Lectio Gioenia

Catania, 17 giugno 2025

Il Tetano:

una malattia paradigmatica fra neurologia e microbiologia

Cesare Montecucco

LAB: Neuroparalysis,
Neuroregeneration

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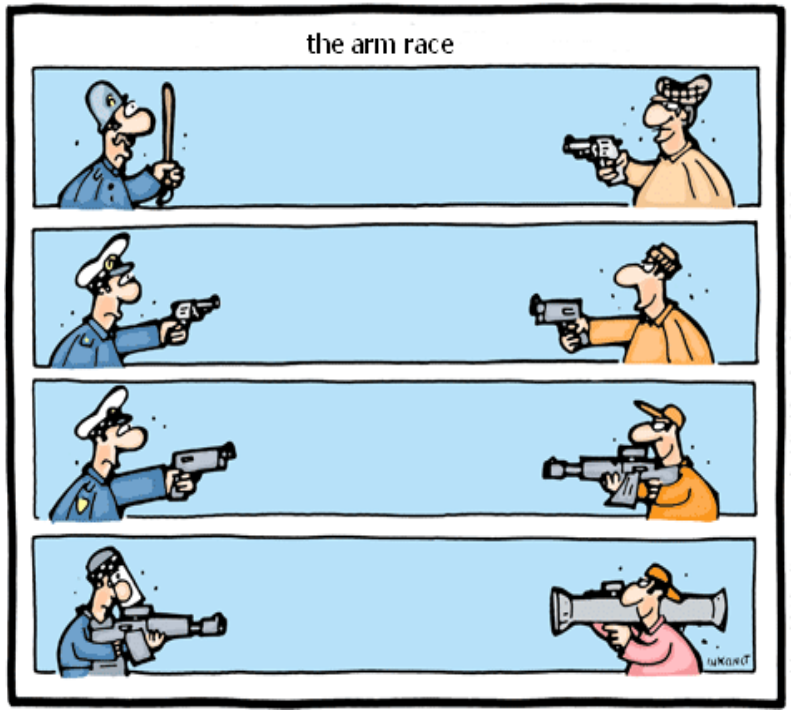
HOST-PATHOGEN INTERACTION IS A PRIVILEGED POINT OF OBSERVATION OF NATURE (1978)

- Virulence factors are the products of a process of **co-evolution** of pathogens with their hosts or preys.
- During evolution a pathogenic organism "**finds out**" how to subvert host physiology to its own advantage,
- Virulence factors are **"tailored"** around key physiological functions of the host.



By studying virulence factors (toxins) we can:

a) discover the molecular pathogenesis of the disease caused by the toxigenic organism;

b) learn more about specific physiological functions of the host;

c) develop novel therapeutics (vaccines, inhibitors etc.);

d) even use the virulence factor itself as a therapeutic;

Host-Pathogen Interaction in our lab over the years

Cholera toxin & Diphtheria Toxin (1979 – 1993)

Tetanus and Botulinum Neurotoxins (1984- present)

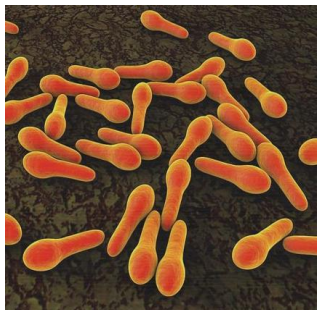
Virulence factors *Helicobacter pylori* (1990 – 2007)

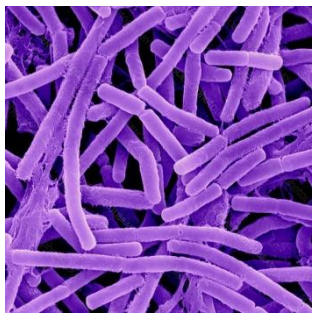
Anthrax and anthrax toxins (1994 – 2010)

Animal Presynaptic Neurotoxins (1998 - present)

since 2008

Peripheral Neurodegeneration and Regeneration



1. **La storia del tetano**
2. **Sieroterapia e vaccini**
3. **La patogenesi del tetano**
4. **Attualità e Sviluppi recenti**

The first partial description of tetanus goes back to 1500 b.c. (Pap. Ebers).

However, the first medical description of the disease and accurate definition of its cardinals symptoms was made by Hippocrates in Kos (Greece) (*the beginning of medical literature*)

Ippocrate

(460 – 377 bC)

τεταηοσ :

Trismus
Risus sardonicus
Opisthotonos

The spastic paralysis of tetanus

Trismus

Risus sardonicus

Opisthotonos

General muscle contractures

Spasms

The patient is conscious but cannot move as skeletal muscles are contracted and work one against the other, causing exaustion.
This paralysis leads to death by respiratory deficit or heart collapse.

Tetanus neonatorum: N x 10^5 neonates die per year because they are born from non vaccinated mothers and the umbilical cord is cut under non sterile conditions

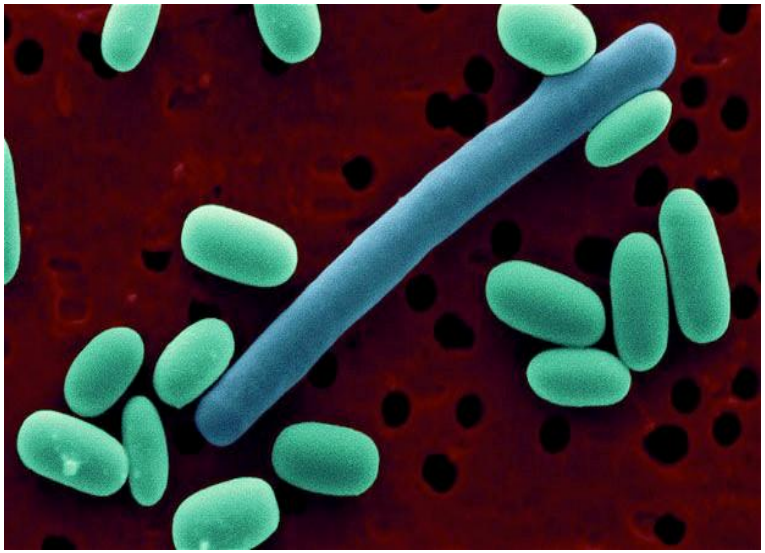
For 24 centuries Tetanus has been considered the prototypic disease of the Nervous system

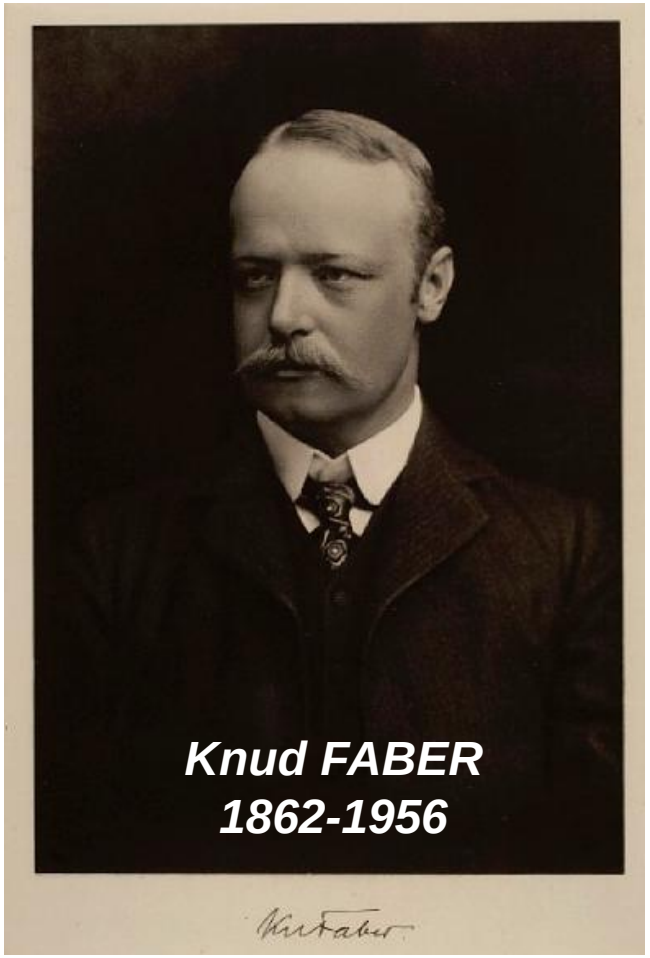
- 1884, **Carle & Rattone** in Italy demonstrated that tetanus had a **microbiological** origin

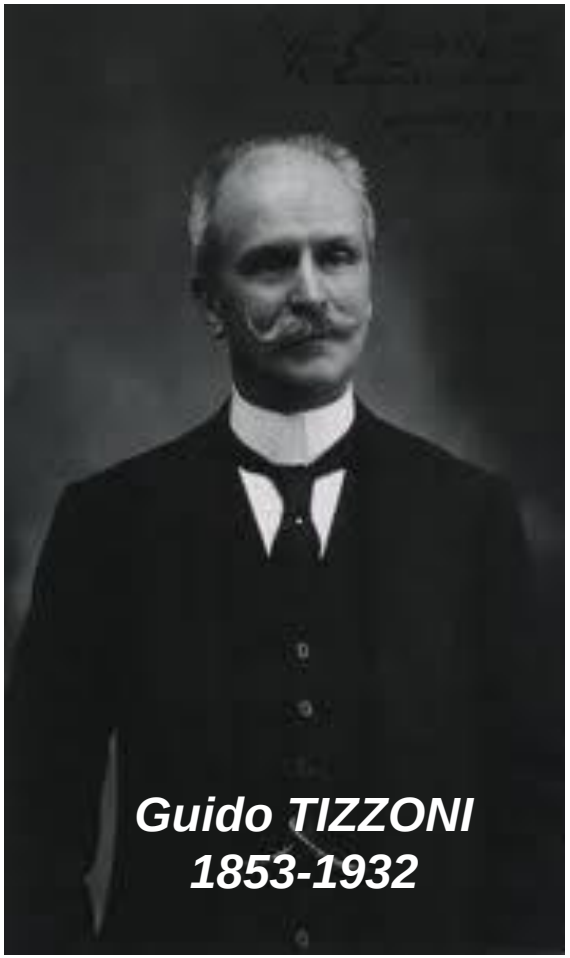
- This **change of paradigm** entrained a series of discoveries:

- 1884, Nicolaier in Gottingen isolated from the ground the bacterium causing Tetanus.

- 1888, Kitasato in Berlin cultivated this bacterium and showed it to be anaerobe and sporogenic. Spores Were found to be ubiquitous.







1889: il tetano è causato da una proteina: la TOSSINA TETANICA

Con lo stesso approccio nel 1895 fu scoperto che la causa del Botulismo è la neurotossina botulinica, una proteina molto simile

Non ci sono dati attendibili sul numero di morti di Tetano nella storia dell'uomo fino alla scoperta del vaccino anti- tetano

Una stima conservativa è che nel corso dei secoli siano morti tetano dell'adulto o *tetanus neonatorum* decine e decine di milioni

La scoperta della proteina tossina tetanica è stata di importanza fondamentale perchè ha dato l'avvio alle ricerche volte a prevenire/trattare il tetano che si sono concretizzate **subito (nel 1890) con la scoperta della** sieroterapia premiata dal primo Premio Nobel per la Medicina nel 1901

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4. **Attualità e Sviluppi recenti**

E. von Behring

S. Kitasato

Dopo iniezioni di tossina tetanica o difterica trattate con iodoformio, gli animali diventavano insensibili alla tossina stessa, diventavano immuni.
Questa immunità è trasmissibile col siero

SIEROTERAPIA (1890)

Prima Guerra Mondiale: 1914-1918: il siero antitetano derivato da cavalli salva molte vite, ma può causare reazioni auto-immmunitarie

Gaston RAMON
(1886-1963)

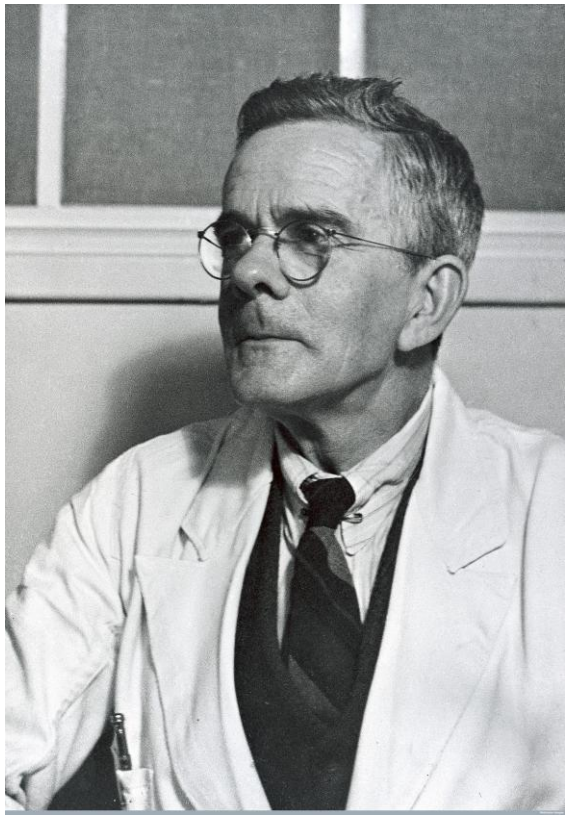
1. Formalina preserva DT e TeNT (1920)

2. La formaldeide inattiva queste tossine, ma ne preserva la capacità d'indurre uno stato d'immunità: **TOSSOIDI sono dei VACCINI** (1924)

Alexander GLENNY (1882-1955).

