The pathophysiological mechanisms of ciguatera

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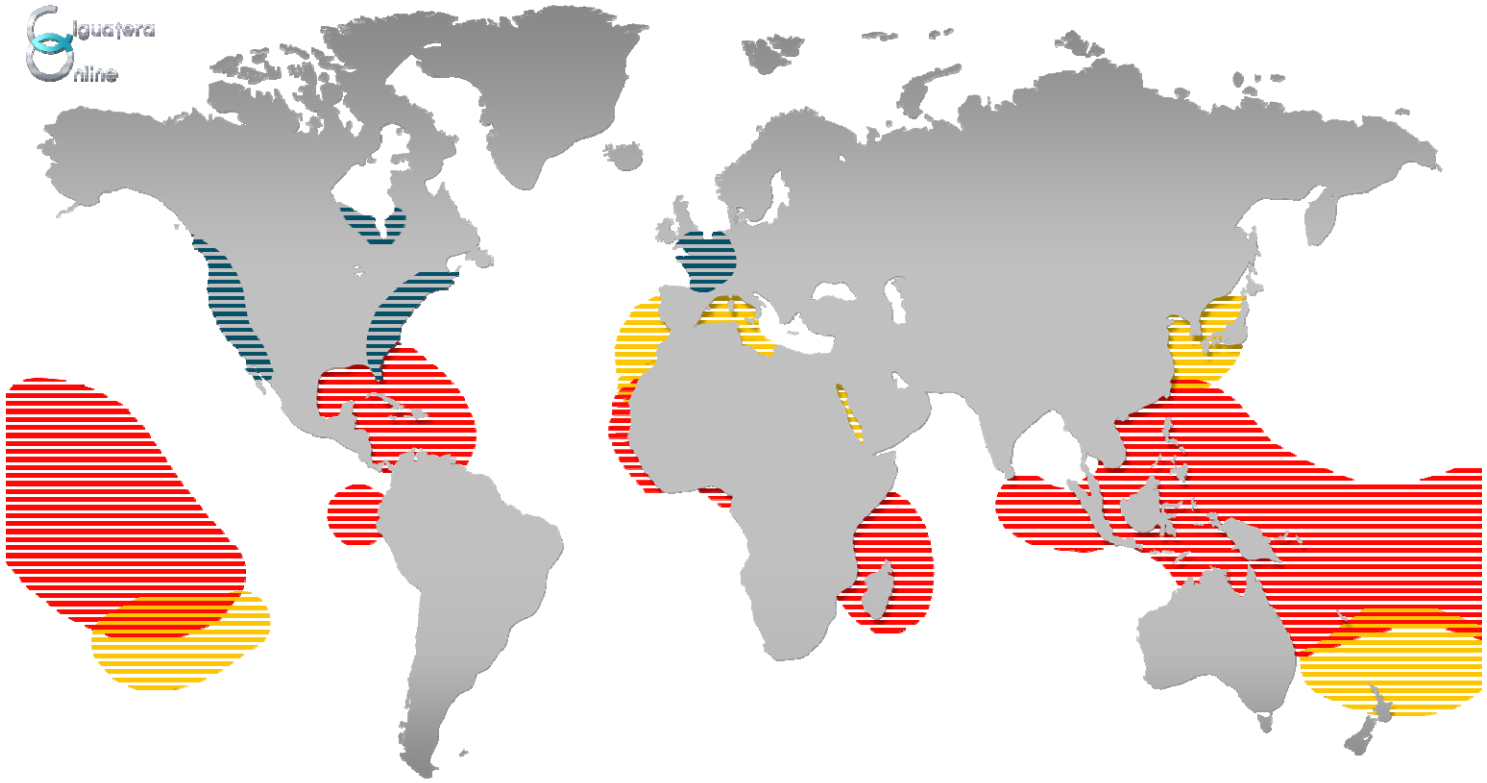
Sensory Neuropharmacology Group

- *Peripheral mechanisms of pain*
- *Neuropharmacology*
- *Analgesic drug discovery*

What is ciguatera?

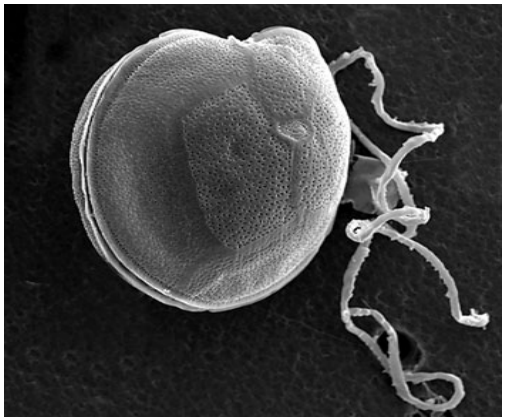
- The most commonly reported marine toxin disease in the world
  - Associated with consumption of reef fish contaminated with ciguatoxin
  - 50 000 people/year (?)
  - Under-recognised in non-endemic areas
    - Mis-diagnosed as multiple sclerosis, chronic fatigue syndrome, bacterial/viral food poisoning
  - Under-reported in endemic areas

