Simposio: Tossine naturali

LA TOSSINA BOTULINICA: DAL PIÙ POTENTE VELENO AD UN FARMACO STRAORDINARIO

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UNIVERSITÀ DEGLI STUDI DI PADOVA

26 Ottobre 2021

LA DOPPIA FACCIA DELLE TOSSINE BOTULINICHE

Le tossine più potenti sinora conosciute

- Dosi letali < 1 ng/kg
- Agenti causali del botulismo
- Incluse nella lista delle sostanze con potenziale bioterroristico

E' un farmaco sicuro ed efficace

- Venduto in molti milioni di dosi per anno
- Molte applicazioni terapeutiche

E' un importante strumento di ricerca in neurobiologia

OUTLINE DELLA PRESENTAZIONE

1) Descrizione del meccanismo d'azione che fa delle tossine botuliniche le sostanze più tossiche in assoluto

2) Applicazioni terapeutiche della tossina botulinica A

3) La tossina botulinica come strumento di ricerca (la nostra esperienza di laboratorio)

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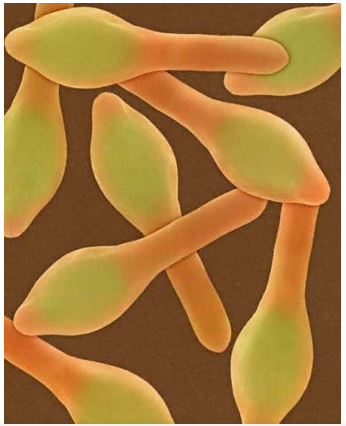
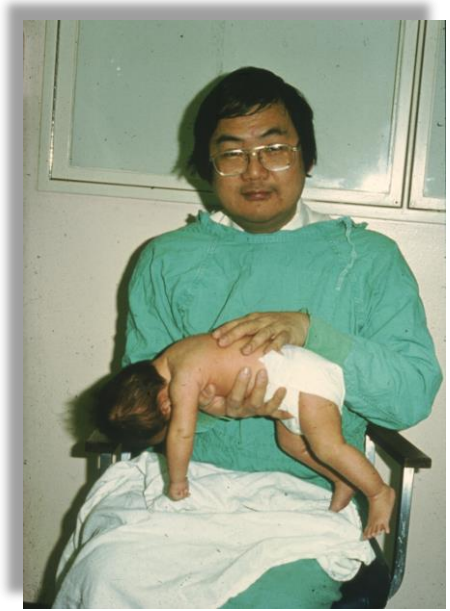
Clostridium spp.

- Batteri Gram-positivi
- Anaerobi obbligati
- Nell'ambiente si trovano sotto forma di spore
- In anerobiosi producono la tossina (7 diversi sierotipi BoNT/A-BoNT/G)