Scopritore degli **adiuvanti** in forma di composti di Alluminio, principalmente Al_2O_3.

Gli attuali vaccini antitetano ed antidifterite sono composti da:



Tossoidi, adiuvante, sali di sodio, acqua distillata

Gli eccezionali risultati pubblicati da Ramon nel 1924, da Glenny non vengono ritenuti di sufficiente rilevanza dalla comunità scientifica ma convincono i medici degli eserciti, seppure con qualche differenza: totalmente l'Esercito italiano, l'Armee Française e la Wermacht. Col risultato che **nessun caso di tetano** si è verificato fra i vaccinati dei loro eserciti durante la seconda guerra mondiale.

Gli inglesi vaccinarono l'80 % delle forze e lasciarono il restante 20 % come controllo, col risultato che il primo gruppo non riportò casi di tetano ed il secondo si.

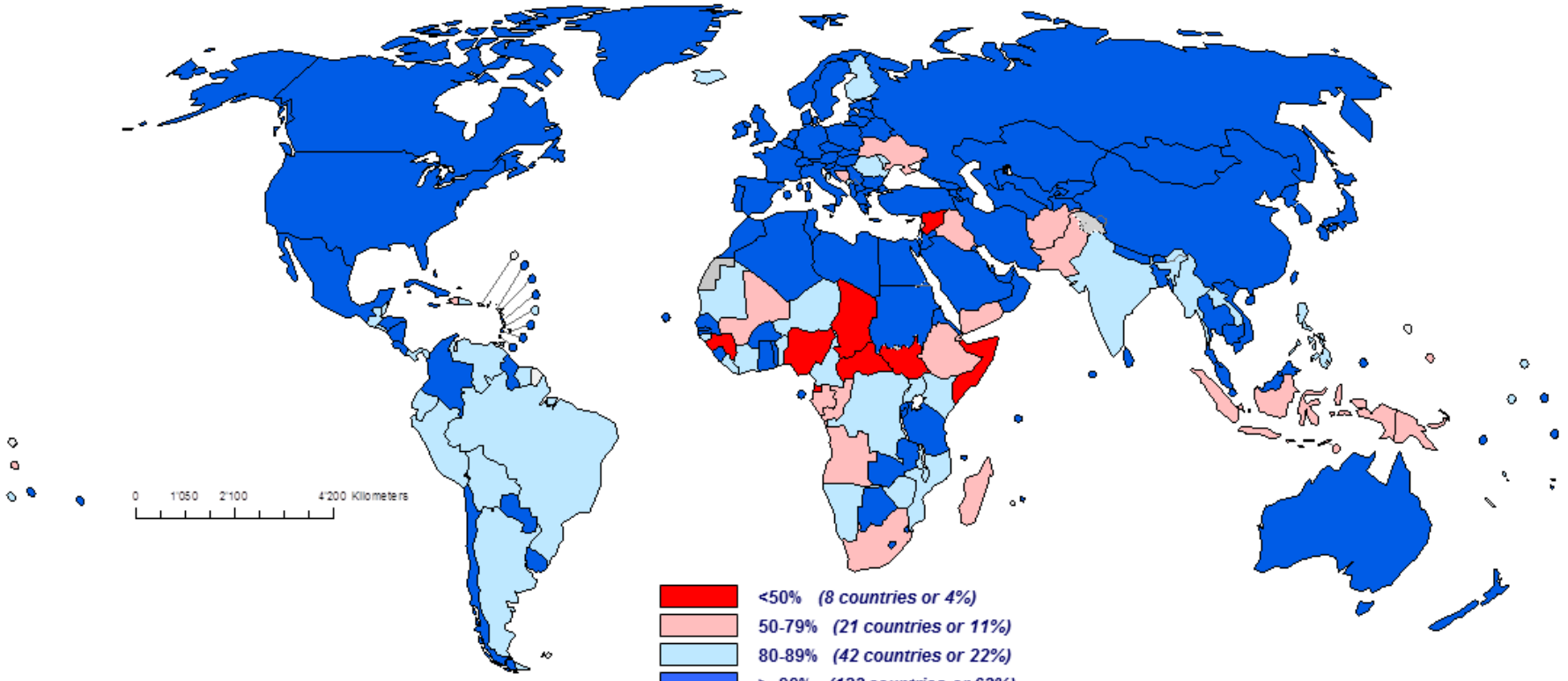
La US Army venne colta impreparata dall'inizio del conflitto, e lo si può capire dato che tutti i lavori importanti sul tetano erano stati fatti in Europa, ma poi a partire dal 1941 anche i soldati americani vennero vaccinati.

La seconda guerra mondiale è stato un esperimento con un campione sperimentale di elevata numerosità e risultati altamente significativi. Tanto significativi da convincere a rendere subito obbligatorie le vaccinazioni antitetano per i civili

Da allora questo vaccino è stato iniettato **in miliardi e miliardi di dosi senza alcun serio effetto secondario**

Purtroppo, la procedura di vaccinazione è complessa perché richiede iniezioni del vaccino ripetute e con scadenze ben definite, cosa che può fare solo un servizio sanitario efficiente

Immunization coverage with DTP3 vaccines in infants , 2017



Source: WHO/UNICEF coverage estimates 2017 revision, July 2018. Map production: Immunization Vaccines and Biologicals, (IVB). World Health Organization. 194 WHO Member States. Date of slide: 15 July 2018.

Le cellule immunitarie della memoria anti-tossina tetanica diminuiscono nel tempo, per cui, dopo i 40/50 anni, si rende necessaria la somministrazione di una dose di vaccino ogni 10 anni

Il tetano in Italia è quasi scomparso (100 casi/anno).
Non sono però scomparse le spore del tetano, che rimangono, come sempre, presenti in numero enorme nell'ambiente, specialmente nei terreni ricchi di deiezioni animali.
Al contempo aumentano i no-vax…..

E' facile predire che, all'aumentare del numero dei non vaccinati, prima o poi si verificheranno casi di tetano in bambini-adolescenti

1. **La storia del tetano**

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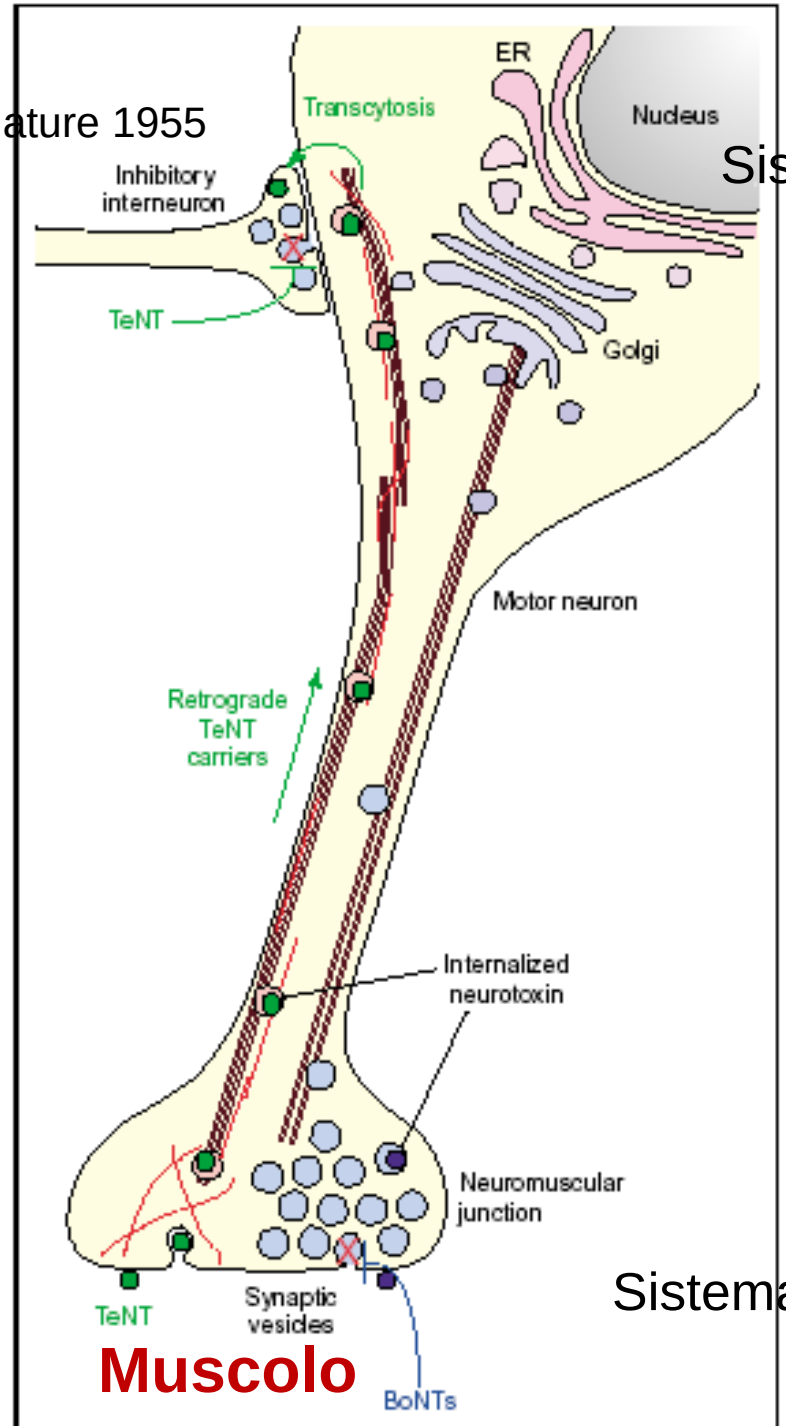
Il tetano si acquisisce in seguito alla **contaminazione di ferite** necrotiche, anche insignificanti, con **spore di *C. tetani***. Tali ferite possono essere le più varie: tagli, incluso quello del cordone ombelicale, contusioni con rottura della pelle, abrasioni, circoncisioni, tatuaggi, iniezioni con siringhe, etc.).

In assenza di ossigeno, dalle spore germinano **batteri** che producono **la tossina tetanica** che diffonde nell'organismo entrando nel midollo **spinale causando la paralisi spastica** caratteristica del tetano. Tra ferita e paralisi intercorre un tempo variabile da 1 a 5 settimane,

Alessandro BRUSCHETTINI (1868-1932) *Sulla diffusione del veleno del tetano nell'organismo* (1892): La Riforma Medica 3: 256-259

Curtis & Eccles, Nature 1955

Sistema Nervoso centrale

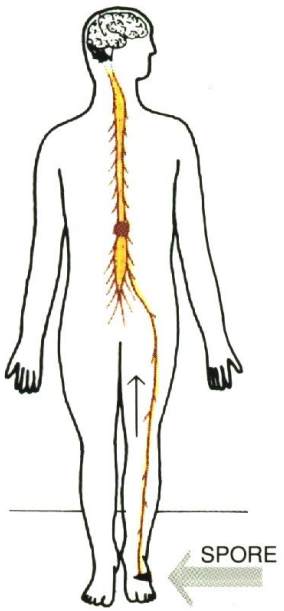


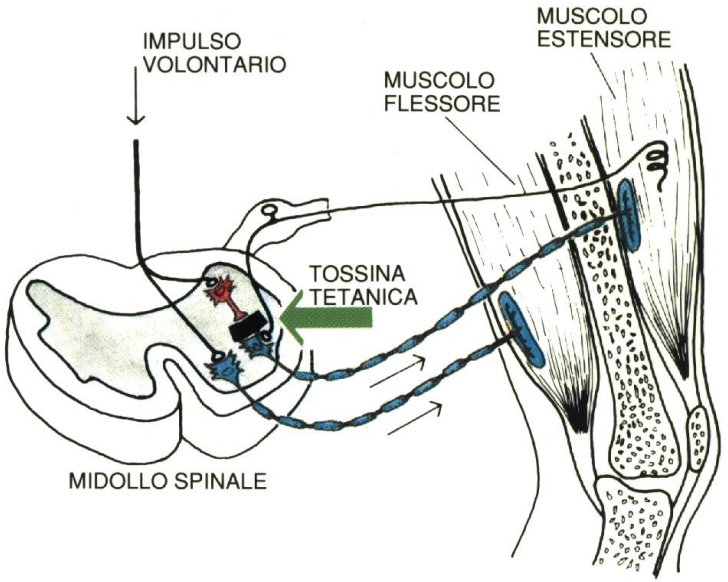
Wellhoner, 1974
Schwab & Thonen, 1977,
Schiavo, 1998-2008