Ciguatera distribution



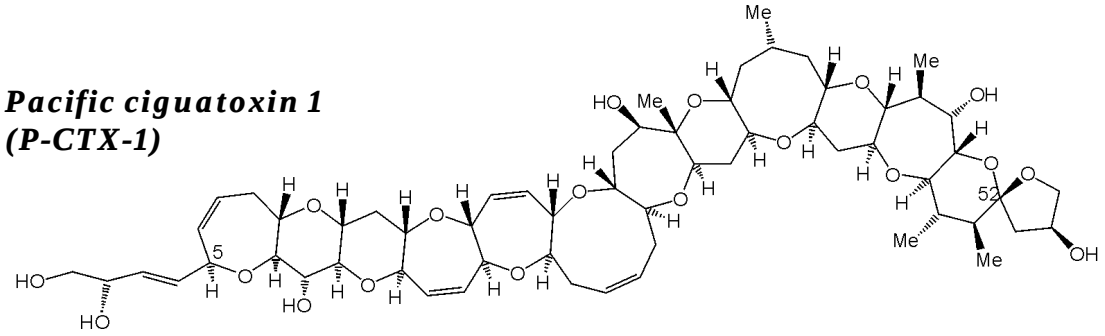
What are ciguatoxins?

- Lipid soluble polyethers
- Resistant to heat, freezing, stomach acid
- Produced by benthic dinoflagellates
  - *Gambierdiscus* spp.
- Named according to origin:
  - Pacific ciguatoxin
  - Caribbean ciguatoxin
  - Indian ciguatoxin

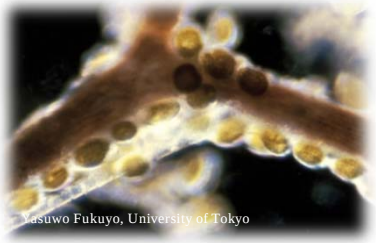


Gambierdiscus toxicus

Pacific ciguatoxin 1 (P-CTX-1)



Bioaccumulation of ciguatoxins



1. ***Gambierdiscus* spp blooms**
   - G. *toxicus*
   - *G. pacificus*
   - *G. polynesiensis*
   - *G. australes*

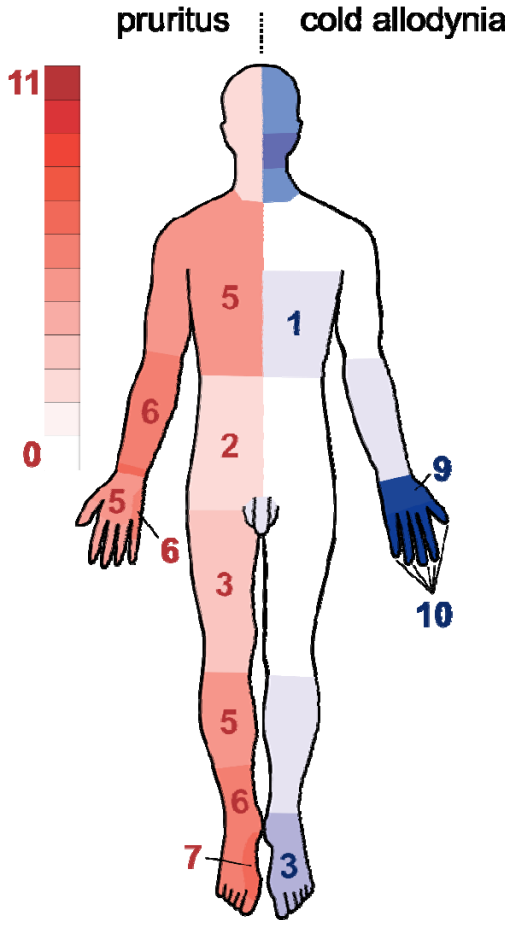
2. ***Herbivores***
   Feed off algae contaminated with *Gambierdiscus* spp

3. ***Carnivores***
   Feed on herbivores contaminated with ciguatoxins

Ciguatera
(> 0.1 micrograms CTX)

Ciguatera symptoms

Symptom	Onset	Frequency	Duration
Gastrointestinal	30 min – 48 h		Up to 1 week
Diarrhoea		50-77%	
Nausea/vomiting		26-82%	
Abdominal pain		43-75%	
Cardiovascular			
Slow heart beat		12%	
Low blood pressure		16%	
Neurological	Delayed (< 3 days)		Weeks-months
Numbness/tingling		64-100%	
Diffuse/localized pain		56-83%	



Mattei et al; Toxicon. 2014 Dec;91:76-83.

Mechanism of action of ciguatoxin

- Ciguatoxin is the most potent known activator of voltage-gated sodium channels
  - Proteins in neurons that are crucial for electrical transmission of signals
  - Nine human isoforms ($Na_V1.1$-$Na_V1.9$)
    - **blocked** by local anaesthetics
  - Crucial for normal function of pain pathways