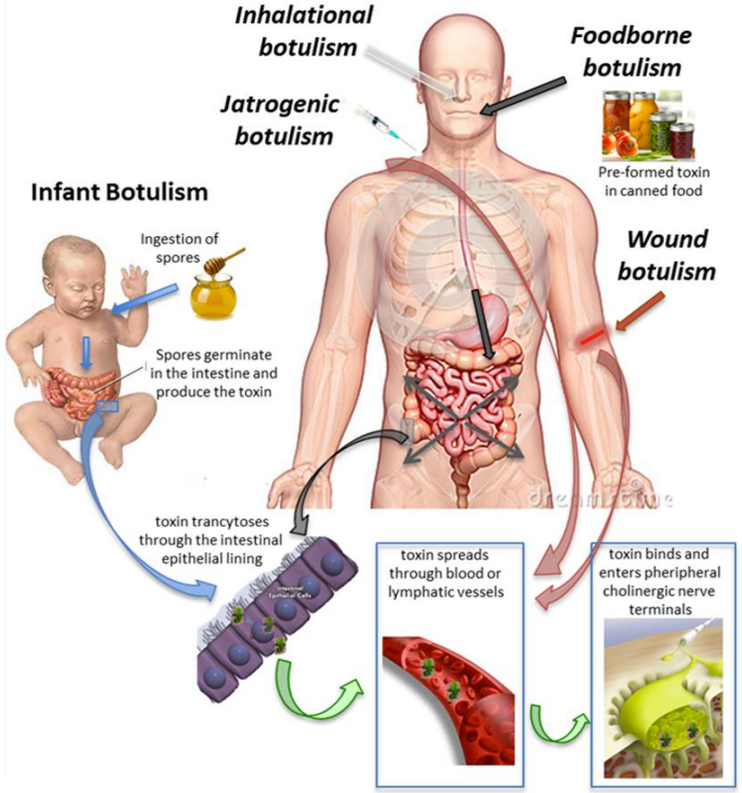


La Sindrome Neuroparalitica del Botulismo

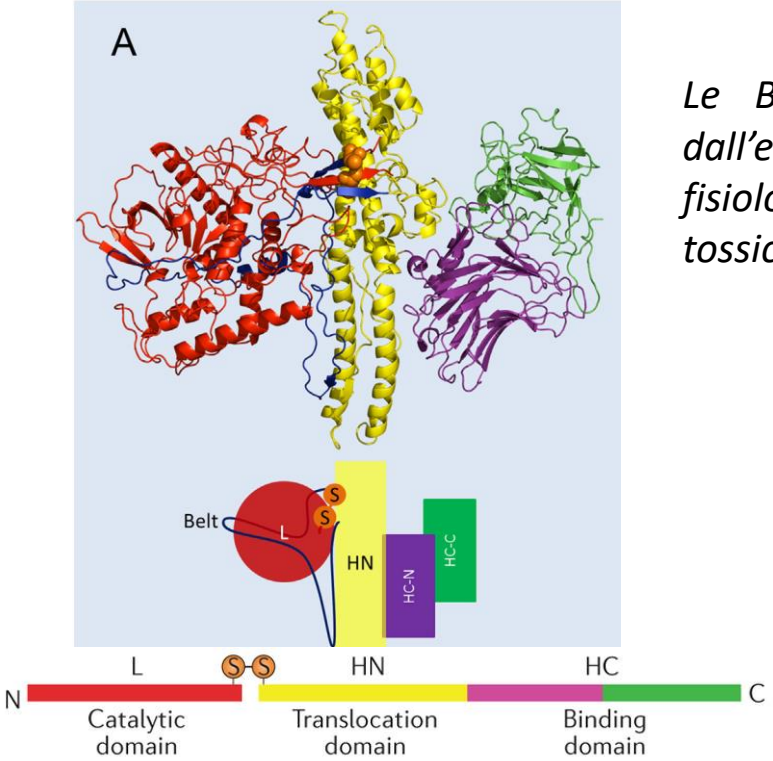
Paralisi discendente dei terminali colinergici somatici ed autonomici



Botulisms in Adult



Architettura molecolare delle tossine botuliniche

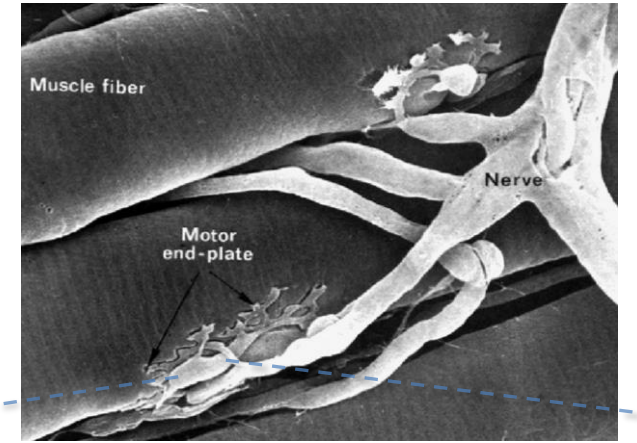


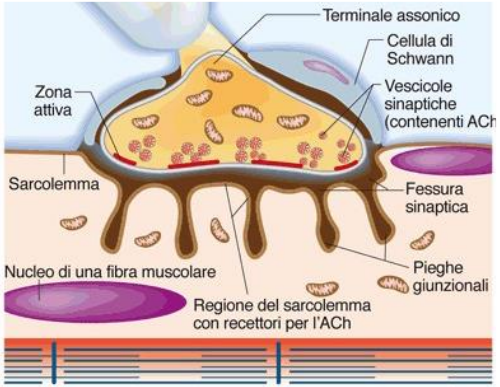
Le BoNTs hanno una struttura che è stata disegnata dall'evoluzione intorno a caratteristiche essenziali della fisiologia del terminale nervoso rendendole le molecule più tossiche sinora conosciute

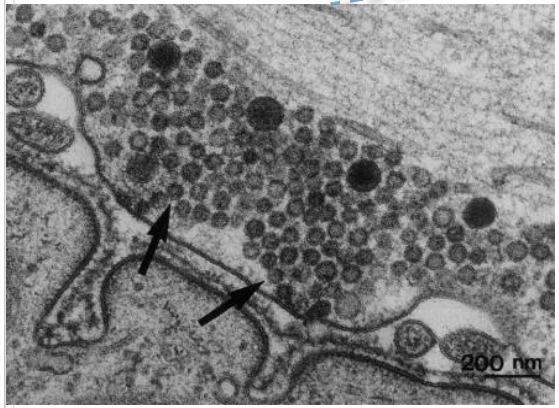
Botulinum Neurotoxin:
A Marvel of Protein Design