Sistema Nervoso periferico

TETANUS

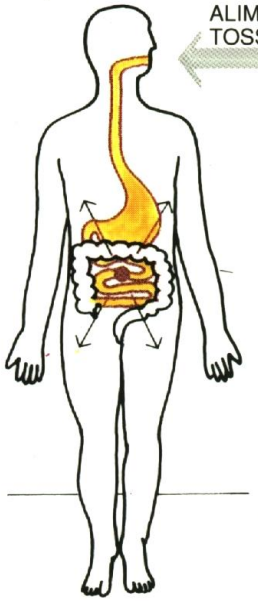
CNS
Spastic paralysis

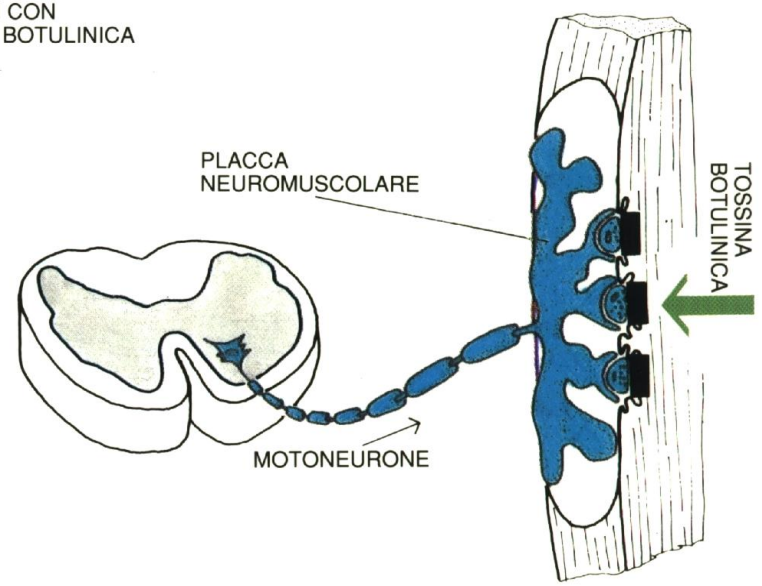


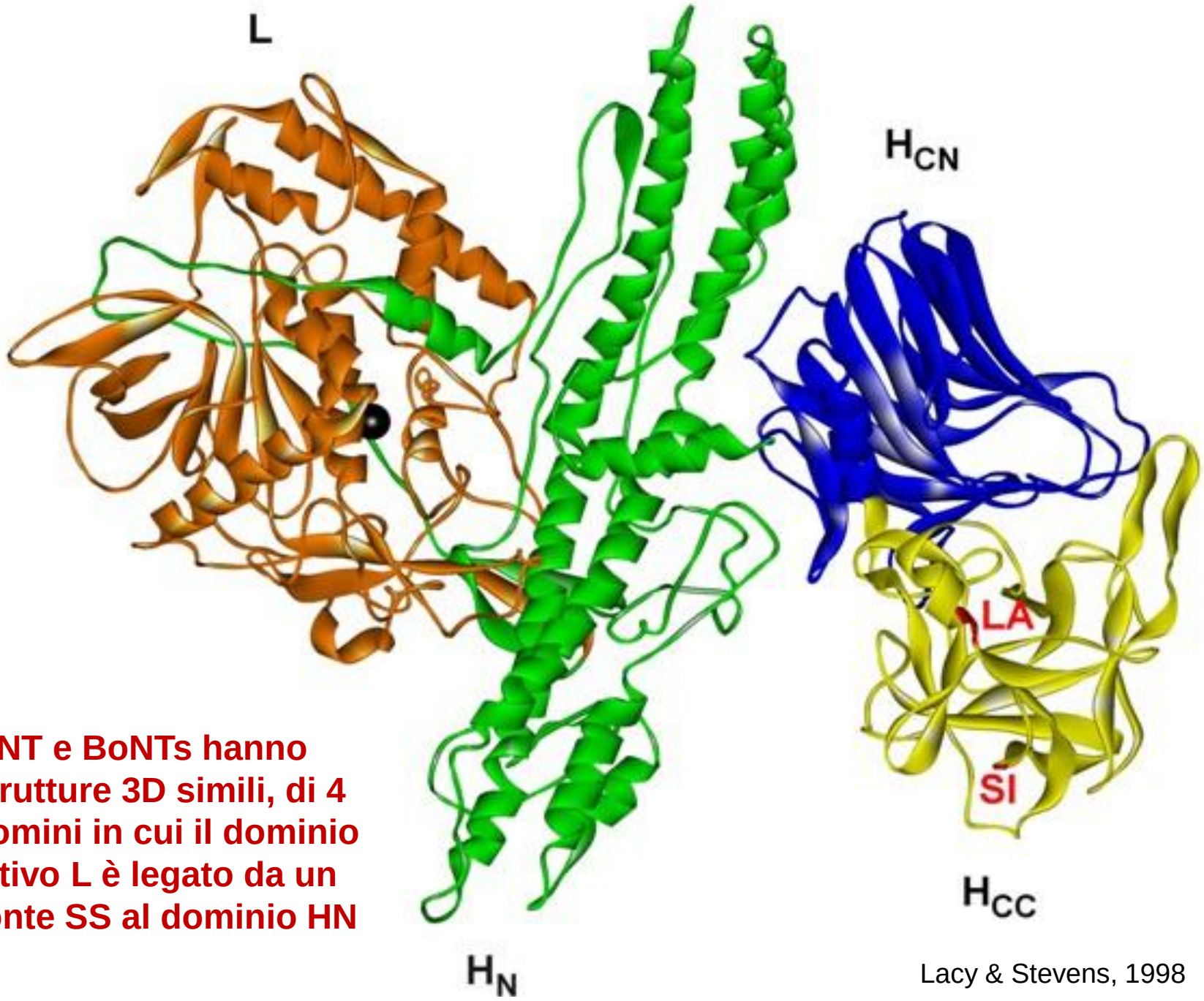


BOTULISM

PNS
Flaccid paralysis



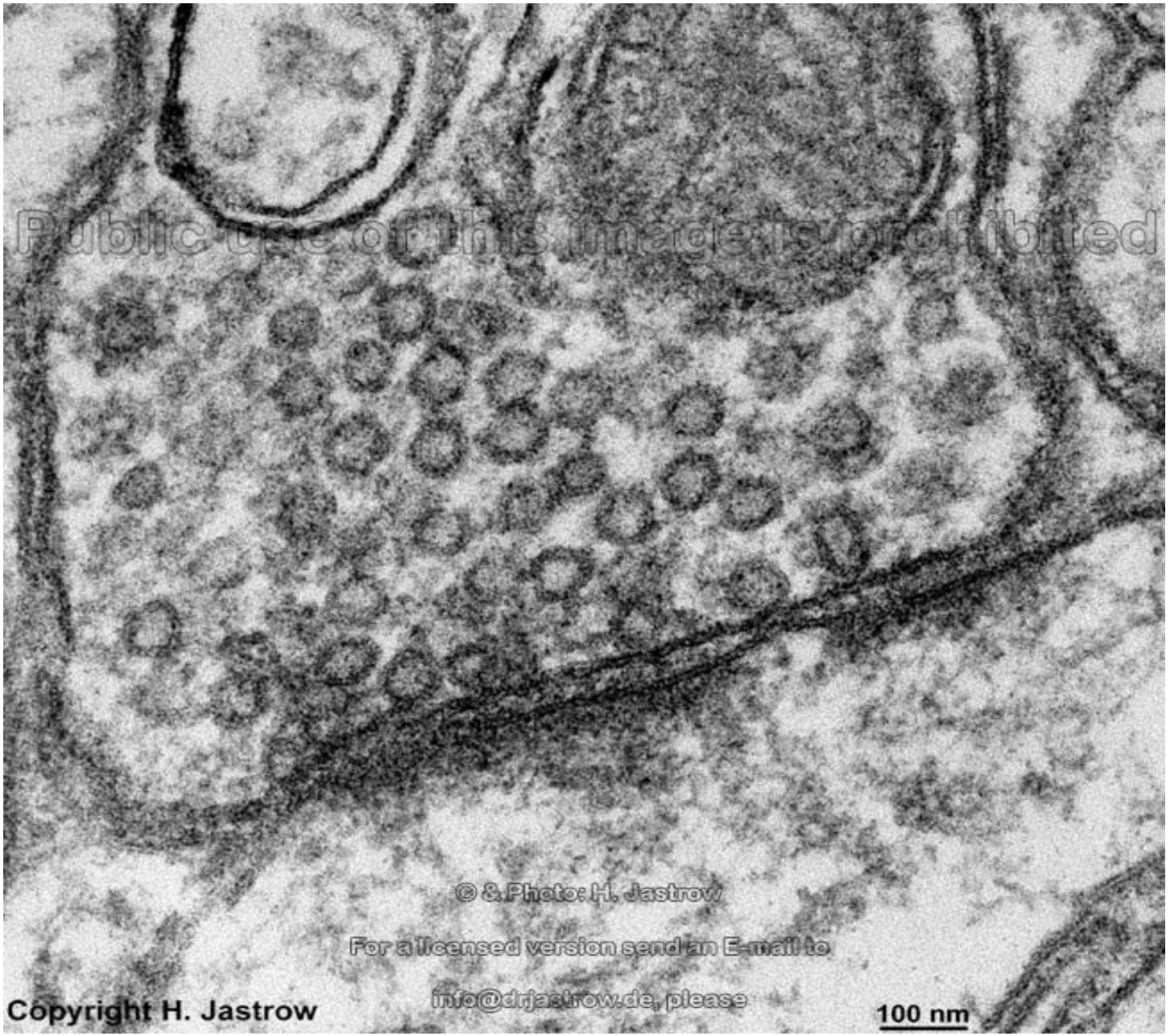




TeNT e BoNTs hanno strutture 3D simili, di 4 domini in cui il dominio attivo L è legato da un Ponte SS al dominio HN

Lacy & Stevens, 1998

Sinapsi CNS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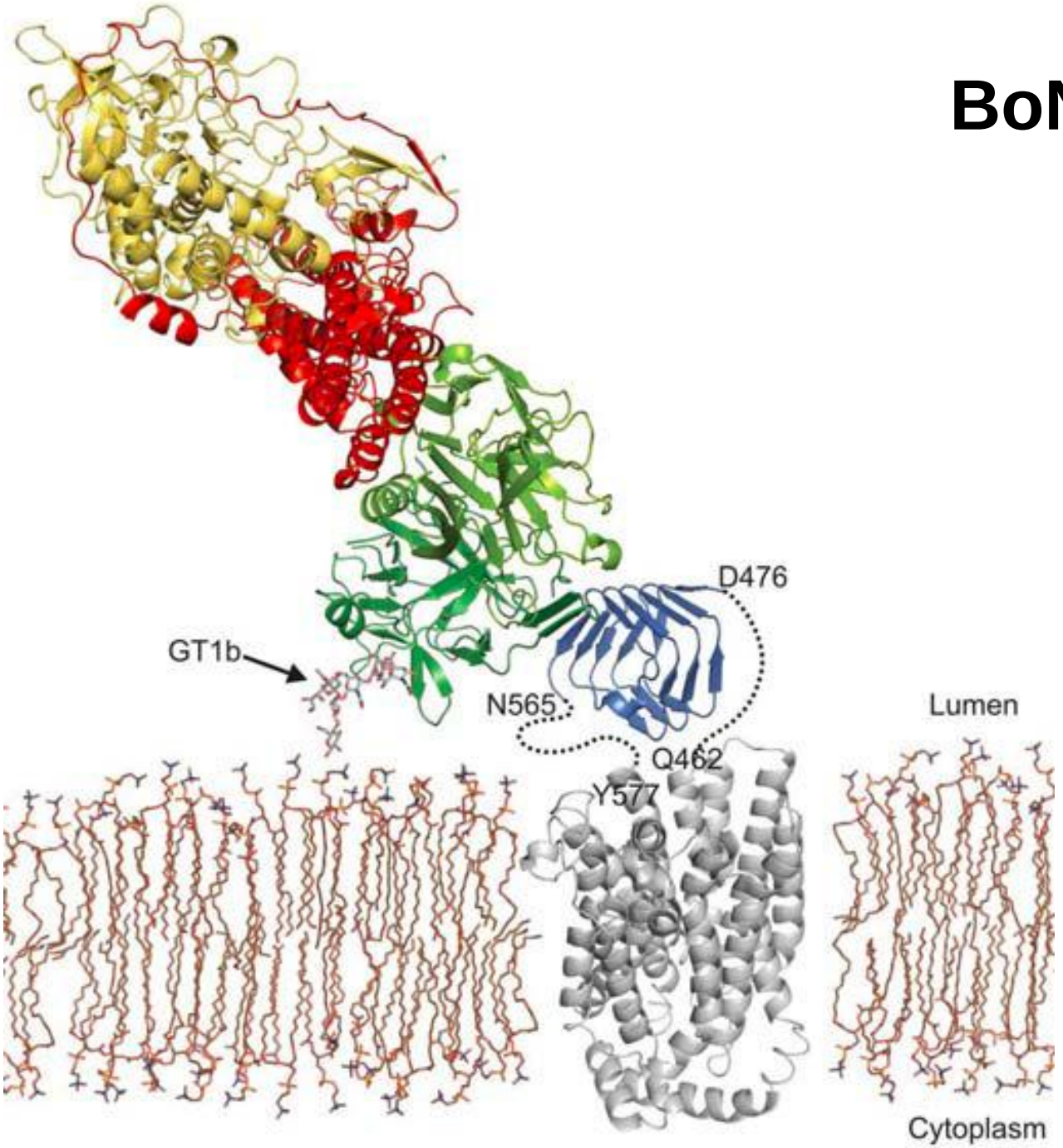
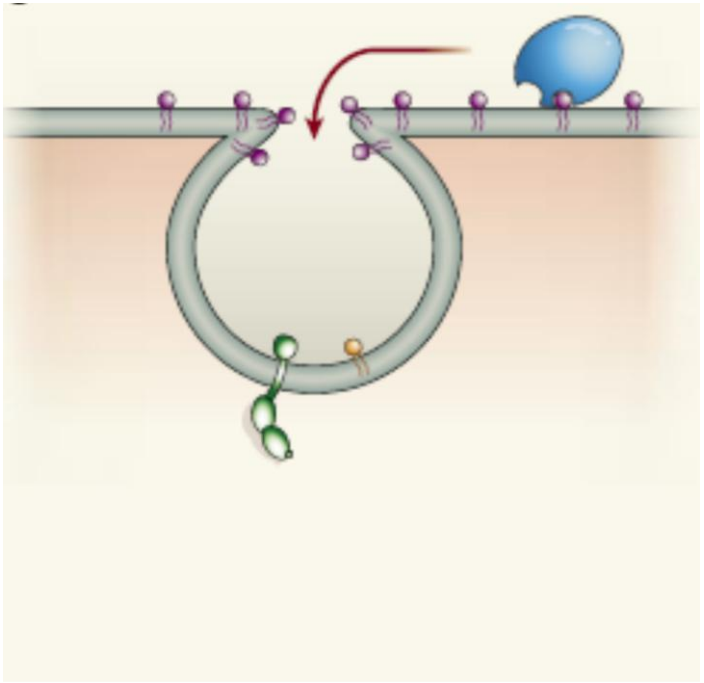
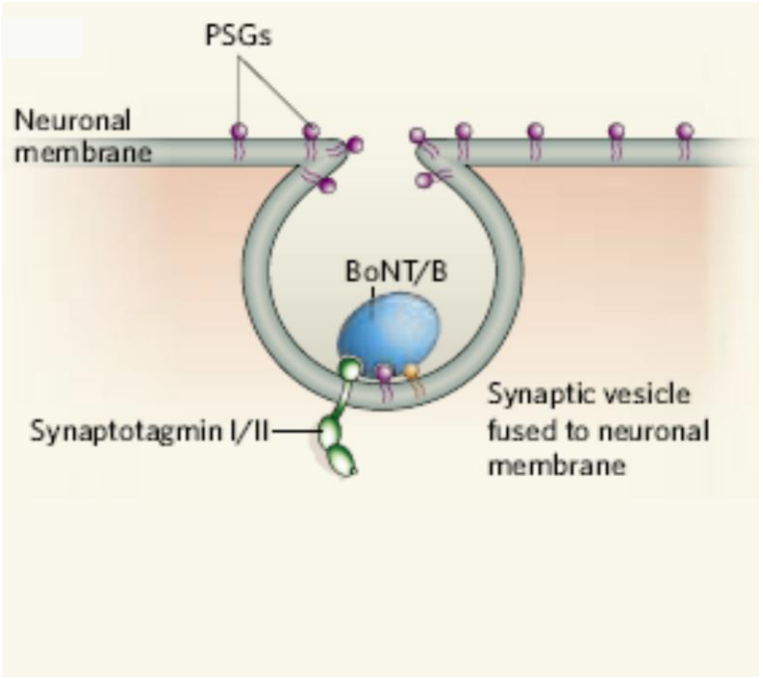
100 nm