Vetter, 2015, 1664

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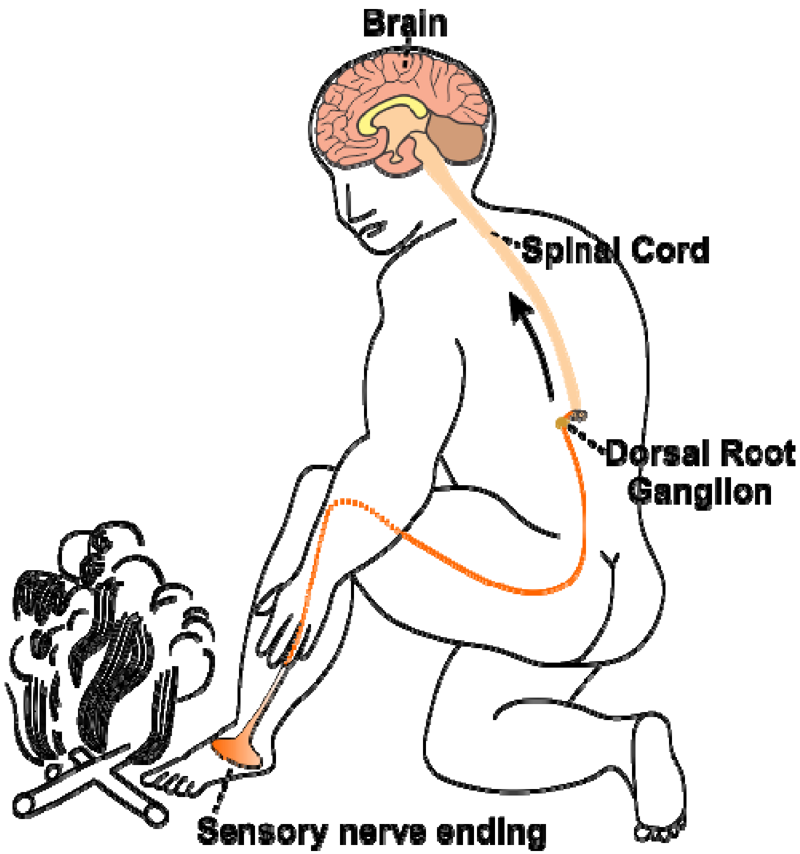
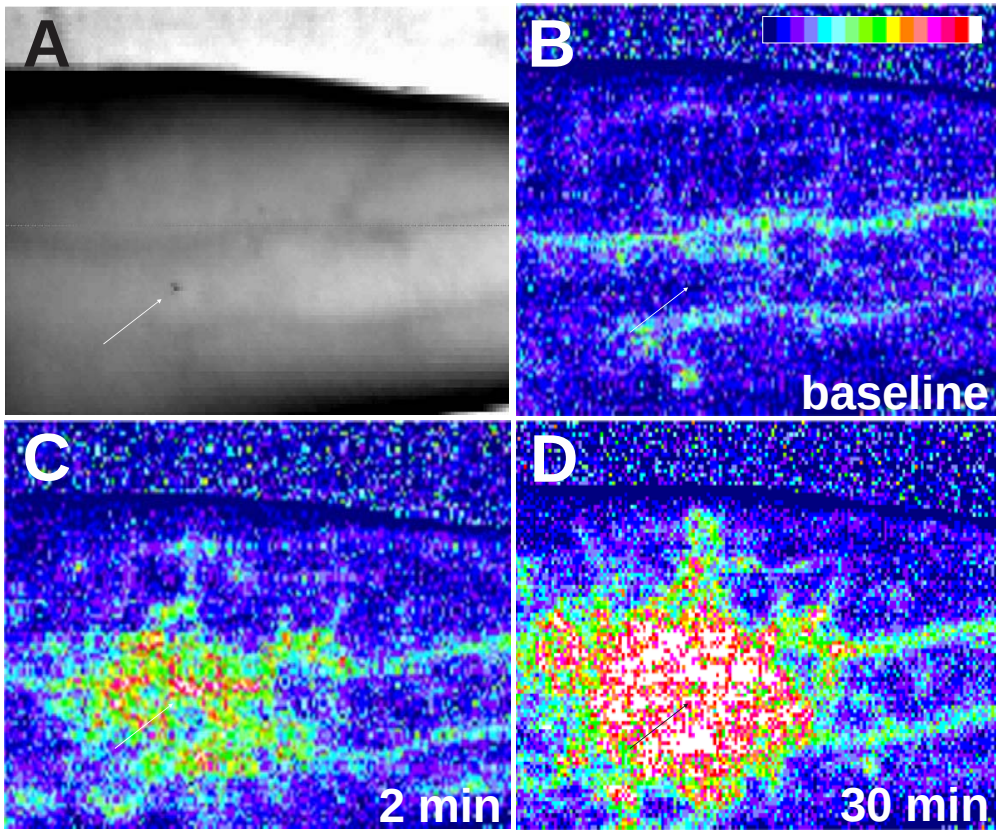


Vetter, 2015
Descartes, 1664

Ciguatoxin directly acts at peripheral nerve endings

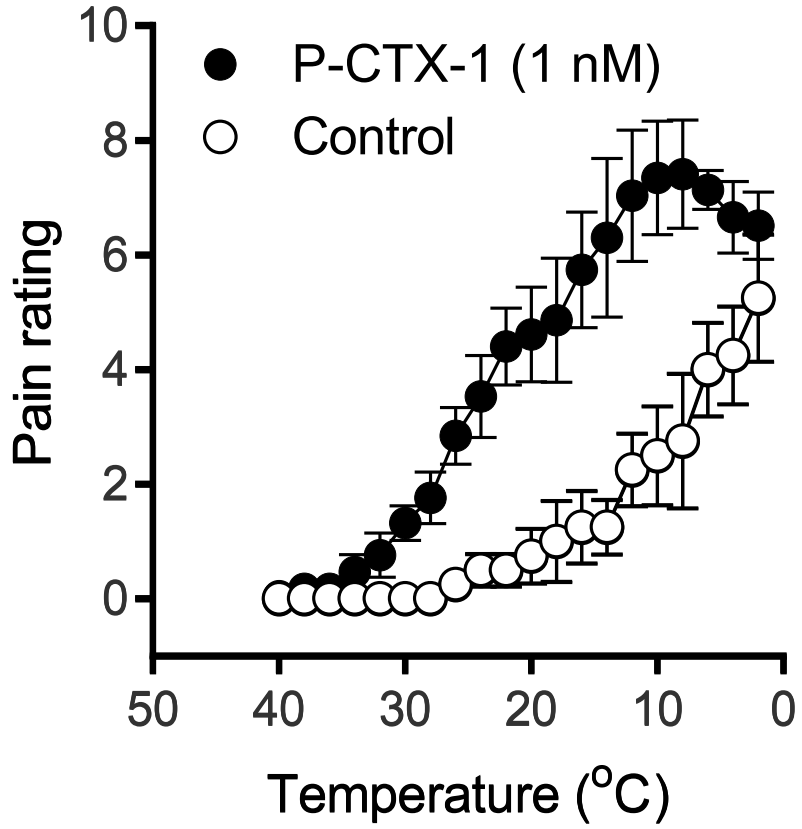
- Intradermal injection of CTX (~ 50 picograms) in humans
  - Burning pain
  - “flare” – similar to bee sting