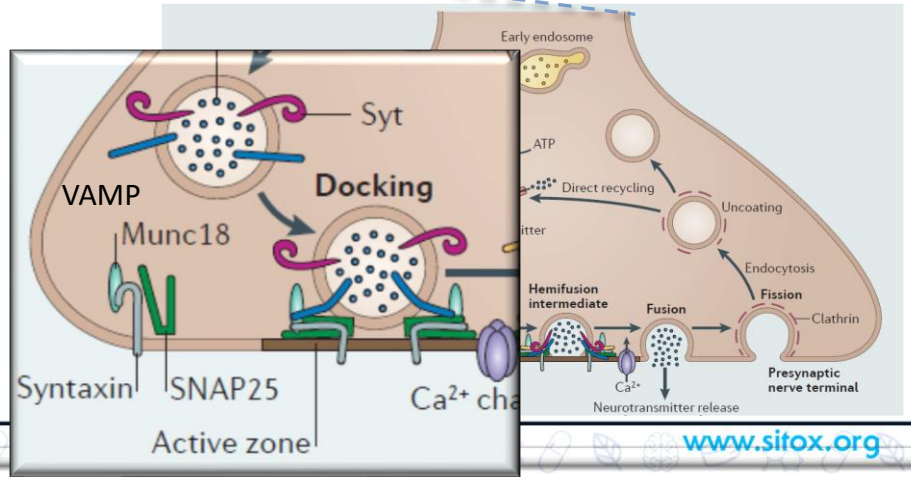
Mauricio Montal

SITO DI AZIONE

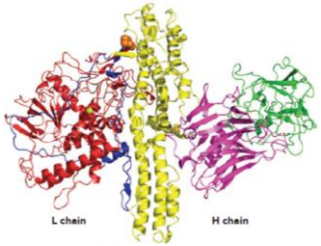


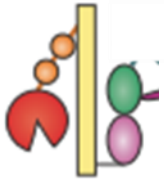




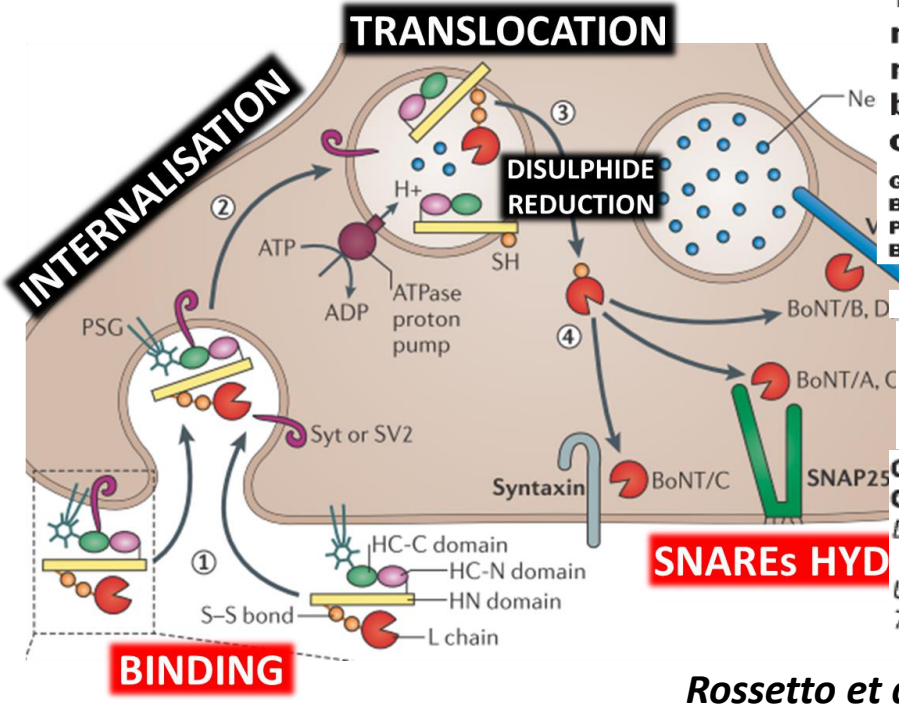


MECCANISMO OF AZIONE MULTI-STEP





TOSSINA



NATURE · VOL 359 · 29 OCTOBER 1992

Tetanus and botulinum-B neurotoxins block neurotransmitter release by proteolytic cleavage of synaptobrevin

Giampietro Schiavo*, Fabio Benfenati†, Bernard Poulain‡, Ornella Rossetto*, Patrizia Polverino de Laureto§, Bibhuti R. DasGupta|| & Cesare Montecucco*

NATURE · VOL 372 · 1 DECEMBER 1994

SNARE motif and neurotoxins

Ornella Rossetto, Giampietro Schiavo Cesare Montecucco*

Dipartimento di Scienze Biomediche and Centro CNR Biomembrane, Università di Padova, 75 Via Trieste, 75 - 35121 Padova, Italy

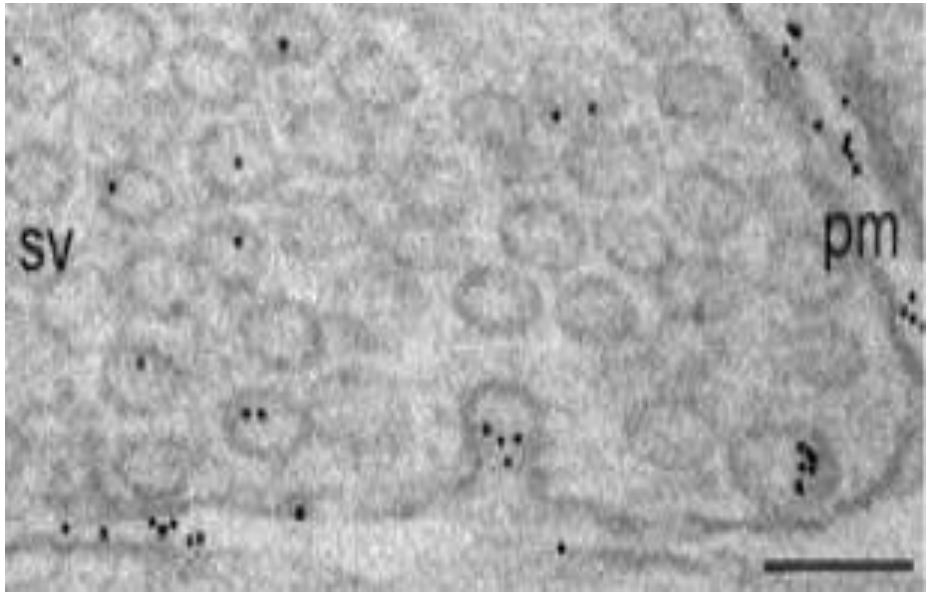
Rossetto et al., Nature Rev. Microbiol 2014