BoNT/A1

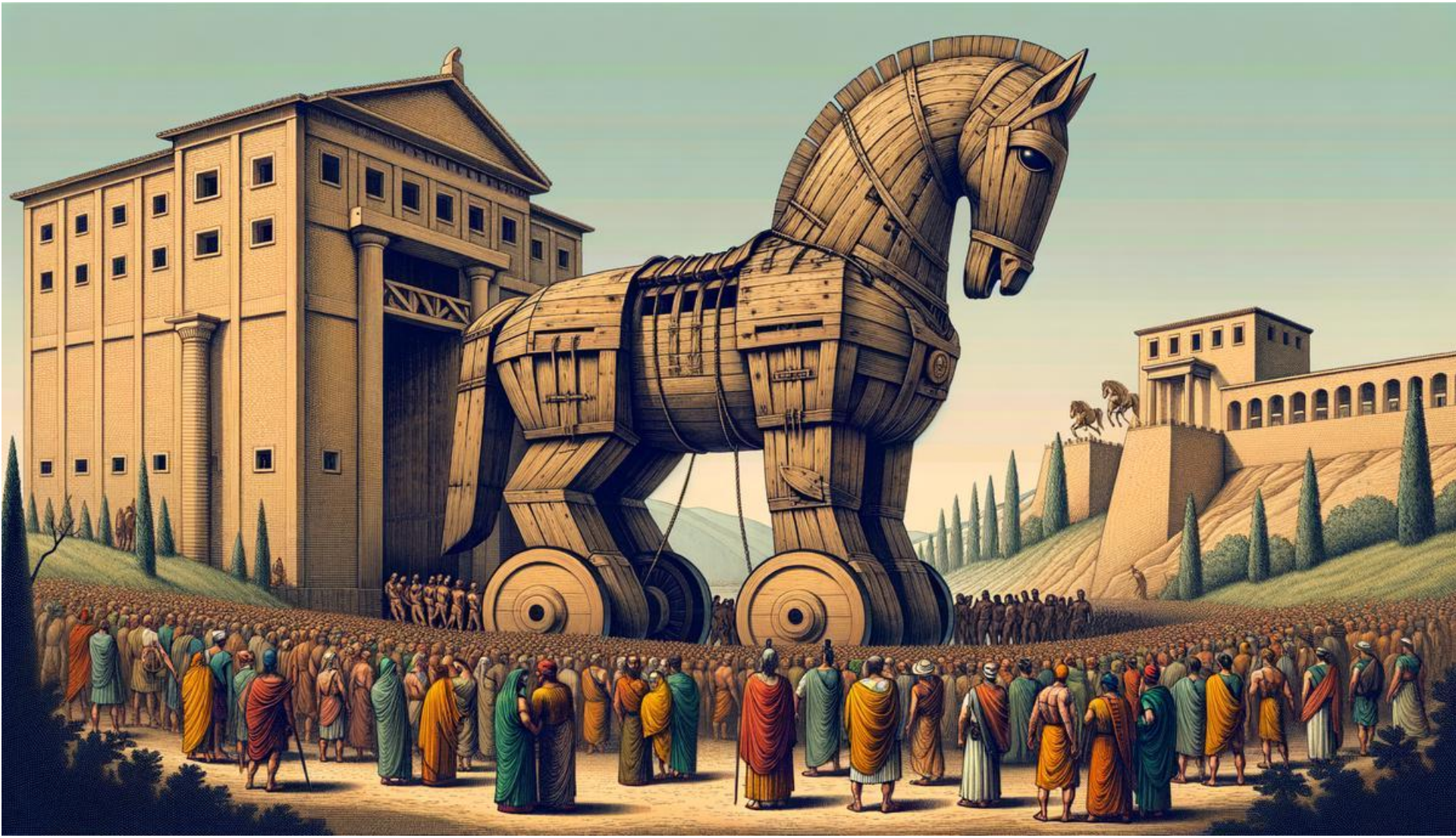


Benoit et al., Nature 2013

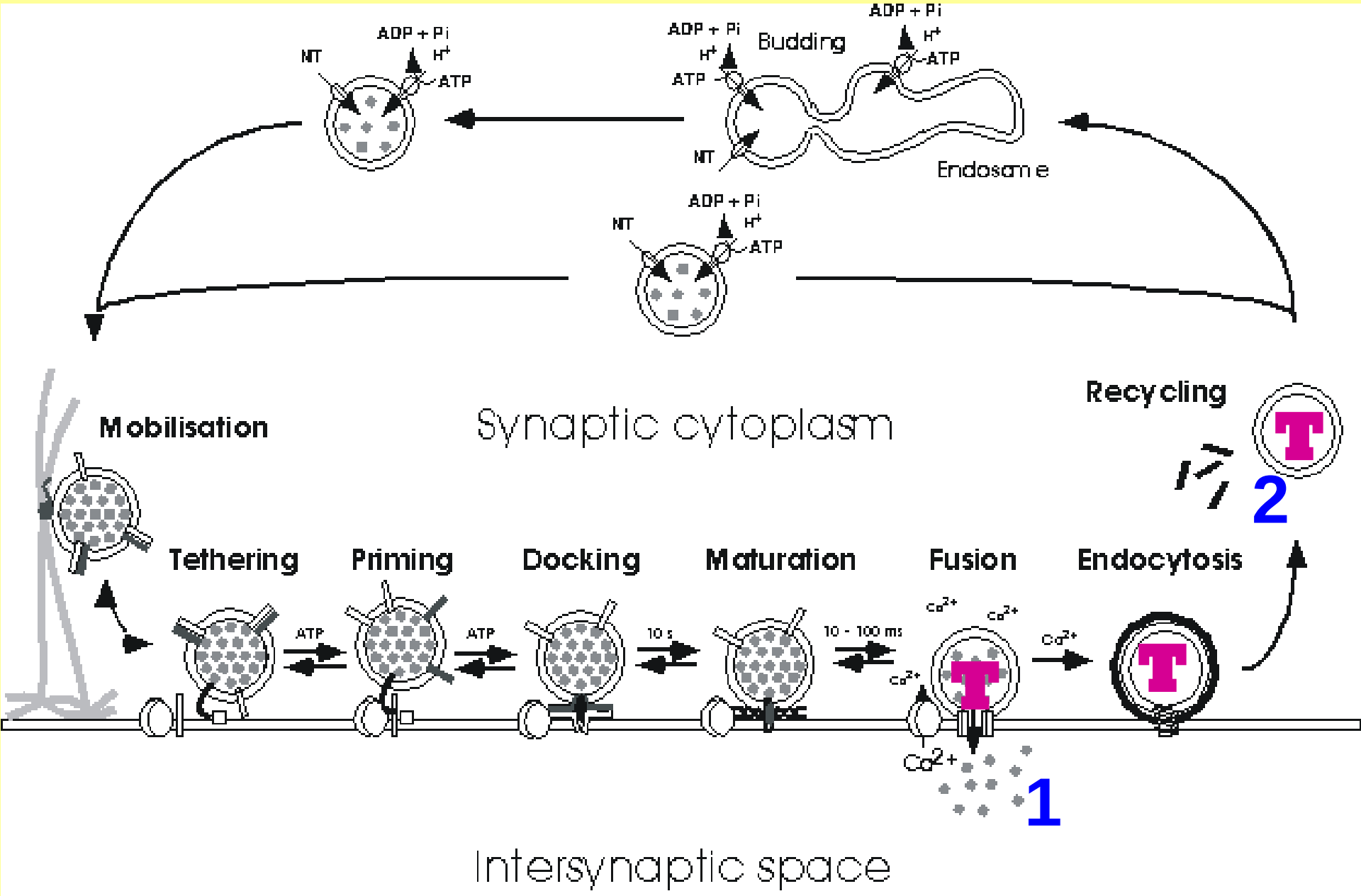


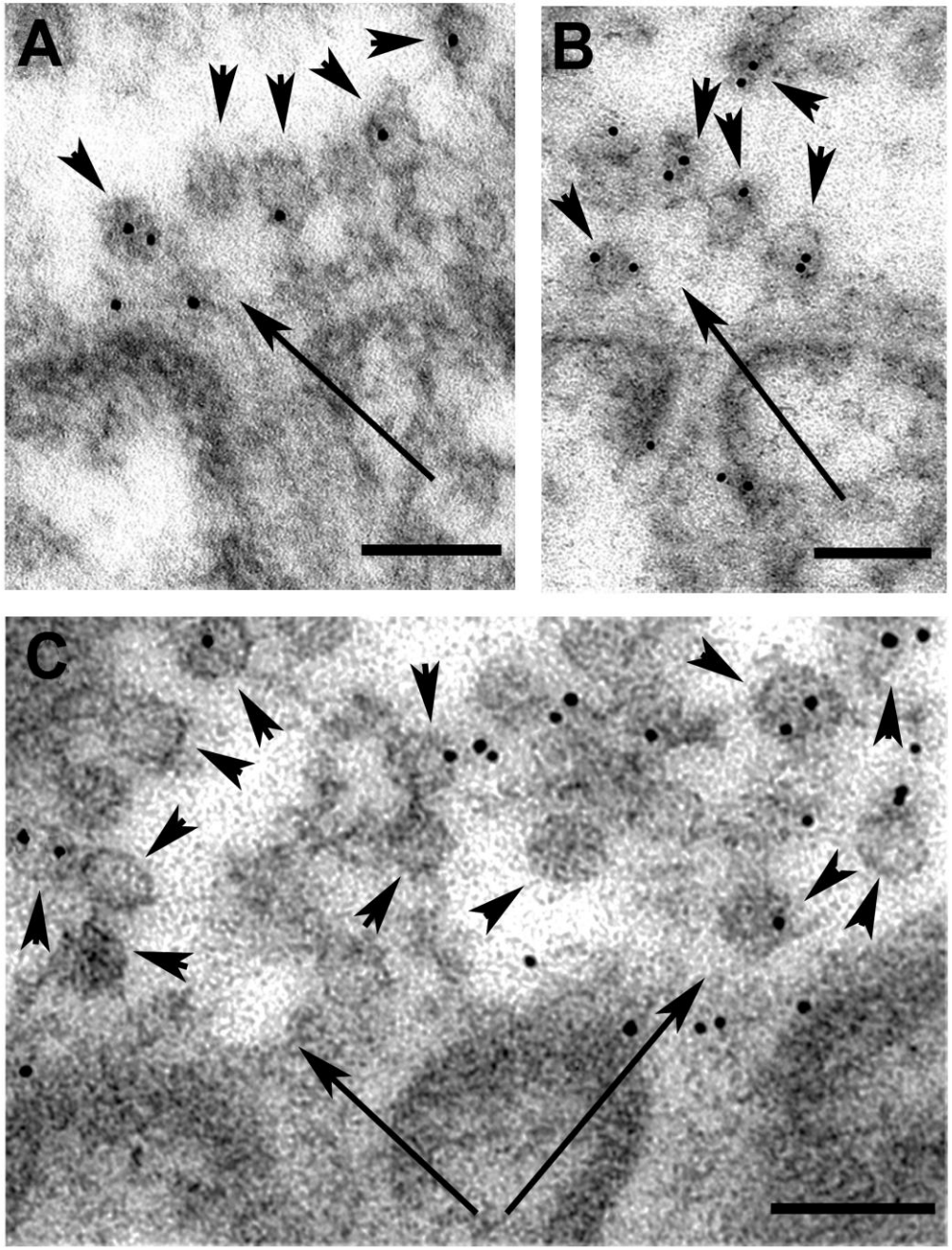


- The PSG receptors ensure neurospecific binding
- The Protein receptors of BoNTs are the lumenal domain of synaptic vesicle glycoproteins, which ensure the endocytosis of BoNTs inside synaptic vesicles



