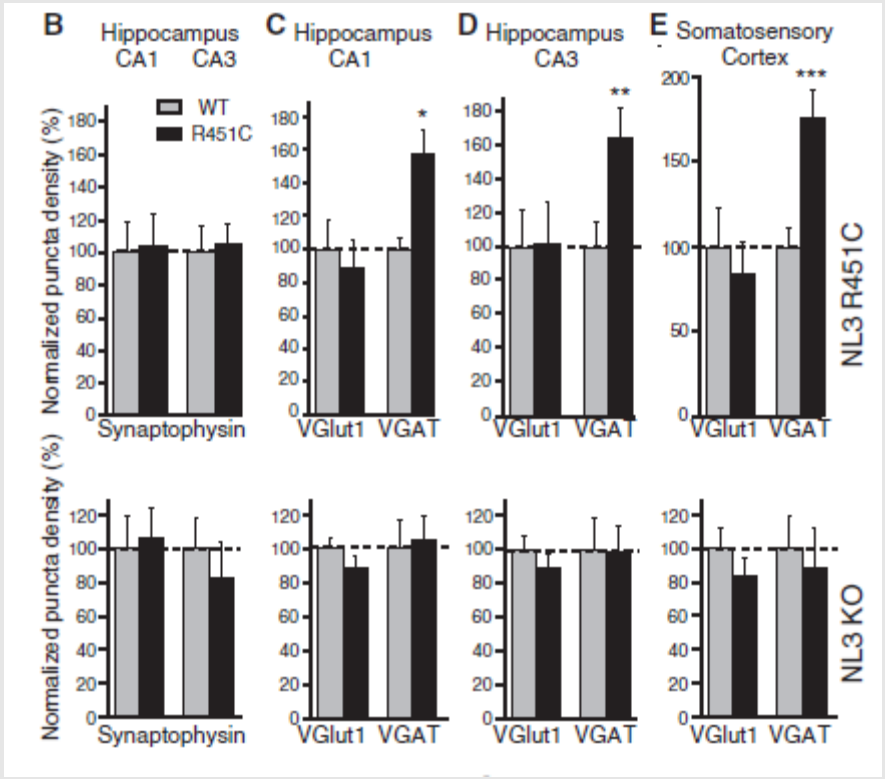


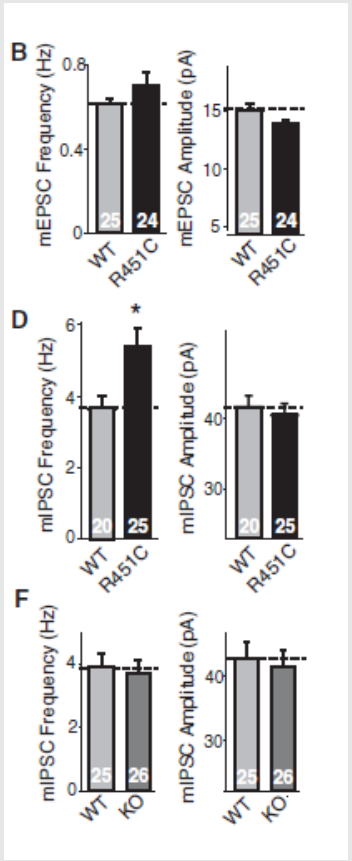
# Neuroligin 3 R451C Knock In mice

## Animal model to study a monogenic form of ASD

- Decrease of Neuroligin3 protein levels
- Autism-like behaviors
- Imbalance of excitatory - inhibitory neurotransmission
- Gain of function phenotype compared to KO