INTERNALISATION

Mol Neurobiol (2013) 48:120–127

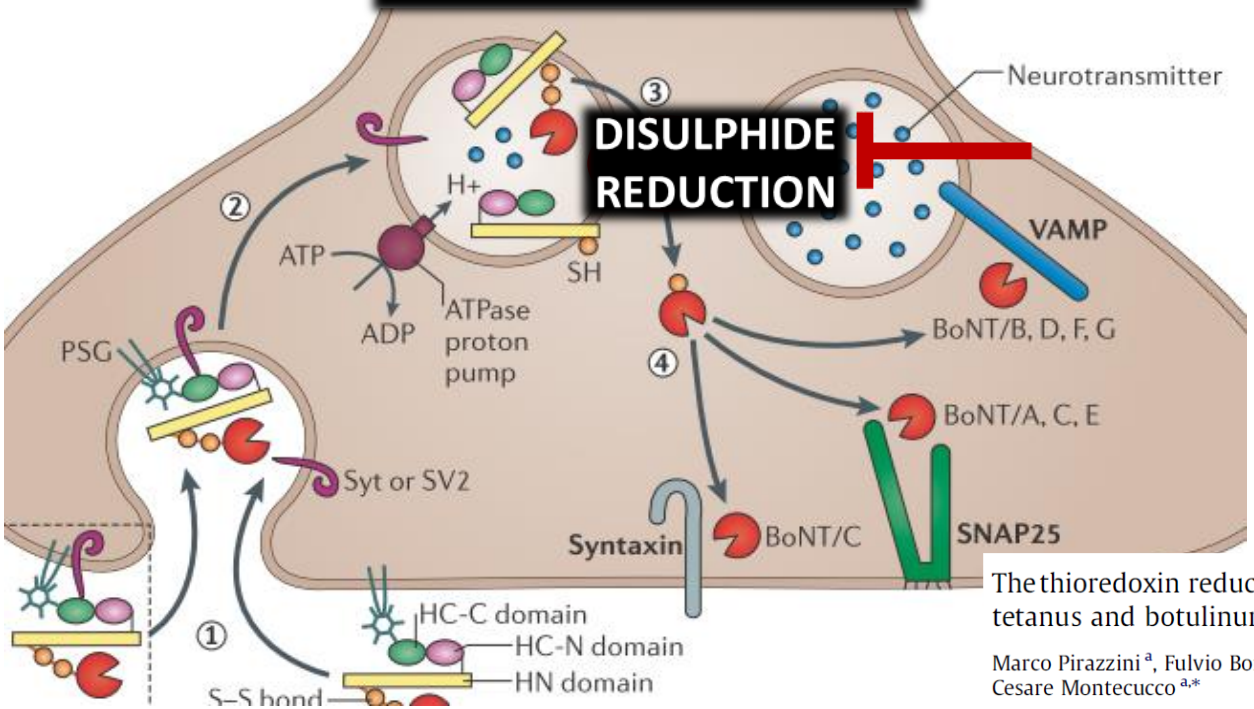
Botulinum Neurotoxin Type A is Internalized and Translocated from Small Synaptic Vesicles at the Neuromuscular Junction

Cesare Colasante • Ornella Rossetto • Laura Morbiato • Marco Pirazzini • Jordi Molgó • Cesare Montecucco



- L'endocitosi della tossina aumenta con attività sinaptica
- 1 max 2 molecule di tossina per vescicola che concorda con il numero di recettori SV2 presenti sulle vescicole sinaptiche (SV)
- BoNTs usano le SV come cavalli di Troia per entrare nel terminale nervoso