The TeNT as Odysseus into the trojan horse…


ADP + Pi
H+
ATP
NT
Budding
Endosome
Mobilisation
Synaptic cytoplasm
Recycling
Tethering
Priming
Docking
Maturation
Fusion
Endocytosis
ATP
10 s
10 - 100 ms
Ca2+
1
2
Intersynaptic space

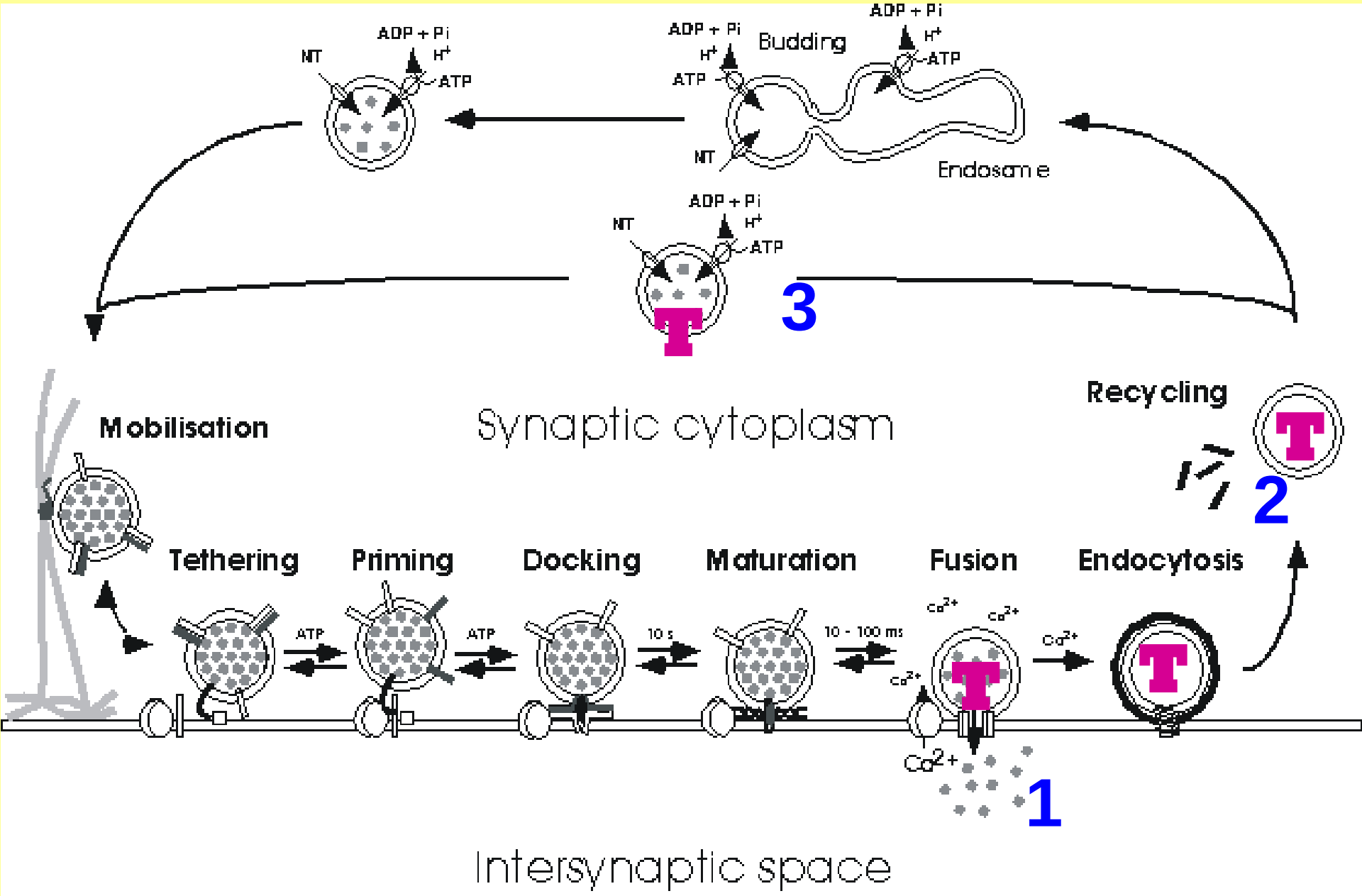


Immuno EM with gold-labelled Ab anti TeNT or anti BoNT

TeNT and BoNTs enter into the nerve terminals via endocytosis of synaptic vesicles.

Synaptic vesicles are the Trojan horses of the neurotoxins

Matteoli et al., PNAS 1996
Colasante et al, Mol. Neurobiol 2013


ADP + Pi
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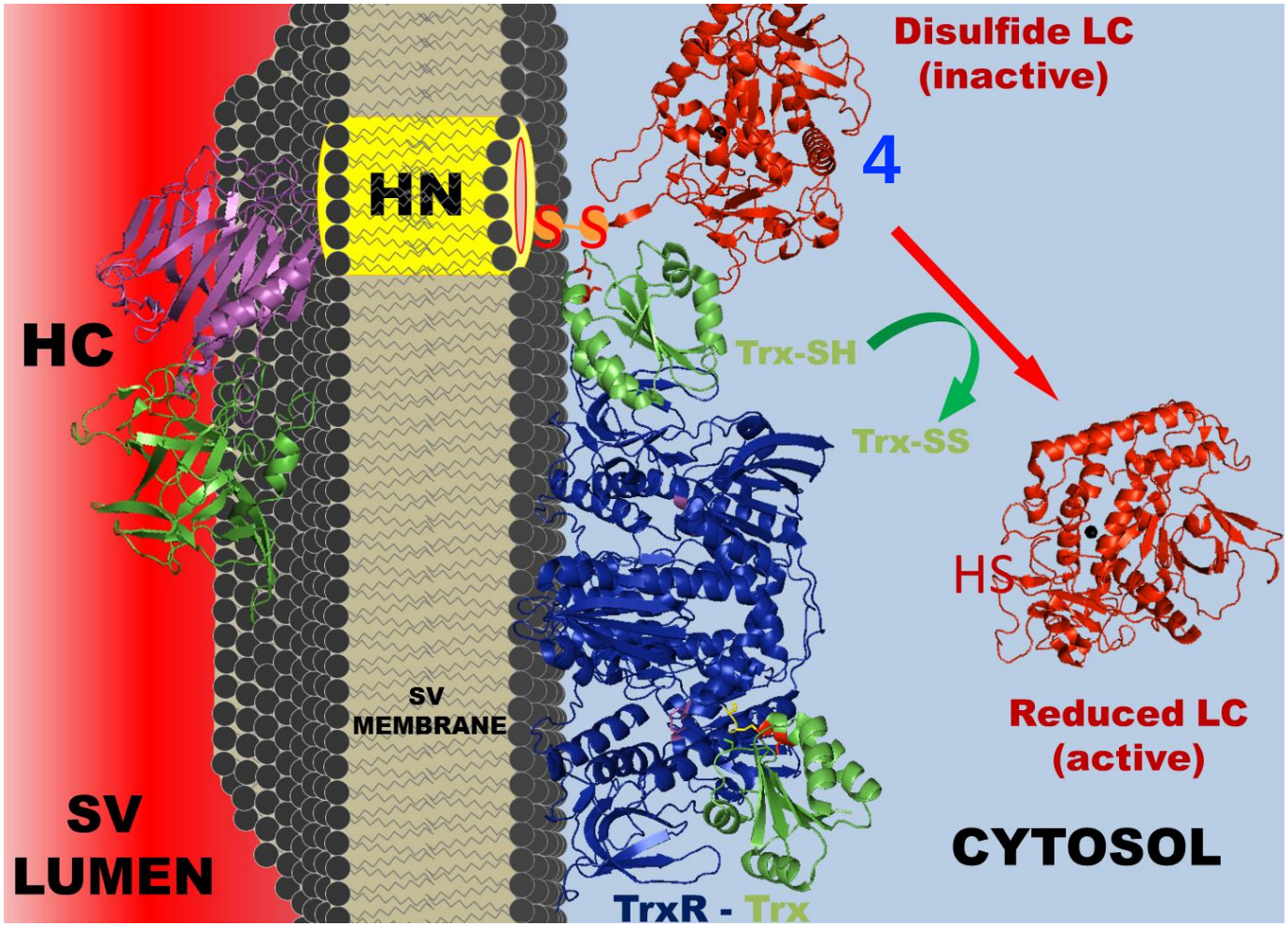
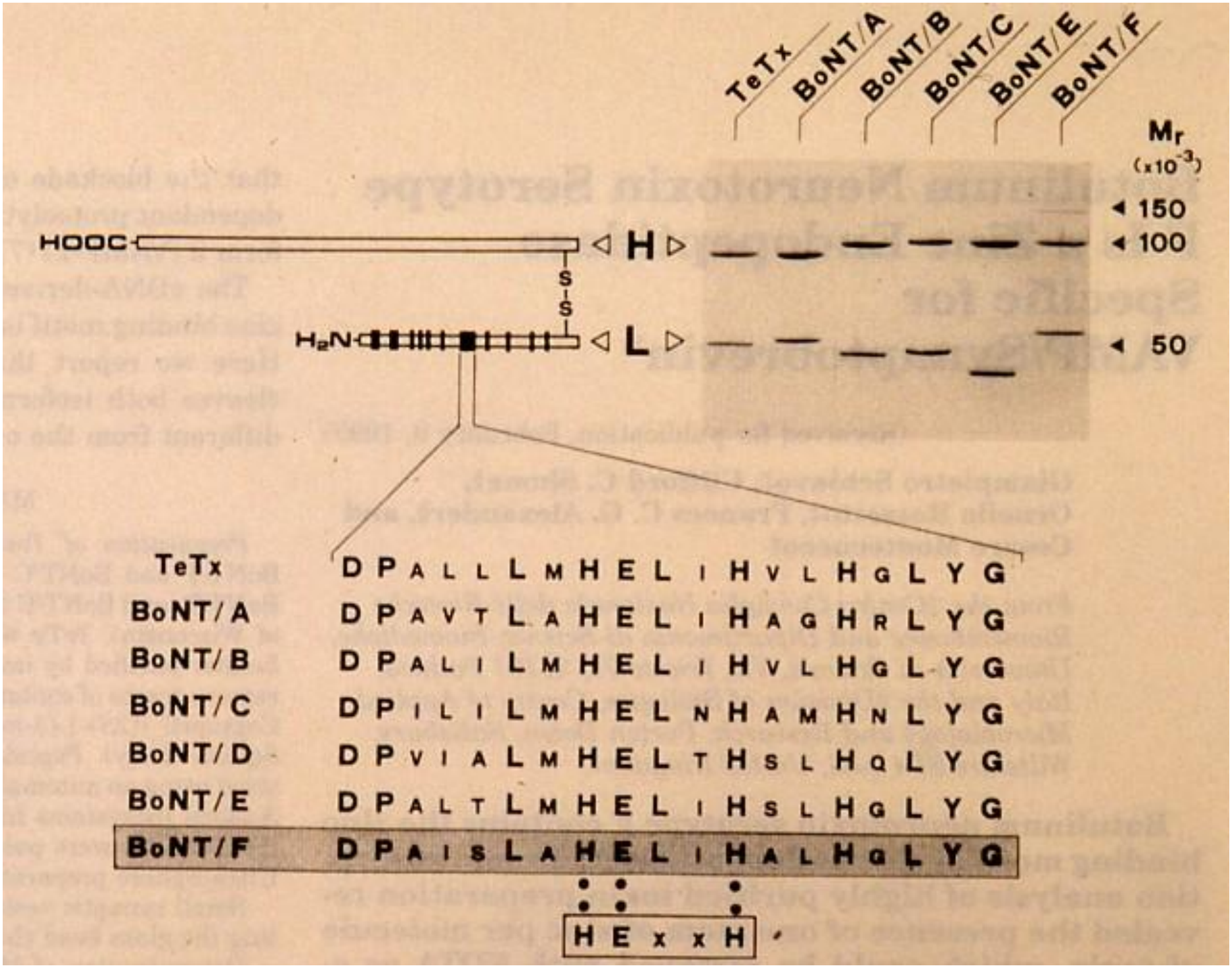
Fusion
Endocytosis
10 s
10 - 100 ms
Ca2+
1
Intersynaptic space

After membrane translocation, the metalloprotease L domain of BoNTs and TeNT is released in the cytosol by Tx-TxReductase.