Pain. 2013 Oct;154(10):1999-2006.

Intradermal ciguatoxin causes cold allodynia

- Local injection of ciguatoxin in the skin causes hypersensitivity to cooling
- “cool” becomes “painful”
- Provides evidence that effects at peripheral nerve endings are responsible for ciguatera symptoms



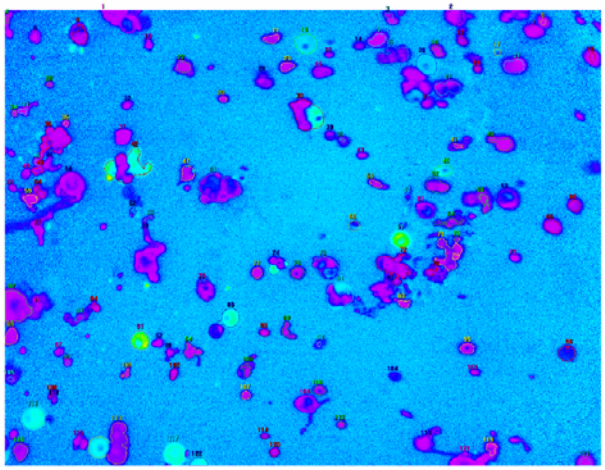
Pain. 2013 Oct;154(10):1999-2006.

Which types of neurons mediate effect of ciguatoxin?

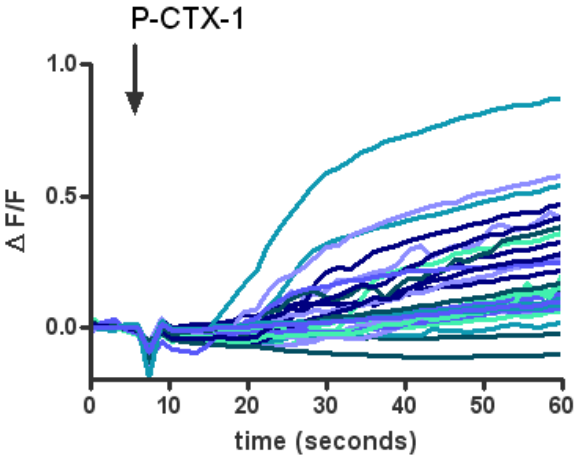
- Sensory neurons are very heterogeneous
  - Different sizes
  - Respond to different stimuli (hot, cold, mechanical)
  - Different functions
- Ciguatera has very unique symptoms
  - Probably mediated by specific subset of sensory neurons

  - Assessed responses of different sensory neurons to ciguatoxin

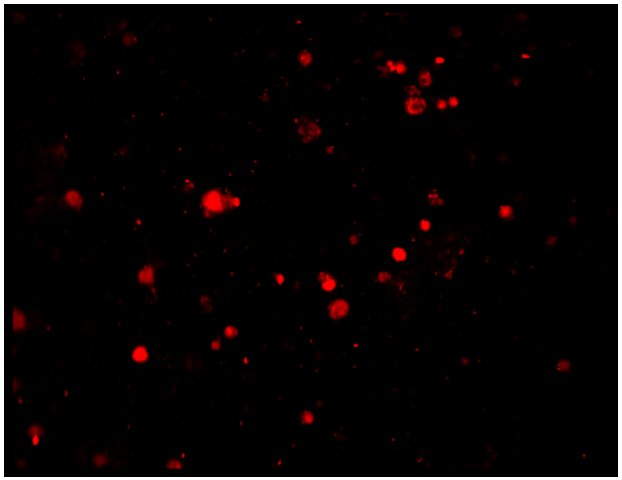
Which types of neurons are activated by ciguatoxin?