TRANSLOCATION

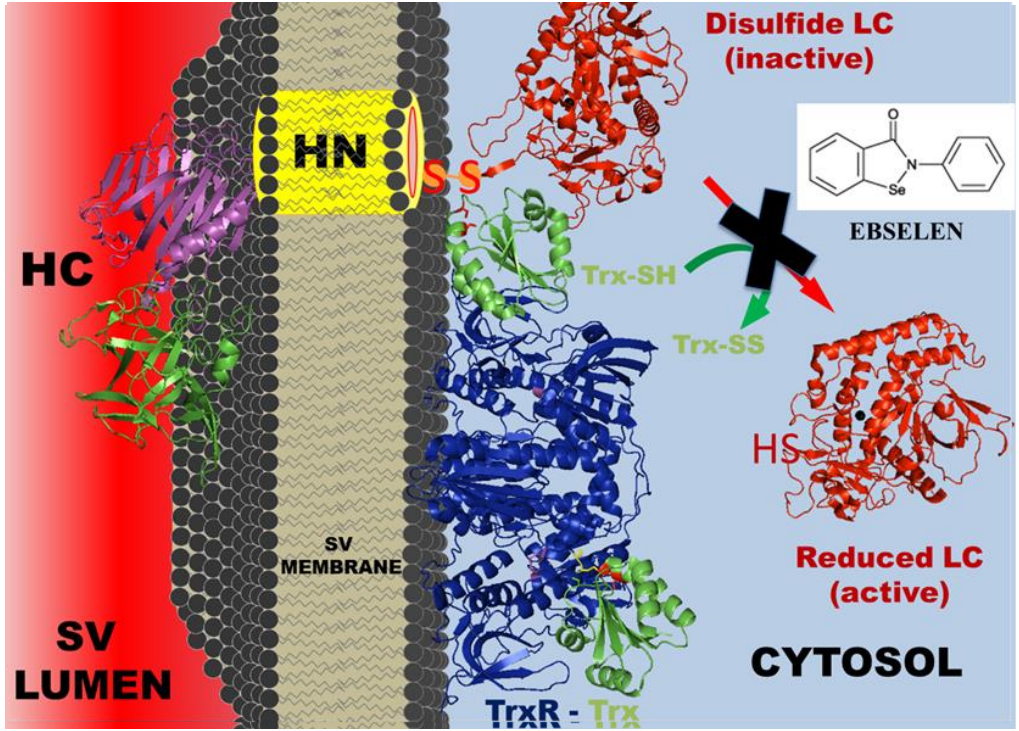


The thioredoxin reductase-thioredoxin system is involved in the entry of tetanus and botulinum neurotoxins in the cytosol of nerve terminals

Marco Pirazzini [a], Fulvio Bordin [a], Ornella Rossetto [a], Clifford C. Shone [b], Thomas Binz [c], Cesare Montecucco [a,*]

Trx-Trx-R costituisce un sistema bersaglio per l'dentificazione di potenziali pan-inibitori delle BoNT

INIBITORI del SISTEMA REDOX TIORESOSSINA-TRX-REDUTTASI PROTEGGONO DALLA PARALISI INDOTTA DA TUTTE LE TOSSINE BOTULINICHE E DALLA TOSSINA TETANICA



Pirazzini et al., Cell Rep. 2014
Rossetto et al., Cell. Microbiol. 2019

Tetanus and botulinum neurotoxins: turning bad guys into good by research

Ornella Rossetto[a], Michela Seveso[a], Paola Caccin[a], Giampietro Schiavo[b], Cesare Montecucco[a,*]

TOXICON 2001

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QUAL E' IL RAZIONALE ALLA BASE DELL'USO DELLA TOSSINA BOTULINICA COME AGENTE TERAPEUTICO

- La tossina botulinica è altamente specifica per i terminali colinergici
- Piccolissime quantità sono sufficienti per bloccare i terminali colinergici che innervano le fibre muscolari ma anche quelli autonomici che innervano le ghiandole
- L'azione della tossina è reversibile
- Diffonde molto poco dal sito di iniezione
- Esistono molte patologie umane caratterizzate da iperattività dei terminali colinergici

BoNT è un farmaco formidabile per trattare moltissime condizioni patologiche umane generate da iperattività dei terminali colinergici

THERAPEUTIC USES

- Cosmetic use
  - Hyperkinetic facial lines (glabellar frown lines, crow's feet)
  - Hypertrophic platysma muscle bands
- Sweating, salivary, and allergy disorders
  - Axillary and palmar hyperhidrosis
  - Frey syndrome, also known as auriculotemporal syndrome (gustatory sweating of the cheek after parotid surgery)
  - Drooling in cerebral palsy and other neurological disorders
  - Nasal allergy
- Strabismus and nystagmus
- Smooth muscle hyperactive disorders
  - Neurogenic bladder – Detrusor hyperreflexia· BPH
  - Achalasia cardia
  - Hemorhoids, Chronic anal fissures
- Focal dystonias - Involuntary, sustained, or spasmodic patterned muscle activity
  - Cervical dystonia (spasmodic torticollis)[3,4]
  - Blepharospasm (eyelid closure)
  - Laryngeal, Limb, Oromandibular, Orolingual, Truncal dystonia
- Spasticity
- Nondystonic disorders of involuntary muscle activity
  - Hemifacial spasm, trismus
  - Tremors, tics, myoclonus.
- Chronic pain and disorders of localized muscle spasms
  - Chronic low back pain
  - Tension headache
  - Chronic migrane headache
  - Medication overuse headache
  - Lateral epicondylitis
  - Knee, Shoulder,Neuropathic pain

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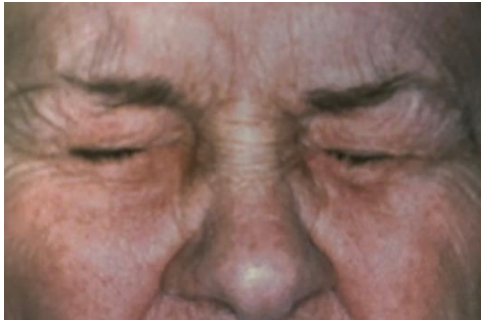

Blefarospasmo

Emispasmo facciale

Spasticità

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Iperidrosi palmare

Iperidrosi ascellare

Botulinum Neurotoxin Serotype A Suppresses Neurotransmitter Release from Afferent as Well as Efferent Nerves in the Urinary Bladder