Many specific inhibitors of TrxR prevent completely toxicity of all BoNTs and of TeNT.

Pirazzini et al., Cell Rep. 2014; Zanetti et al, 2015


TeTx
BoNT/A
BoNT/B
BoNT/C
BoNT/E
BoNT/F
Mr (x10⁻³)
150
100
50
HOOC
H
S
S
H₂N
L
TeTx DPALLLMHELIHVLHGLYG
BoNT/A DPAVTLAHELIHAGHRLYG
BoNT/B DPALILMHELIHVLHGLYG
BoNT/C DPILILMHELNHAMHNLYG
BoNT/D DPVIALMHELTHSLHQLYG
BoNT/E DPALTLMHELIHSLHGLYG
BoNT/F DPAISLAHELIHALHGLYG
HExxH

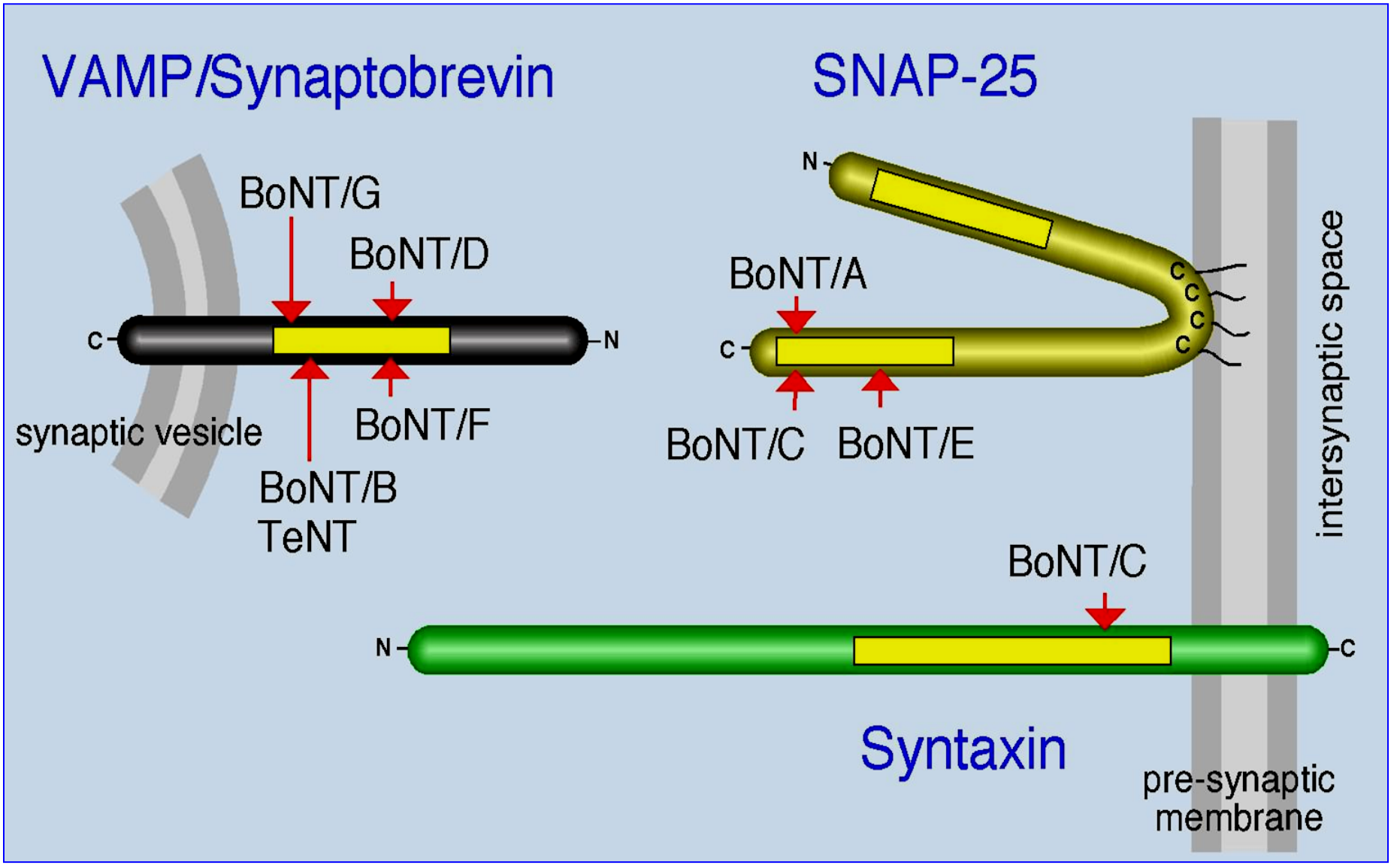
Biochem. J. (1991) **275**, 389–391 (Printed in Great Britain)

Homologues of insulinase, a new superfamily of metalloendopeptidases

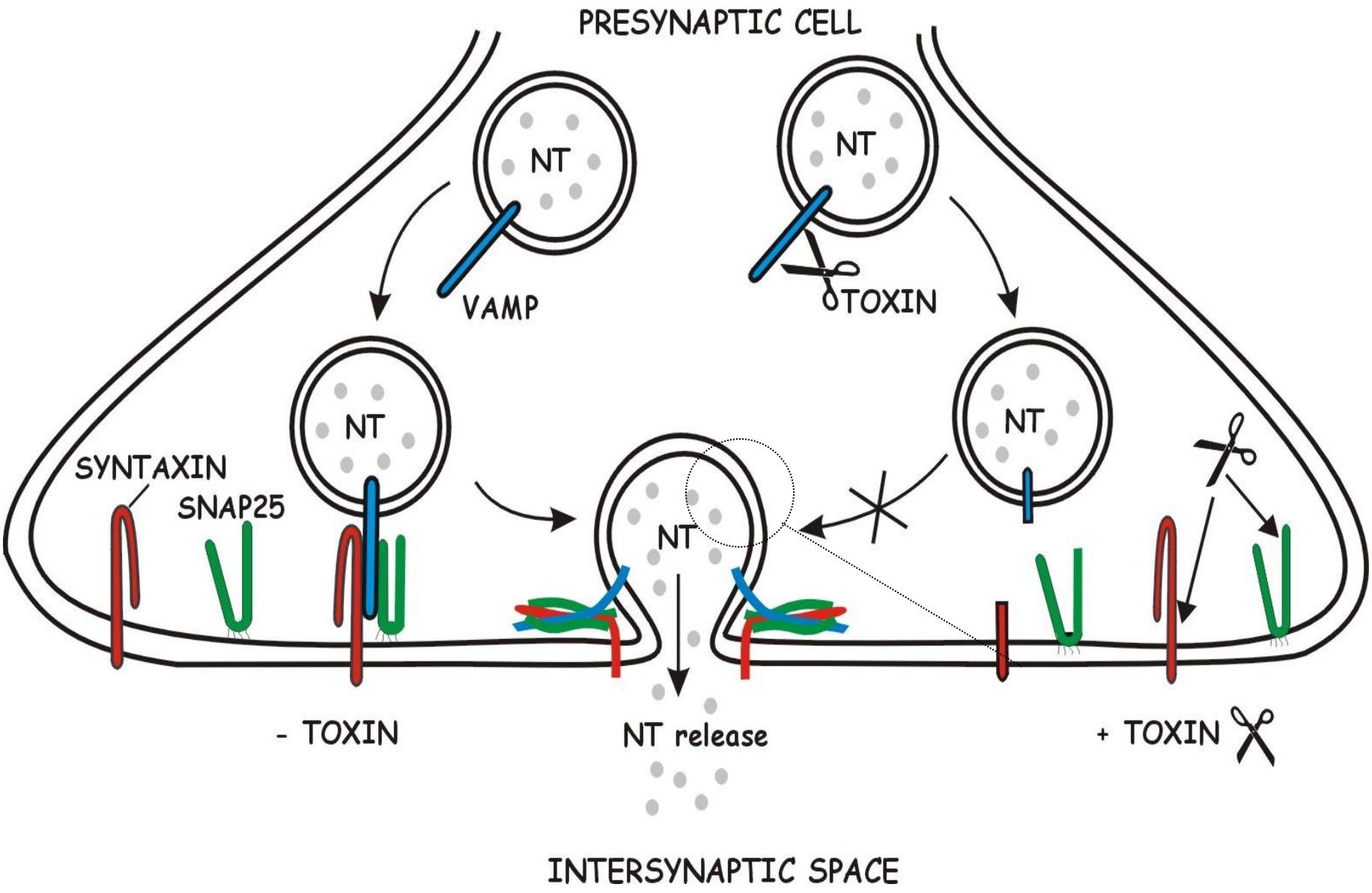
Neil D. RAWLINGS* and Alan J. BARRETT
Department of Biochemistry, Strangeways Research Laboratory, Worts Causeway, Cambridge CB1 4RN, U.K.

On the basis of a statistical analysis of an alignment of the amino acid sequences, a new superfamily of metalloendopeptidases is proposed, consisting of human insulinase, *Escherichia coli* protease III and mitochondrial processing endopeptidases from *Saccharomyces* and *Neurospora*. These enzymes do not contain the 'HEXXH' consensus sequence found in all previously recognized zinc metalloendopeptidases.

Tetanus and Botulinum neurotoxins could be Zinc metalloproteases



Schiavo et al. 1992: **TeNT**. Blasi et al. 1993: BoNT/A, /C ; Schiavo et al. 1992-1993 BoNT/A, /B, /D, /E, /F; Schiavo et al. 1994 BoNT/G; Schiavo et al. 1995: BoNT/C**.**



SNARE COMPLEX

Rothman et al, 1993

These finding really made a paradigmatic change because:

a) It provided a molecular understanding of the paralysis caused by tetanus and botulinum neurotoxins.

b) It showed that the the different paralysis of botulism and tetanus (flaccid vs spastic) are not due to a different mechanism of action of the different toxins, but to the different neurons targeted by the different toxins

The demonstration that VAMP is essential for neurotransmission provided the final experimental evidence that the Katz'quantal release hypothesis for neurotransmitter release is correct.

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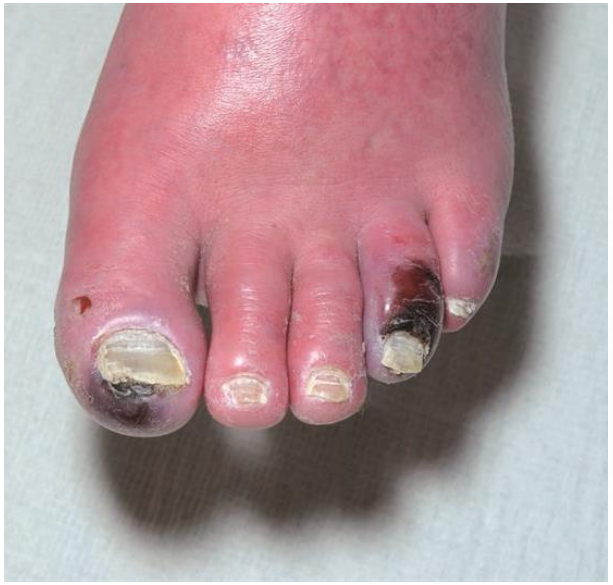
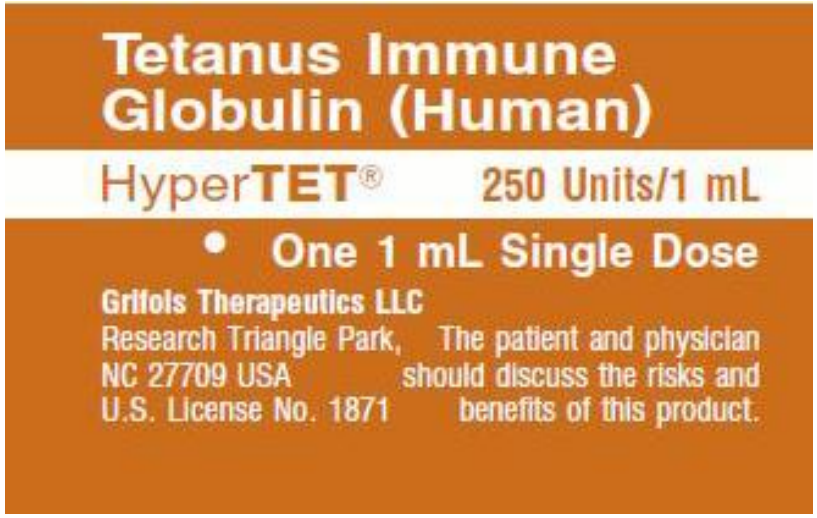


In the hospital emergency room, wounds are cleaned and disinfected and **TIGs are injected to prevent tetanus**

Hyperimmune horse sera are used in poor countries because of their lower cost though they can generate dangerous **hypersensitivity reactions**



The currently used serotherapy with TIG, prepared from hyperimmune donors, is affected by several drawbacks:

1. variation of neutralizing power from lot to lot.