Grow sensory neurons in dish



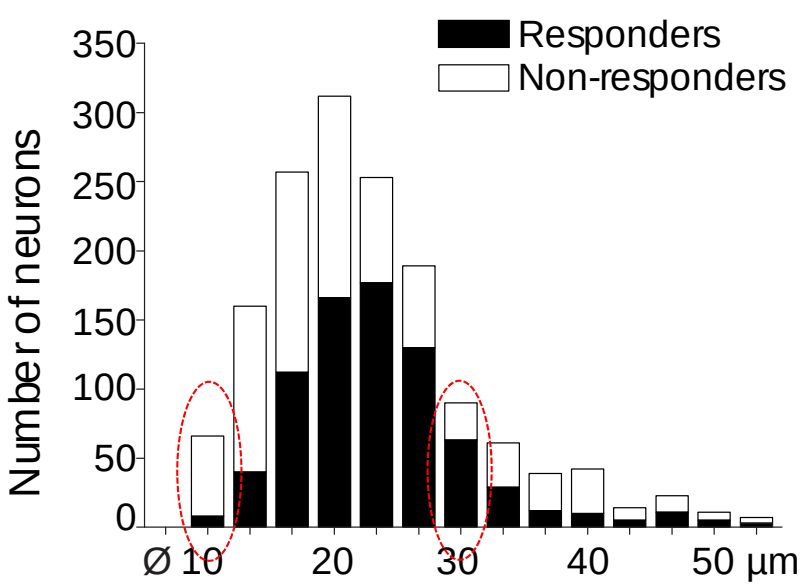
Measure response to ciguatoxin

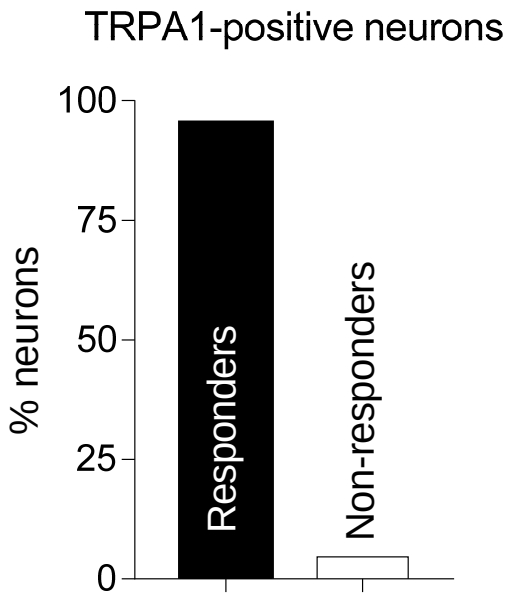


Identify neuronal subpopulations

Neuronal populations activated by ciguatoxin

- P-CTX-1 activates a subset of neurons
  - Varying sizes
  - Nearly all neurons expressing TRPA1 responded to ciguatoxin