Smooth muscle hyperactive disorders

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- Achalasia cardia
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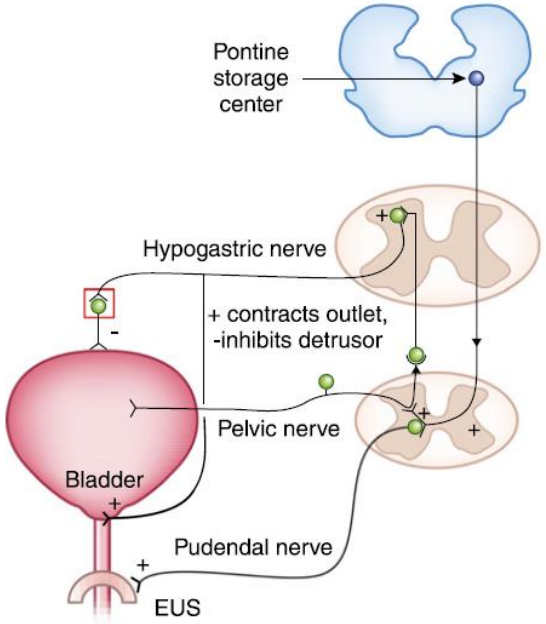

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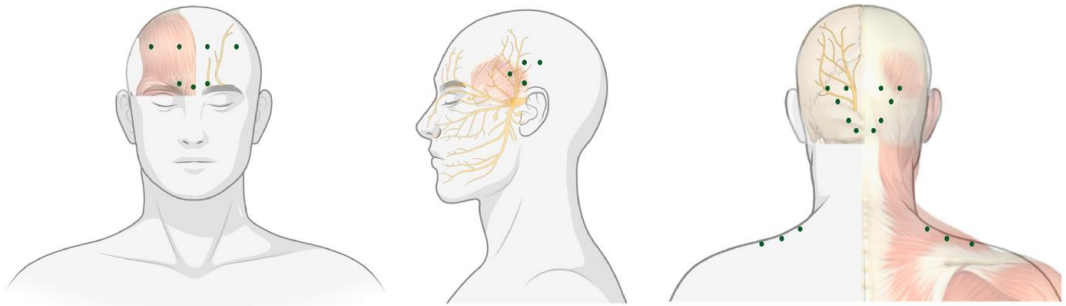


Figure 1. The PREEMPT protocol for injection of onabotulinumtoxinA (stylised)—green dots represent injection sites [12].

EFFETTI CENTRALI DELLA BoNT/A-EVIDENZE DI TRASPORTO RETROGRADO

The Journal of Neuroscience, 2008

Long-Distance Retrograde Effects of Botulinum Neurotoxin A

Flavia Antonucci,[1] Chiara Rossi,[1] Laura Gianfranceschi,[2] Ornella Rossetto,[3] and Matteo Caleo[1]

PLoS Pathog 2012

Botulinum Neurotoxins A and E Undergo Retrograde Axonal Transport in Primary Motor Neurons

Laura Restani[1,2], Francesco Giribaldi[1], Maria Manich[1,3], Kinga Bercsenyi[1], Guillermo Menendez[1], Ornella Rossetto[4], Matteo Caleo[2], Giampietro Schiavo[1]*

Central origin of the antinociceptive action of botulinum toxin type A

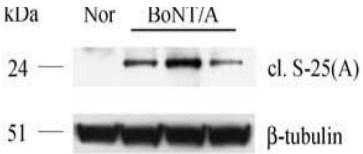
Lidija Bach-Rojecky[a], Zdravko Lacković[b,*]

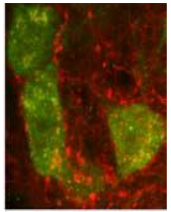
Pharmacology, Biochemistry and Behavior 94 (2009)

PAIN 2014

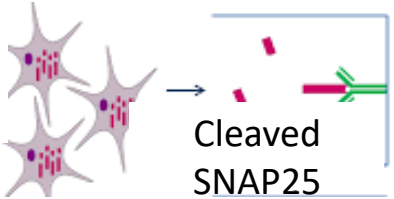
Botulinum toxin type A selectivity for certain types of pain is associated with capsaicin-sensitive neurons

Ivica Matak[a], Ornella Rossetto[b], Zdravko Lacković[a,*]





After BoNT/A injection at the center of the whisker pad, cleaved SNAP-25 was detected in the facial nucleus, confirming migration of toxin along motoneurons



Antibody specific for BoNT/A-cleaved SNAP25

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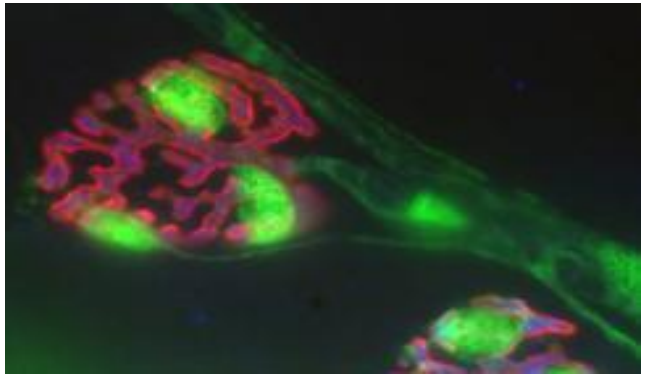
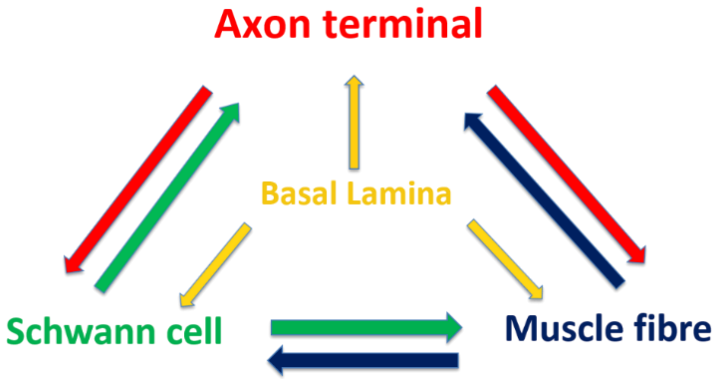
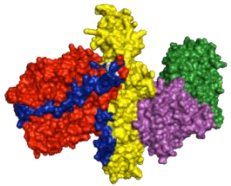
LA TOSSINA BOTULINICA (E ALTRE TOSSINE) COME TOOLS PER STUDIARE LA PLASTICITA' E LA RIGENERAZIONE DELLA GIUNZIONE NUROMUSCOLARE