2. risk of contamination due to the blood source of IgG.

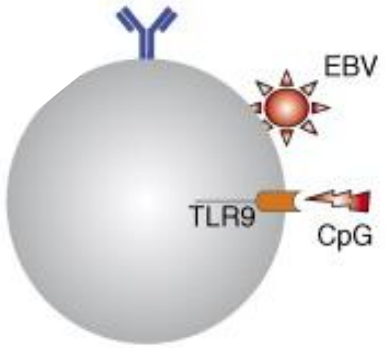
3. need of injecting relatively large amounts of IgG (as an hyper-immune does not contain > 1 % of antigen-specific IgG).

4. the risk of anaphylactic reactions or serum sickness in the case of the equine antisera used in low-income countries.

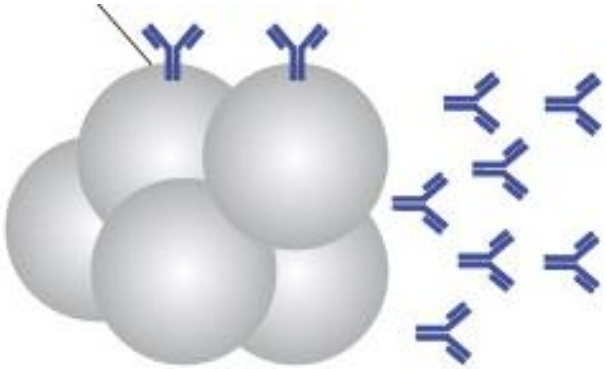
All these problems can be overcome by the injection of small amounts of human monoclonal antibodies of high affinity

Generation of HumAbs from pathogen specific memory B cells (Lanzavecchia , Nature Med. 2004)

Immunized human volunteers with TeNT toxoid

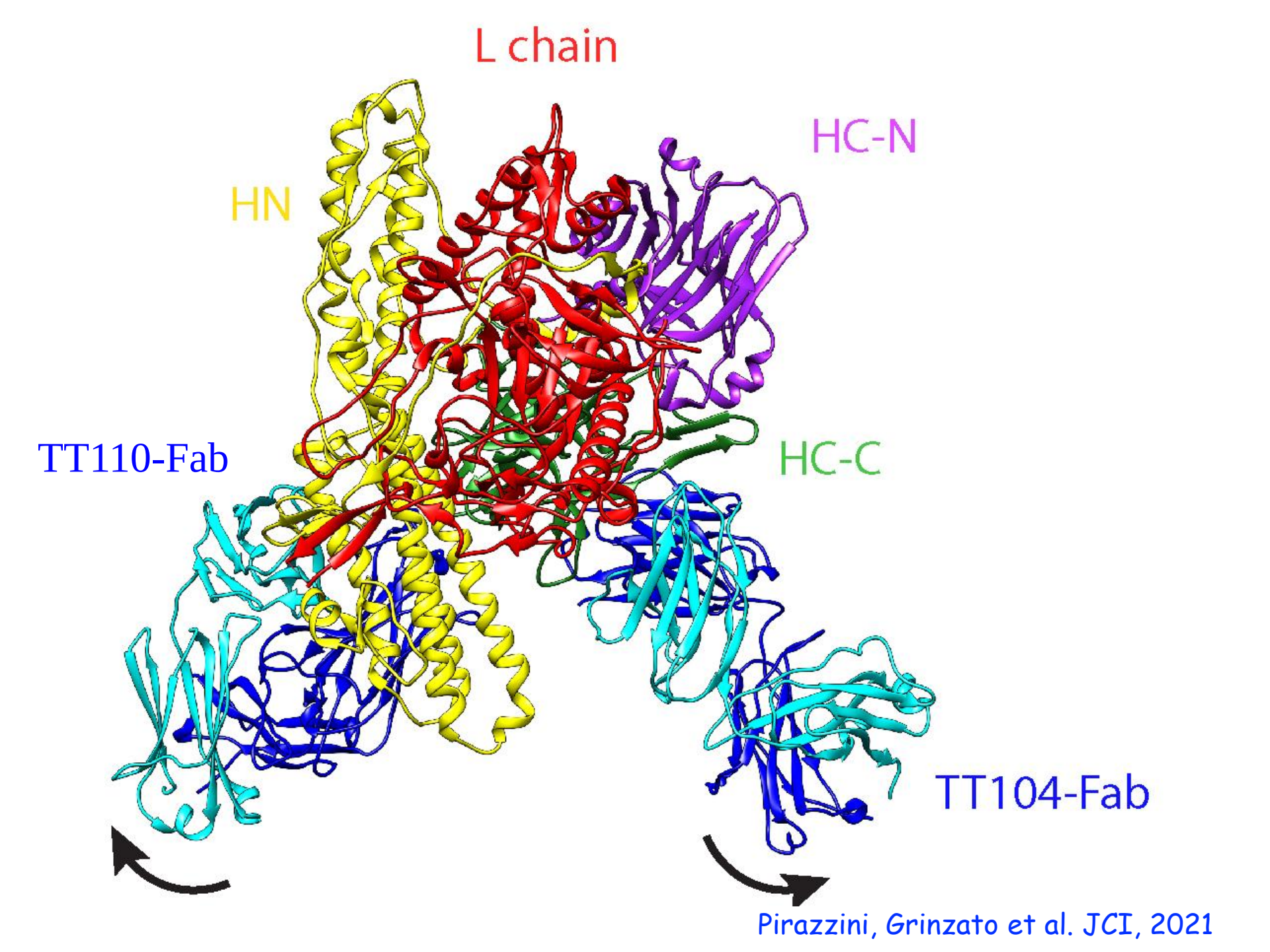


Isolated memory B cells from blood and Immortalized them with EBV



Hundreds of B cell clones specific for TeNT were isolated.

The IgG1 genes of specific clones were then sequenced and subcloned in HEK-293 cells for HumAbs production



Pirazzini, Grinzato et al. JCI, 2021

Conclusions

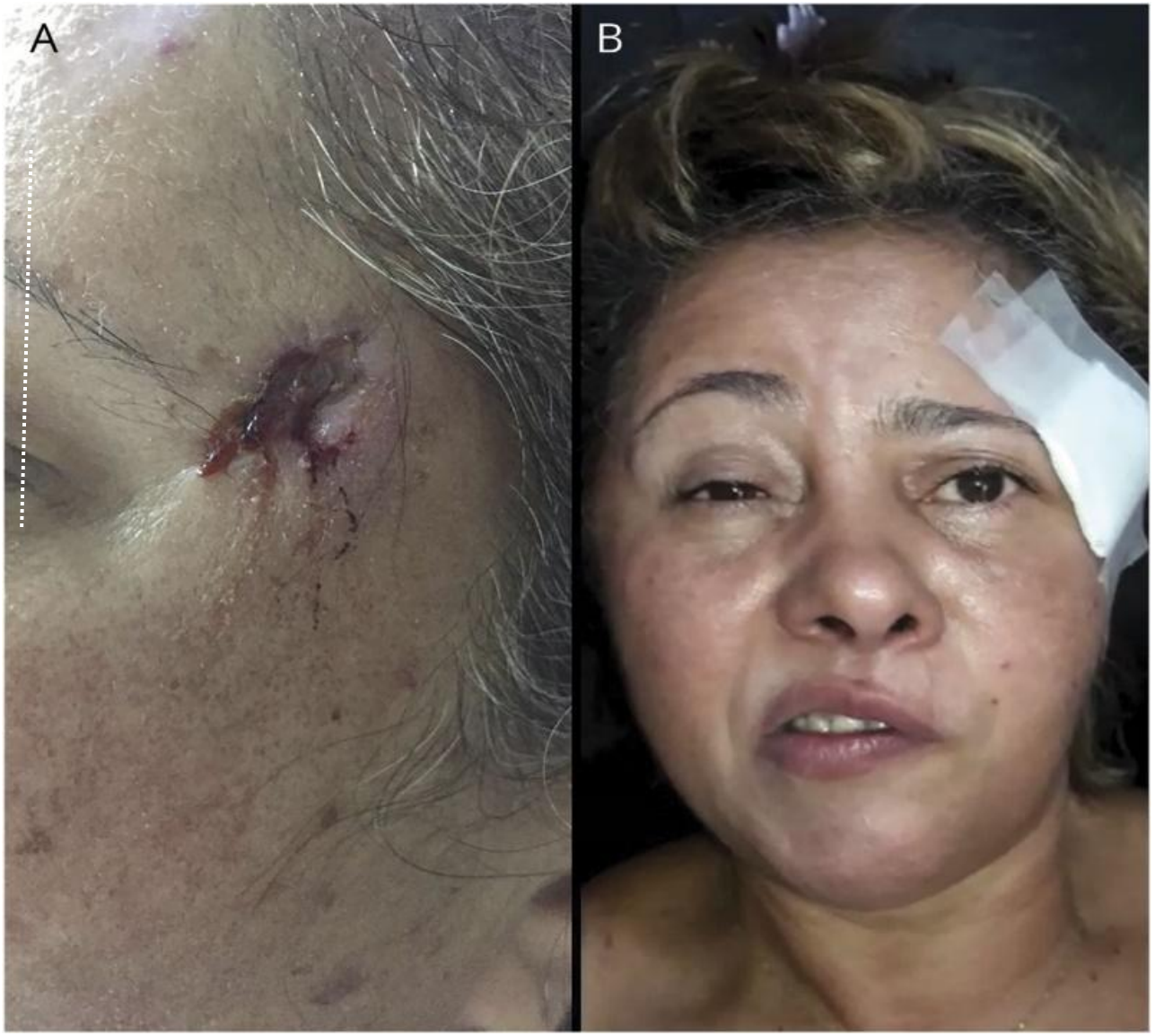
1. Our anti-TeNT humAbs are the most potent humAbs anti TeNT ever reported.

2. Meet all requirements for being considered for the prophylaxis of tetanus.

3. They perform as well as TIG in serotherapy, but without the drawbacks of TIG.

4. **Fabs 1.2 microgr / Kg versus TIG 5 milligr / Kg**.
   This opens the possibility of performing a novel and more effective treatment of symptomatic tetanus by injecting the Fab into the spinal cord, which is the very site of action of TeNT

Cephalic tetanus



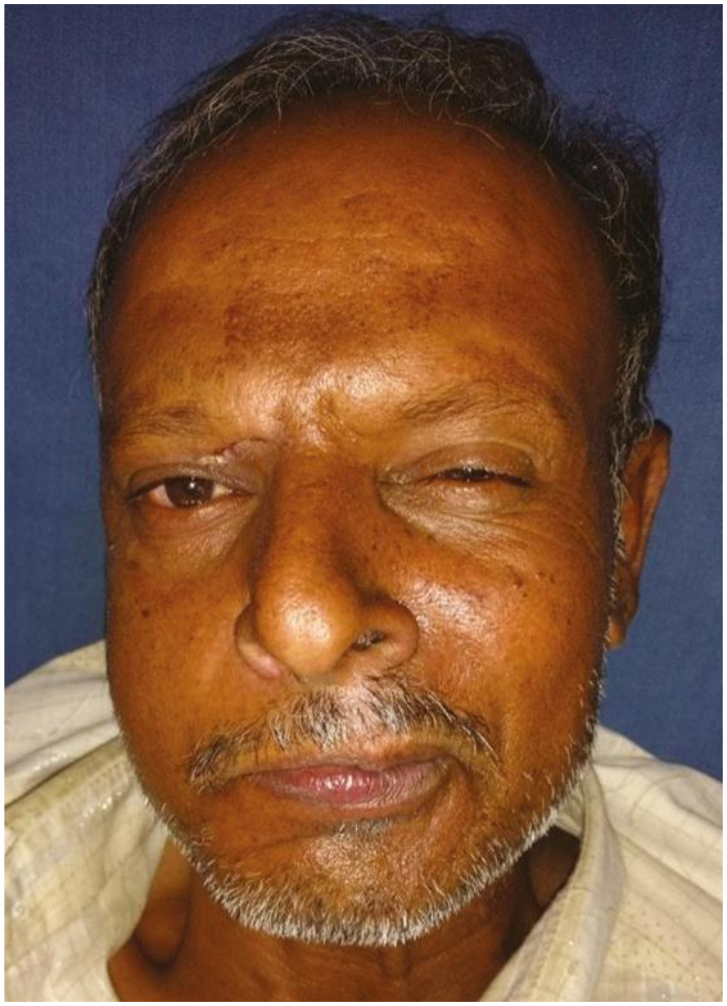
Nascimento et al., Neurology 2019

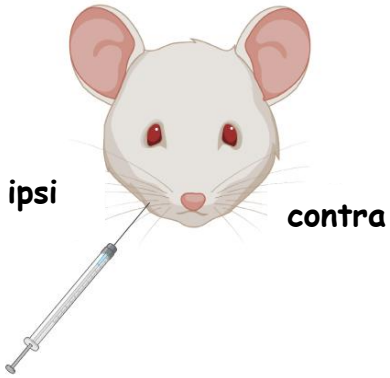
Cephalic Tetanus is caused by infections of craniofacial wounds or inner ear or gingivae