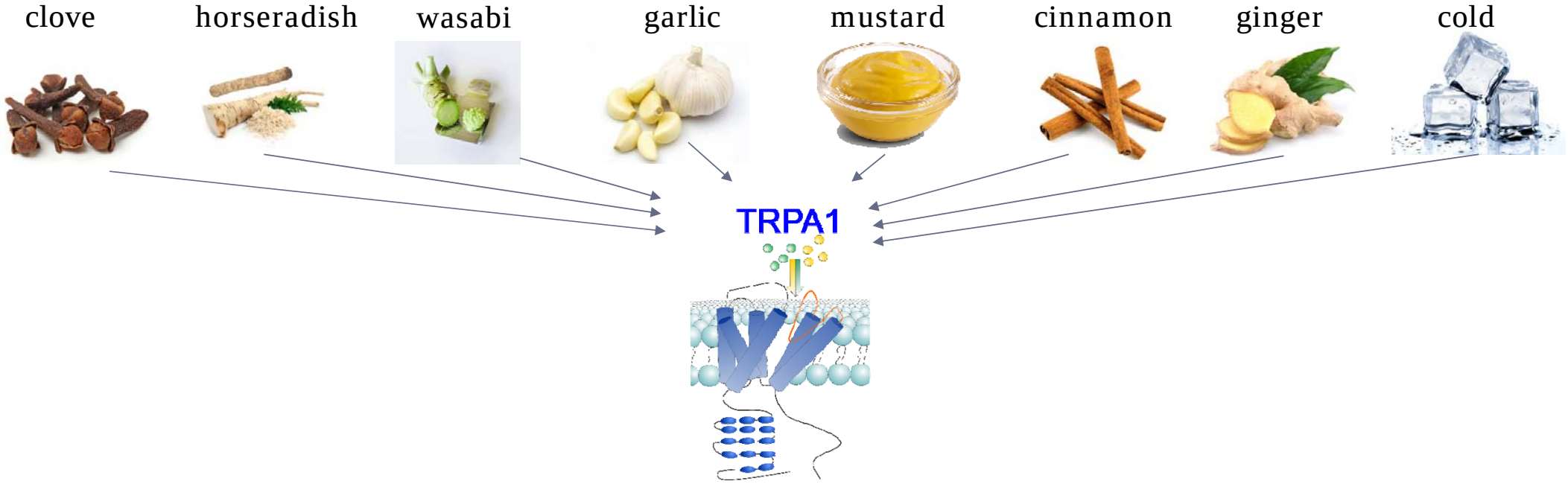




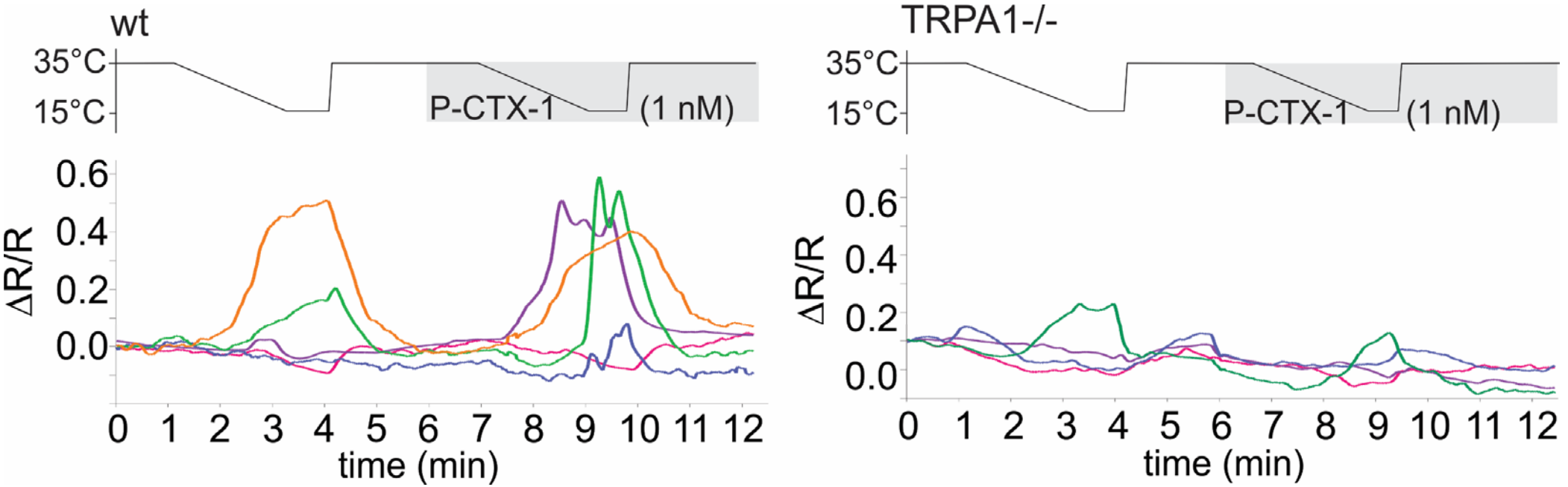
EMBO J. 2012 Oct 3;31(19):3795-808.

Ciguatoxin-induced cold pain involves TRPA1

- TRPA1 (transient receptor potential ankyrin 1)
  - Protein expressed in sensory neurons
  - Involved in sensing noxious chemicals & noxious cold



Ciguatoxin does not cause cold sensitivity in sensory neurons without TRPA1

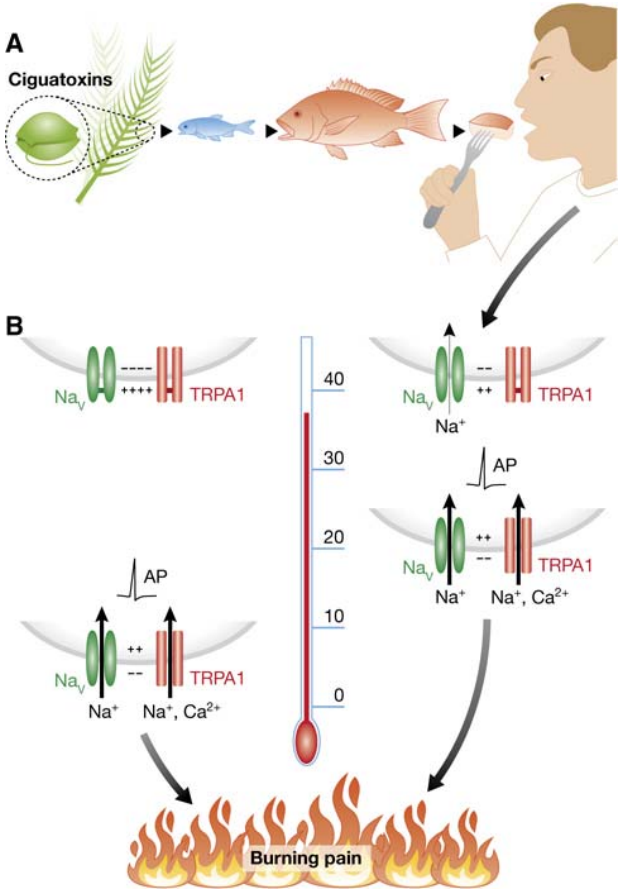


EMBO J. 2012 Oct 3;31(19):3795-808.

Mechanism of ciguatoxin-induced cold pain

- Ciguatoxin-induced cold pain
  - Increased excitability of sensory neurons
    - Voltage-gated sodium channels
  - Activation of cold-sensitive TRPA1 channels