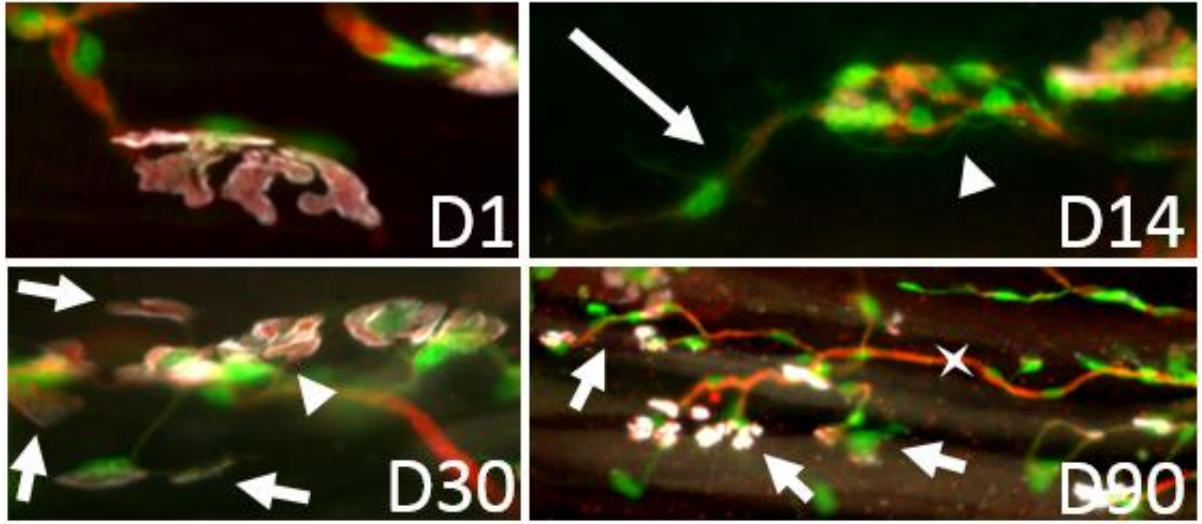
- plp-GFP
- α-BTX-555
- VAchT-370







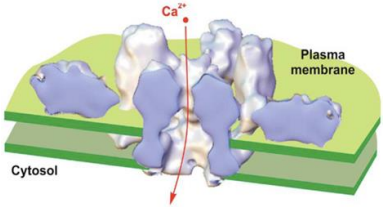
BoNTs causa una denervazione funzionale persistente della giunzione neuromuscolare che attiva un remodelling con formazione di sprouts e nuovi contatti sinaptici



QUALI SONO I DETERMINANTI MOLECOLARI DI QUESTA PLASTICITA' SINAPTICA?

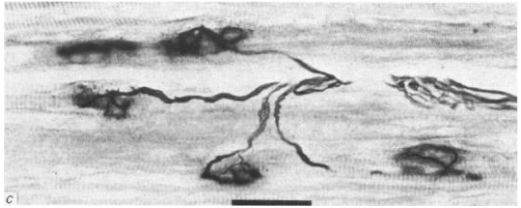
α-Ltx

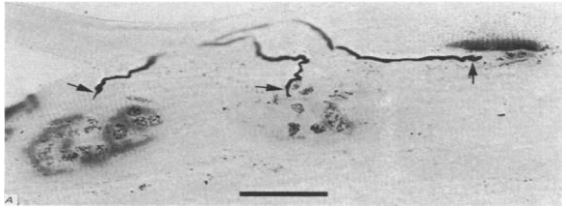
MASSIVE CALCIUM INFLUX



Pore forming toxin

DEGENERATION

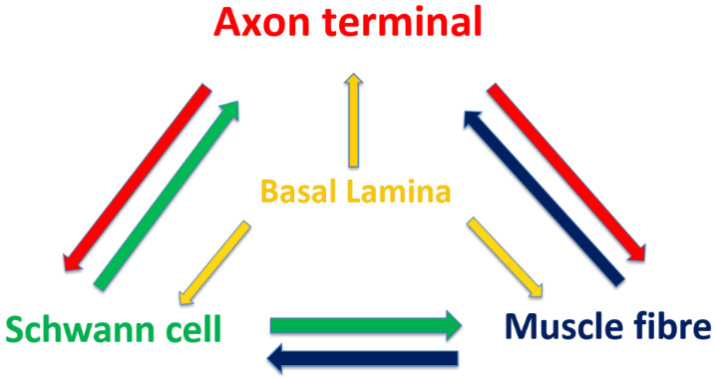




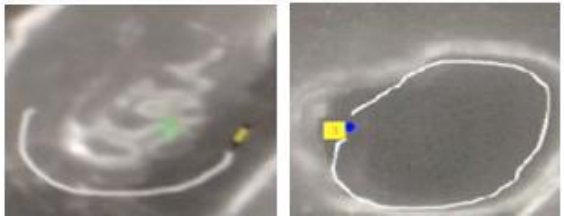
REGENERATION

within 1 week in mice

COME STUDIAMO I SEGNALI MOLECOLARI DELLA NMJ?