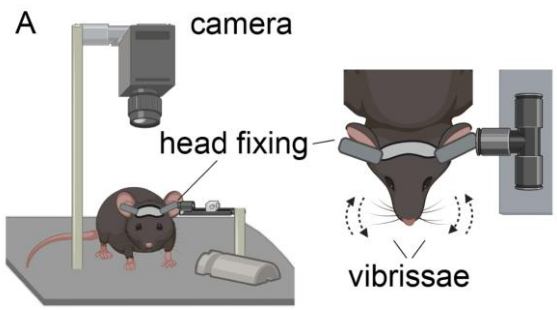
Clinically particular for two reasons:

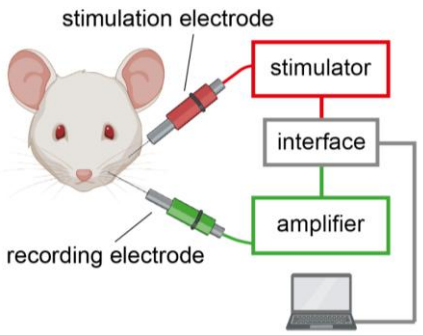
1. Patients first develop a cranial nerve palsy that precede (up to a week) spastic paralysis

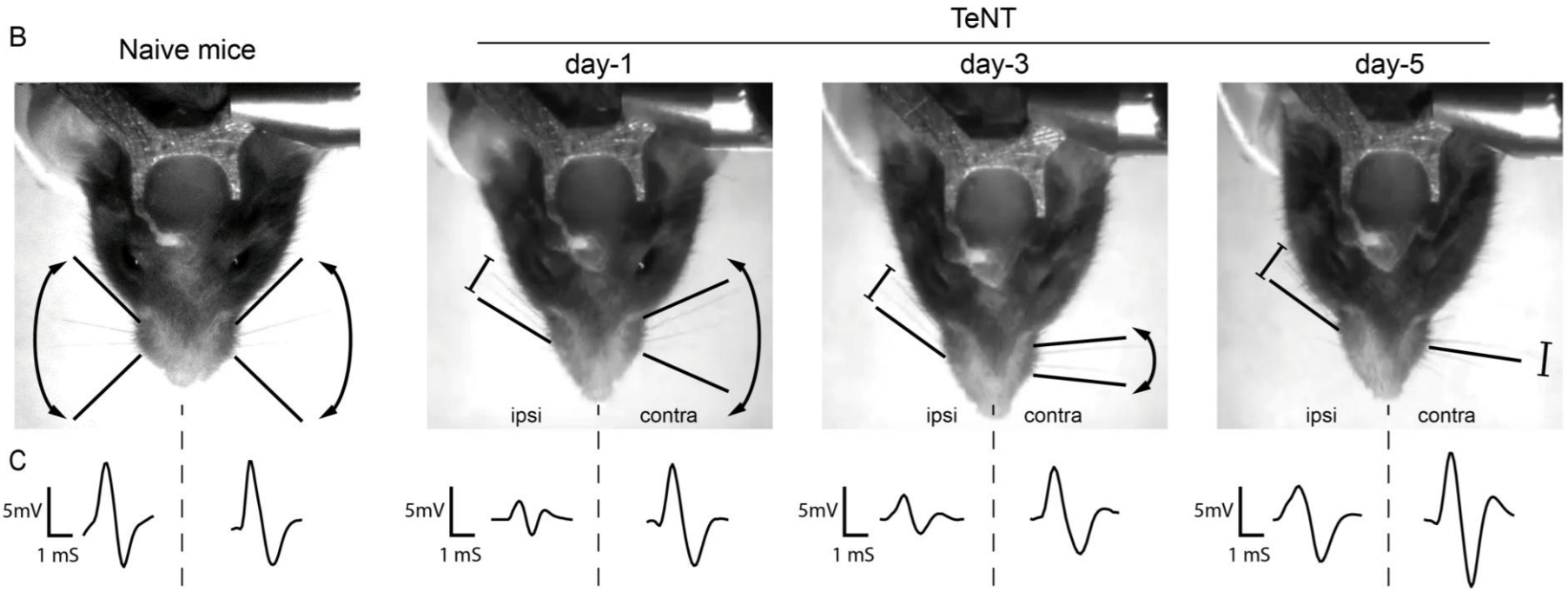
2. After spasticity appears there is a rapid progression to very dangerous cardiorespiratory symptoms even without generalized tetanus



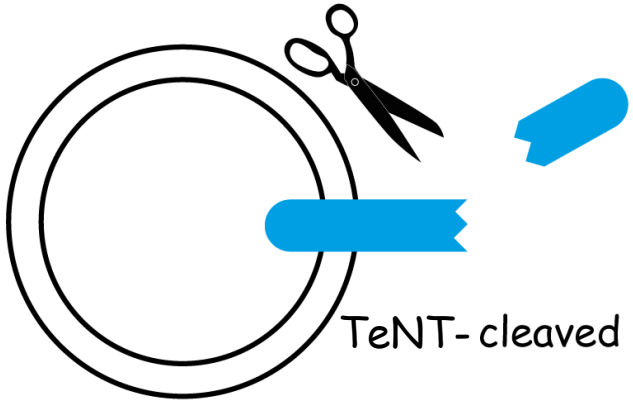


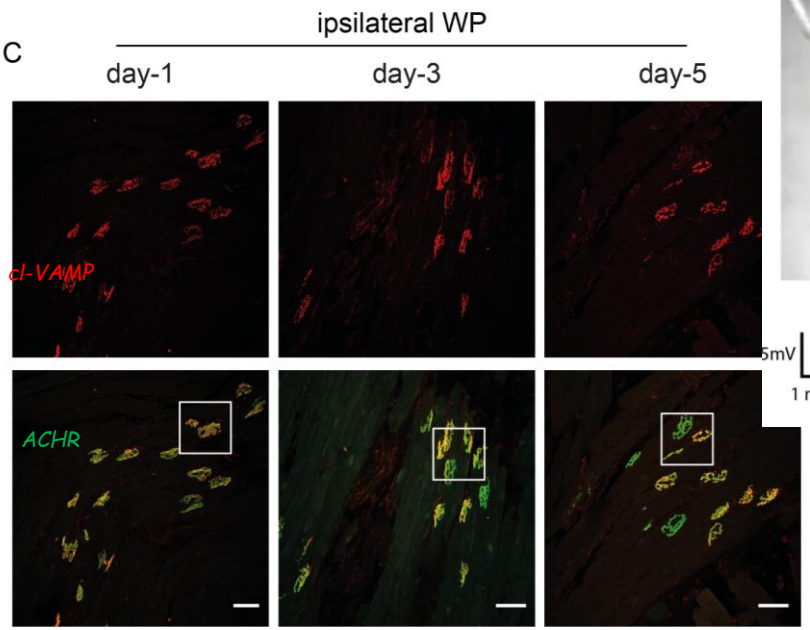


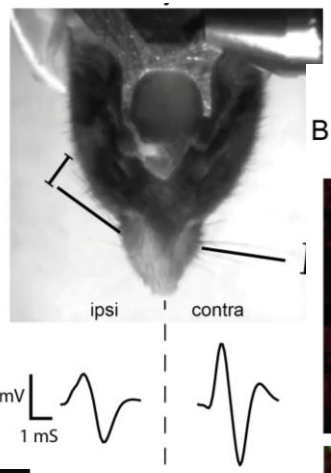


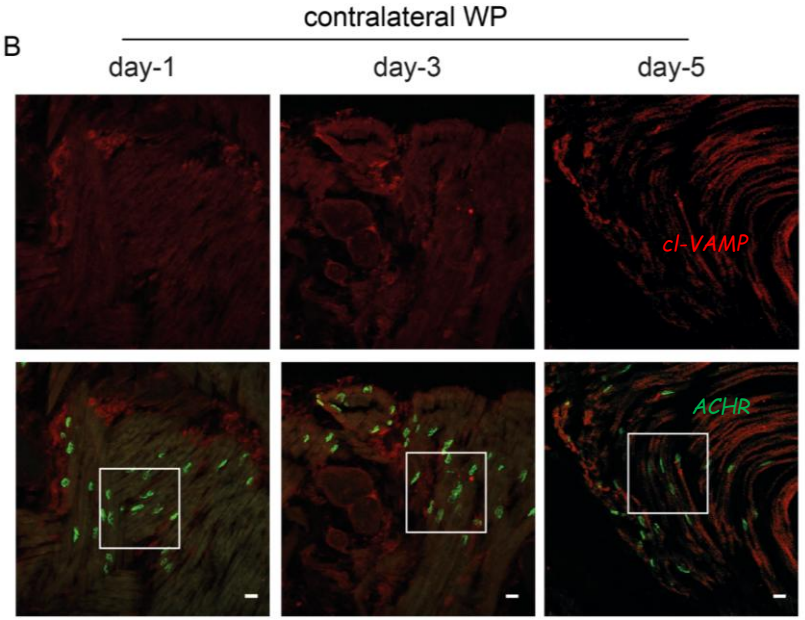


Fabris et al. JCI 2023









Flaccid paralysis

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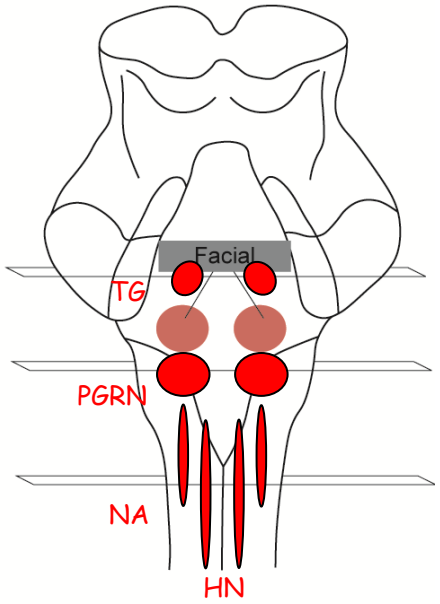
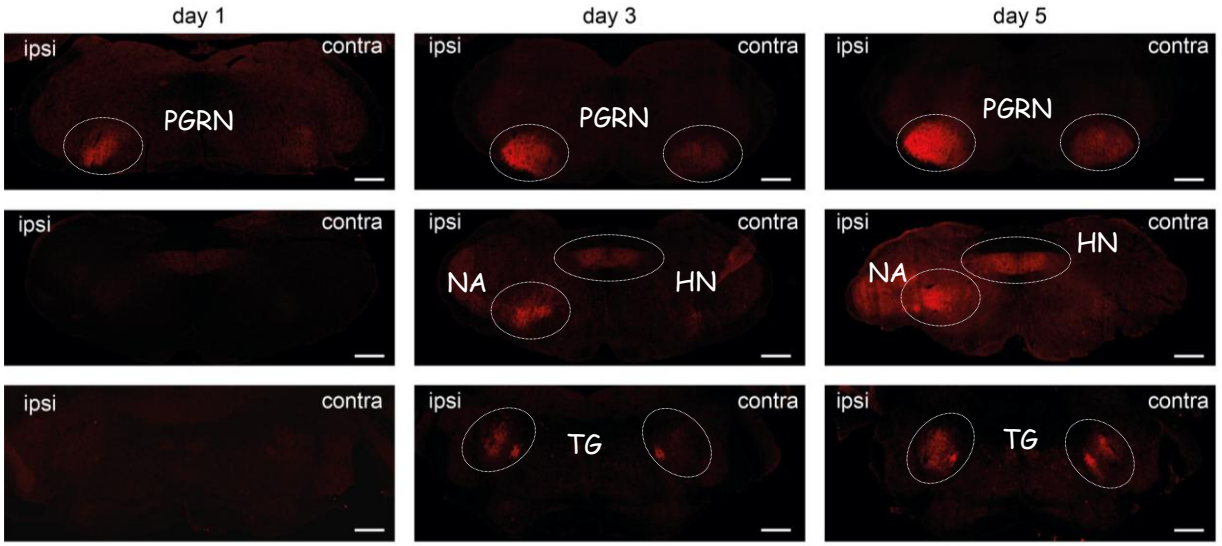
TeNT rapidly spreads among brainstem nuclei controlling deglutition, cardiovascular functions and respiration





(PGR) Para Gigantocellular Reticular: Respiration and cardiovascular function

(TG) Trigeminal: afferent sensory fibers from the WP

(HN) Hypoglossal: Mastication and Deglutition

(NA) Ambiguus: Upper airways control

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- TeNT causes a local, peripheral, faccid paralysis via cleavage of VAMP within facial nerves.

- In the case of head wounds this translates in a facial palsy that misleads diagnosis ***delaying the diagnosis of tetanus.***

- Meanwhile TeNT rapidly reaches and spreads among brai-stem nuclei essential for survival, including those controlling respiration and swalloving, causing death.

- This lead us to suggest that patients presenting a local paralysis should be injected anti-tetanus Immuno Globulins (TIG) to neutralize the possibility of a local paralysis due to TeNT.

Fabris et al. JCI 2023,
Fabris et al. Am. J. Pathol., 2024


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