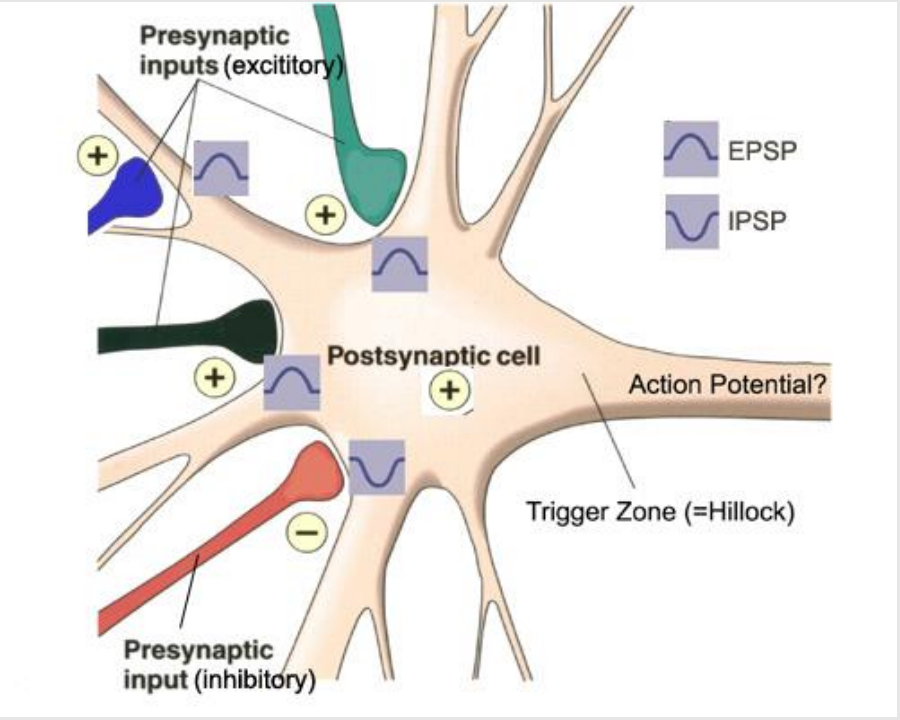



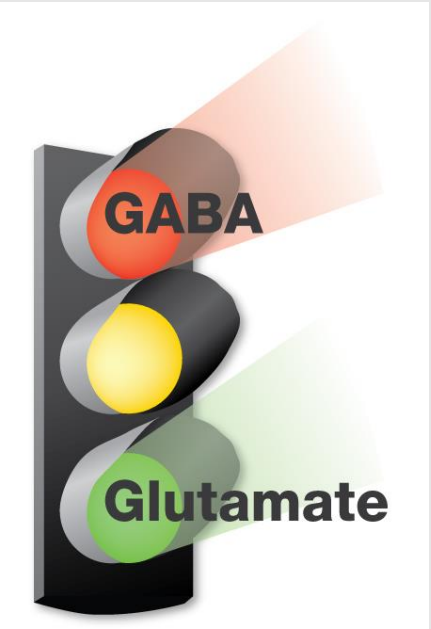

Vesicular GABA transporter **VGAT**

Vesicular glutamate transporter 1 **VGLUT** 1,

Tabuchi et al., 2007
Etherton et al., 2011

# The excitatory/Inhibitory balance is altered in ASDs



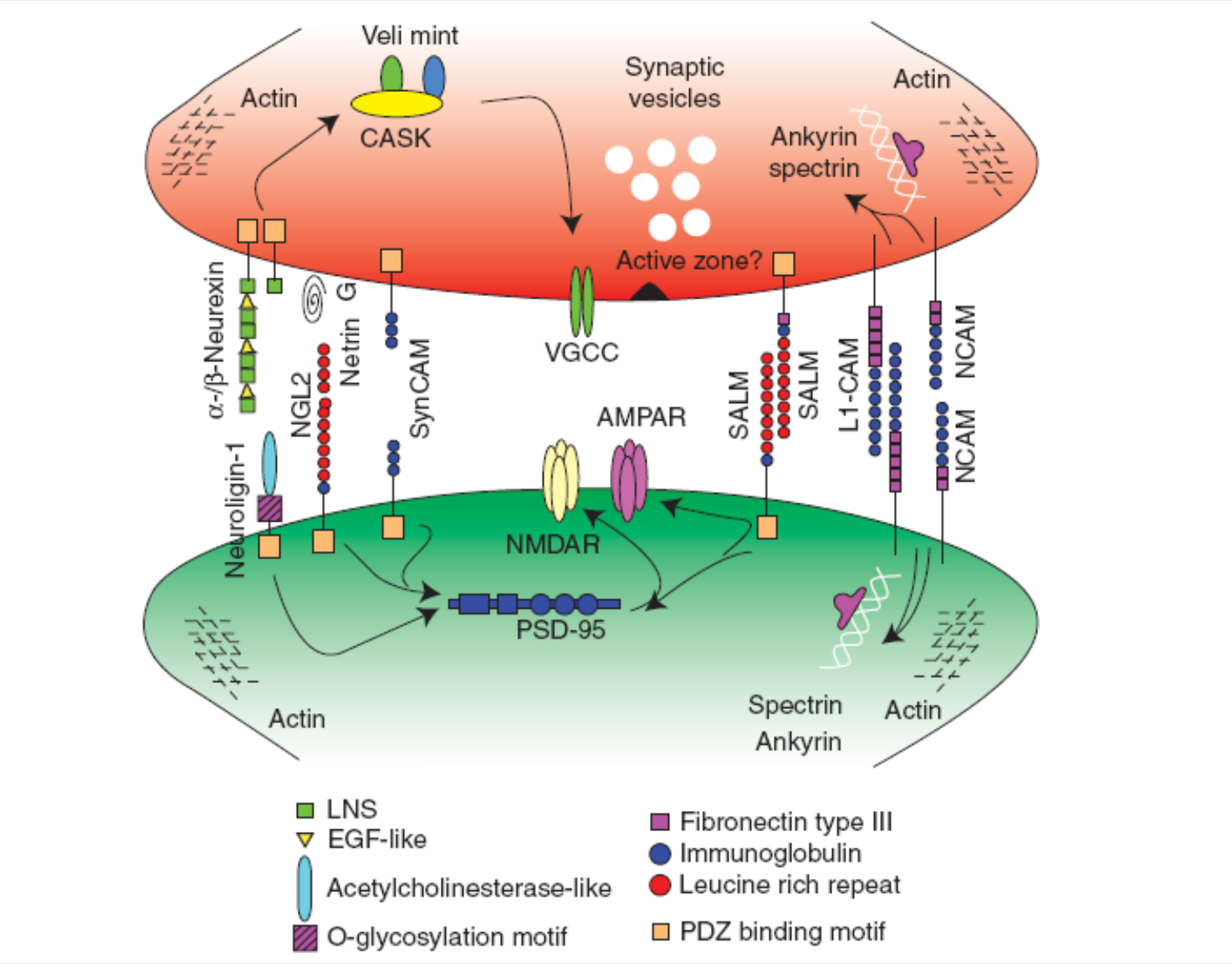




Neuronal excitability relies on the summation of excitatory and inhibitory signals a process regulated by the number of excitatory vs inhibitory (E/I) contacts received by a single neuron.

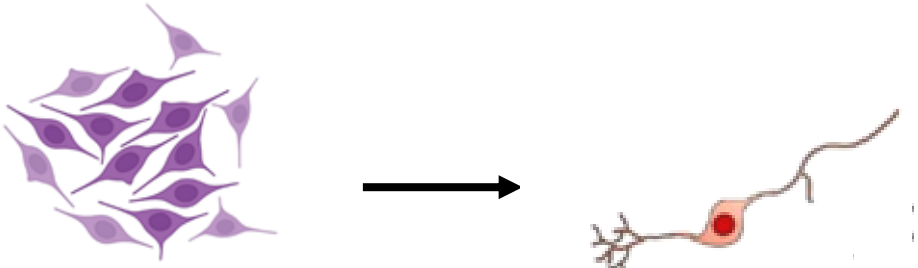
Impaired glutamatergic and GABAergic transmission, as has been reported in several mouse models of ASD, can