Transcriptomics restricted to NMJ collected by laser-capture microdissection (LCM)



transcriptomic profiling of NMJ → Computational biology

↓

Candidate hits

↓

Functional characterization

	A	B	C	D	E	F	G	H	I	J	K	L
1	test_id	gene_id	gene	locus	sample_1	sample_2	status	value_1	value_2	log2(fold_c	test_stat	p_value
2	XLOC_00233	XLOC_00233	Mon2	chr10:12299	Ctrl	4Hours	OK	29,1474	0	#NOME?	#NOME?	5,00E-05
3	XLOC_01041	XLOC_01041	Nolc1	chr19:46075	4Hours	24Hours	OK	63,8325	0	#NOME?	#NOME?	5,00E-05
4	XLOC_02141	XLOC_02141	Smarca4	chr9:216161	Ctrl	96Hours	OK	0	14,8201	inf	#NOME?	0,0001
5	XLOC_00666	XLOC_00666	Slc39a14	chr14:70303	Ctrl	24Hours	OK	0	115,724	inf	#NOME?	0,00015
6	XLOC_01218	XLOC_01218	Cwc22	chr2:778956	4Hours	24Hours	OK	0	15,6738	inf	#NOME?	0,00015
7	XLOC_01306	XLOC_01306	Arhgef2	chr3:886162	4Hours	96Hours	OK	0	31,0794	inf	#NOME?	0,00015
8	XLOC_01296	XLOC_01296	Ccdc169,Spg	chr3:551121	Ctrl	96Hours	OK	0	45,3318	inf	#NOME?	0,00025
9	XLOC_02012	XLOC_02012	Rnh1	chr7:141160	4Hours	24Hours	OK	0	17,5514	inf	#NOME?	0,00035
10	XLOC_00863	XLOC_00863	Adamts10	chr17:33524	Ctrl	4Hours	OK	204,606	0	#NOME?	#NOME?	0,0004
11	XLOC_00196	XLOC_00196	Rnf146	chr10:29313	Ctrl	96Hours	OK	0	32,7395	inf	#NOME?	0,0008
12	XLOC_00262	XLOC_00262	P4ha2	chr11:54100	Ctrl	24Hours	OK	0	86,1978	inf	#NOME?	0,001
13	XLOC_01394	XLOC_01394	Prkacb	chr3:146729	Ctrl	24Hours	OK	0	11,6317	inf	#NOME?	0,0012
14	XLOC_00834	XLOC_00834	Runx1	chr16:92601	24Hours	96Hours	OK	21,8446	0	#NOME?	#NOME?	0,00155
15	XLOC_00254	XLOC_00254	Ebf1	chr11:44618	Ctrl	4Hours	OK	31,3095	0	#NOME?	#NOME?	0,0022
16	XLOC_00840	XLOC_00840	Pde10a	chr17:85268	Ctrl	24Hours	OK	18,1828	0	#NOME?	#NOME?	0,0025
17	XLOC_00534	XLOC_00534	Homer1	chr13:93304	24Hours	96Hours	OK	0	12,4601	inf	#NOME?	0,00255
18	XLOC_01083	XLOC_01083	Ablim1	chr19:57033	24Hours	96Hours	OK	4,51912	237,518	5,71585	1,91289	0,00255
19	XLOC_01537	XLOC_01537	Mib2	chr4:155654	4Hours	96Hours	OK	0	25,8462	inf	#NOME?	0,00275
20	XLOC_02356	XLOC_02356	Trmt2b	chrX:134222	Ctrl	24Hours	OK	0	13,2908	inf	#NOME?	0,0029
21	XLOC_01148	XLOC_01148	Sema6d	chr2:124089	Ctrl	4Hours	OK	16,6776	1,00431	-4,05363	-1,21566	0,00385
22	XLOC_00308	XLOC_00308	Rara	chr11:98937	24Hours	96Hours	OK	239,357	0	#NOME?	#NOME?	0,0041
23	XLOC_02356	XLOC_02356	Pcdh19	chrX:133582	4Hours	24Hours	OK	15,745	0	#NOME?	#NOME?	0,00415
24	XLOC_00733	XLOC_00733	Eef1d	chr15:75894	Ctrl	4Hours	OK	222,283	0	#NOME?	#NOME?	0,00425
25	XLOC_00489	XLOC_00489	Gtf2a1	chr12:91555	Ctrl	24Hours	OK	0	56,9361	inf	#NOME?	0,0043

"I veleni possono essere usati come mezzi di distruzione della vita o come agenti per il trattamento di malattie"

Claude Bernard

I veleni possono essere utilizzati anche come mezzi per studiare processi fisiologici delle cellule e dei tessuti

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