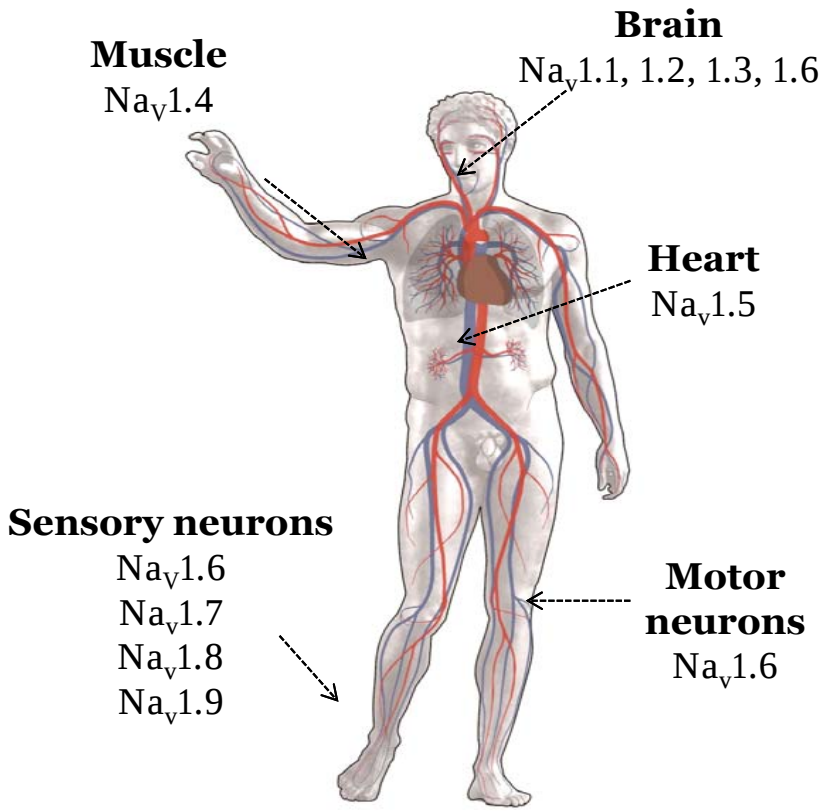
- Blockers of voltage-gated sodium channels or TRPA1 channels might be beneficial for treatment of ciguatera



EMBO J 2012 31:3785–3787

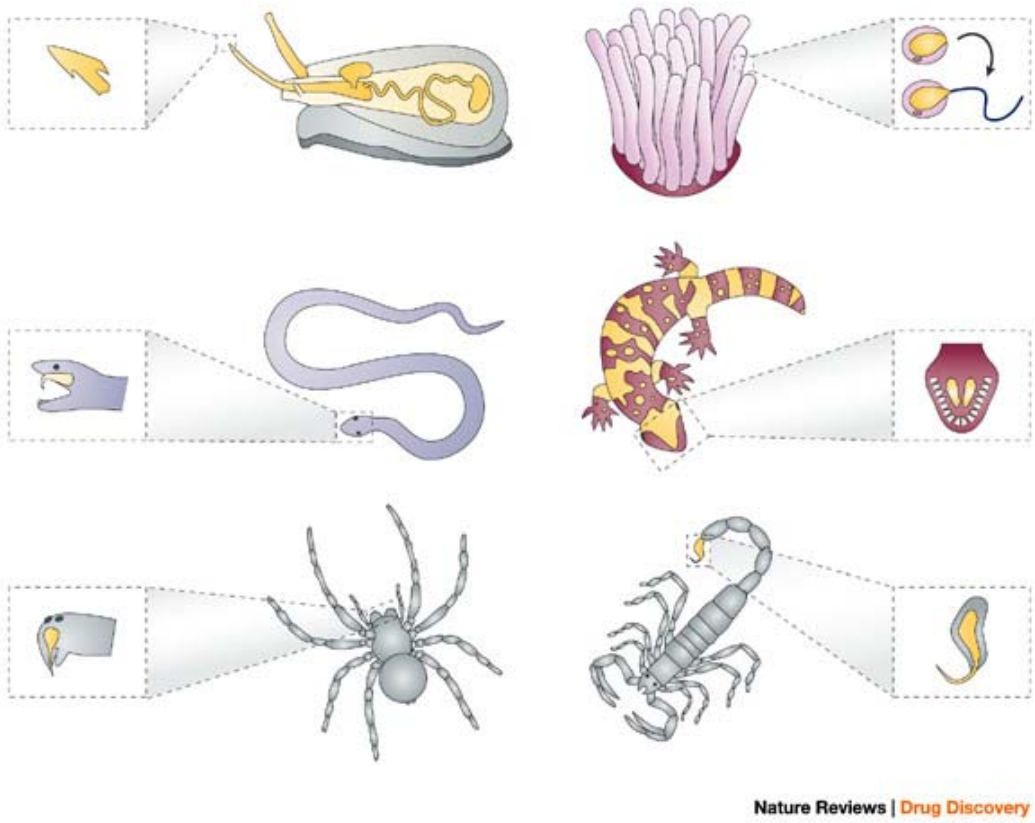
Which sodium channels need to be blocked?

- 9 isoforms:
  - $Na_V1.1$ – brain
  - $Na_V1.2$ – brain
  - $Na_V1.3$ – brain
  - $Na_V1.4$ – skeletal muscle
  - $Na_V1.5$ – heart
  - **$Na_V1.6$ – sensory/motor neurons, brain**
  - **$Na_V1.7$ – sensory neurons**
  - **$Na_V1.8$ – sensory neurons**
  - **$Na_V1.9$ – sensory neurons**
- Need selective blockers:
  - Venoms and toxins!



Venoms as sources of subtype selective sodium channel blockers

- Venoms have evolved to rapidly incapacitate prey/predators
- highly complex mixtures of bioactive compounds
  - small molecule, peptide, protein
  - 100s or 1000s of individual components
  - Highly potent & highly selective
  - Thousands of venomous species