result in ASD-like behaviors that can be alleviated by modulators of AMPA receptor (AMPAR), NMDA receptor (NMDAR) and GABAAR.

# IN UNA SINAPSI ECCITATORIA MOLECOLE DI ADESIONE DIVERSE COESISTONO E PARTECIPANO ALLA FORMAZIONE DI COMPLESSI SINAPTICI CHE MANTENGONO LA SINAPSI ALTAMENTE ORGANIZZATA

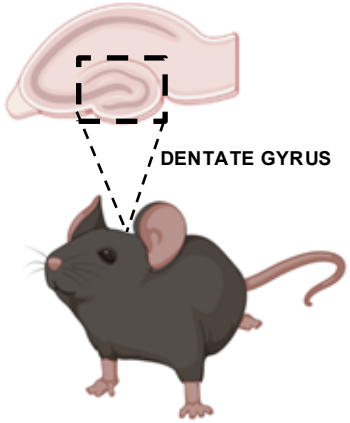


# Hippocampal Neural Stem Cells from R451C Neuroligin3 mice

**Endogenous** expression of R451C Neuroligin3



**Adult neural progenitors cell**




**KI mouse R451C Neuroligin3:**
-Highly reduced levels of Neuroligin3
-Deficits in social behavior
-Altered neurotransmission



LAB CACCI

*Diamanti et al., 2023*