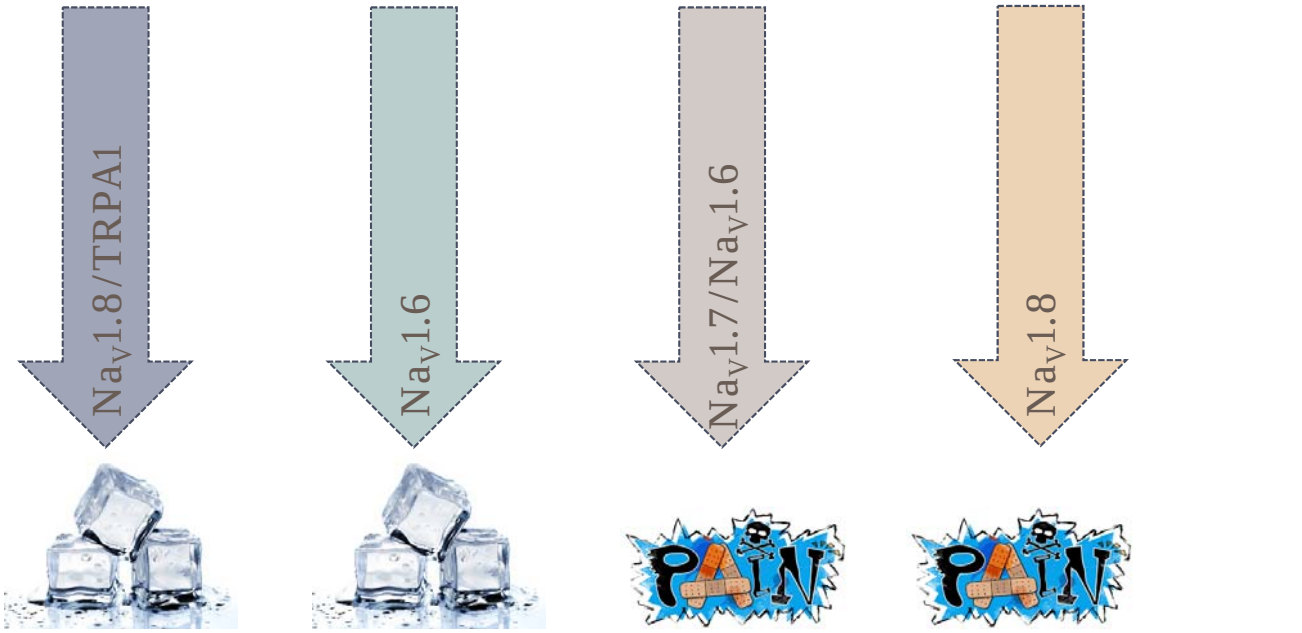
Lewis et al Nature Reviews Drug Discovery 2, 790-802 (October 2003)

Which sodium channels need to be blocked?

	Tetrodoxin (TTX)	μ-conotoxin GIIIA	μ-theraphotoxin Pn3a
			
$Na_V1.6$	✓	✓	✗
$Na_V1.7$	✓	✗	✓
$Na_V1.8$	✗	✗	✗
$Na_V1.9$	✗	✗	✗

Multiple sodium channel isoforms mediate the symptoms of ciguatera

Different sensory neurons & sodium channels mediate different symptoms



Ciguatera

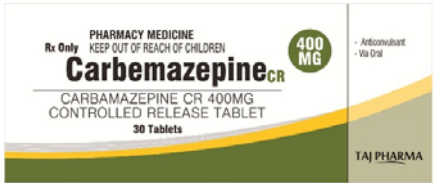
Treatment approaches for ciguatera

- No validated treatments available
  - Intravenous mannitol (to reduce neuronal swelling)
    - Within 48 h – no proven efficacy in clinical trials
  - Cholestyramine (bile-acid binding resin)
    - No clinical trials
  - Which sodium channel blockers might be useful?

Repurposing existing drugs

- Several drugs with the required channel blocking activity already exist:

amitriptyline



carbamazepine

flupirtine

lamotrigine

mexiletine

phenytoin

topiramate

Pain. 2013 Oct;154(10):1999-2006.

Repurposing existing drugs

- Several drugs with sodium channel blocking activity already exist:

lamotrigine

flupirtine

phenytoin

- Flupirtine, lamotrigine and phenytoin may be useful to treat ciguatera
  - Clinical studies need to be carried out to validate therapeutic effect

Pain. 2013 Oct;154(10):1999-2006.

Acknowledgements

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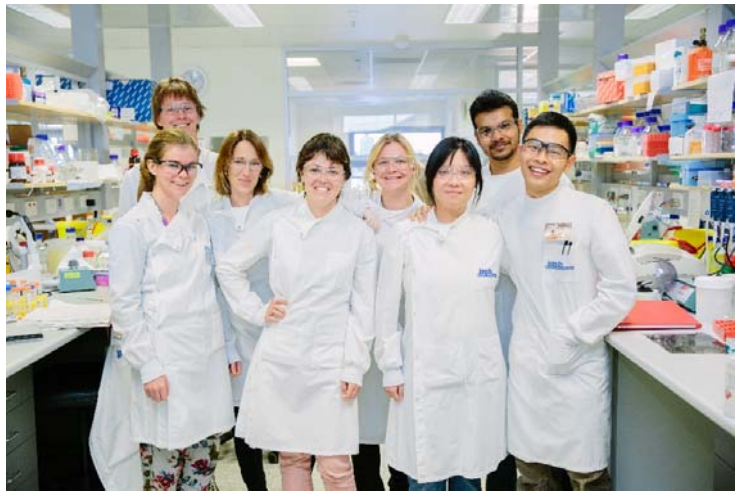

Collaborators

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  - Dr Edouard Delcroix & selection committee

Summary

- Specific subtypes of sensory neurons mediate diverse symptoms of ciguatera
- Ciguatoxin acts through different mechanisms in each subtype
- Treatment with existing drugs (eg. Flupirtine, lamotrigine) may be useful for ciguatera