"...he heard himself pronounced dead, and remembered being placed in a coffin and buried..."

-Revelations of a voodoo zombie

Assigned Reading:

Ion-Selective Channels Allow Rapid Movement of ions across membranes
The Neuronal Na⁺ Channel is a Voltage-Gated ion Cannel
Defective Ion Channels Can Have Adverse Physiological Consequences

Ion-Selective Channels Allow Rapid Movement of Ions across Membranes
Gated Ion Channels Are Central in Neuronal Function
Defective Ion Channels Can Have Severe Physiological Consequences
Voltage-Gated Ion Channels Produce Neuronal Action Potentials

Ethnobiologist Wade Davis claims that "Zombies" are truly produced by Haitian voodoo sorcerers, or "bokor" by the administration of sub-lethal doses of Tetrodotoxin (TTX) extracted from Pufferfish. Innocent victims are not chosen at random for "zombification". Rather, a formal sentence imposed upon perceived wrongdoers and malefactors by the secret societies—a direct offshoot of similar powerful societies in West Africa.

Davis' claim is not universally accepted, but, if it is true, then zombification is based on the more widely encountered syndrome "fugu intoxication".

Pertinent references to the controversial TTX-Zombie connection:

"The ethnobiology of the Haitian zombi"

Voodoo Science

Zombification

Davis has also written several popular accounts of his Haitian field work:

W. Davis (1985)
The Serpent and the Rainbow
Simon and Schuster, New York

W. Davis (1988)
Passage of Darkness
Univ. of North Carolina Press, Chapel Hill
Fugu Intoxication and the Year of Eating Dangerously

In Japan, the culinary "delicacy" fugu, is prepared from Pufferfish by specially licensed chefs, and can cost as much as $400 per serving. Tetrodotoxin has long been known to exist in high concentrations in the ovaries, liver, skin, and other tissues of many pufferfishes of the family Tetraodontidae (whence the toxin's name), particularly in species of the genus Fugu.

Pufferfishes are most dangerous just prior to and during their reproductive season, when, you guessed it, their flavor is said to be most delicious. The toxin is not inactivated by cooking, and despite the great care taken in the preparation of fugu, fatal poisonings are not uncommon. Authorities estimate that between 100 and 200 people become seriously intoxicated each year. About half of them die, even with treatment.

Symptoms of fugu intoxication usually appear within an hour after eating the toxic fish. One victim in a 1996 California incident became ill while still eating his third bite. Symptoms include weakness, dizziness, pallor, drastically lowered blood pressure and body temperature. In severe cases the victims turn blue and are completely paralyzed. The eyes become fixed, dilated, and glassy and the patient is unable to move or speak. Vital signs may cease to be apparent even to an experienced physician. In other words, the victim appears to be dead. Amazingly, the victim of TTX intoxication remains fully conscious throughout this period, probably until shortly before death. Convulsions and respiratory failure follow within six to 24 hours of intoxication in terminal cases. TTX affects myelinated, or sheathed, peripheral nerves but does not appear to cross the blood-brain barrier. Thus, although the nerves innervating the heart and lungs are affected, consciousness and mental functions are typically unimpaired.

Several cases in which victims of fugu intoxication were erroneously declared dead by competent physicians have been described in the Japanese medical literature.
Structure and Biosynthesis of TTX

TTX is a medium-sized molecule with non-aromatic 6-membered rings. The rings are fused in an unusual 3-D “superstructure” which implies that, for a molecule of its size, TTX should have a limited range of conformational states.

A protonated **GUANIDINIUM** group protrudes from the superstructure and is thought to mimic a hydrated Na ion.

The model for TTX biosynthesis shown below envisions that the guanidinium group is derived from the R-group of Arginine.
**Mode of Action**

TTX is > 10,000 times more toxic than cyanide. Such high toxicity is unusual in a non-enzymatic toxin. (LD-50 in the mouse is 10 nanograms!)

TTX blocks neuronal action potentials (AP) by inhibiting the “Voltage-Gated” Sodium Ion Channel (SIC).

In the propagation of an AP each SIC opens for approximately 1 millisecond in response to local membrane "depolarization". This is long enough to allow passage of some 7,000 Na⁺. Furthermore, the channel is very selective for Na⁺ relative to K⁺.

**Structure of the Voltage-Gated Na Ion Channel**

The structure and mode of action of the SIC has been difficult to elucidate, which is not unusual for integral membrane proteins. Numerous studies have used TTX as a “probe” to help elucidate SIC structure and function.

The SIC is a single polypeptide chain folded into 4 X 6 trans-membrane alpha helical segments. The helices aggregate into four similar clusters oriented around a central channel. Study Fig. 11-50a/12-26a in the text. The diagram shows the SIC viewed in the plane of the membrane (extracellular is up), but unrolled to lie flat on the page. The 4th helix in each of the 4 domains (dark blue) has many positively charged amino acids, and these are thought to be the "voltage sensor". The model suggests that when the local membrane voltage depolarizes, conformational shifts in these 4 helices are transmitted to the gate, causing it to open.

Fig. 11-50a/12-26a in the text shows the SIC rolled back up into its true conformation—be sure you understand the relation between parts a. and b. of the Figure. The hydrophilic central channel contains 4 "loops" with similar sequences of aspartic acid, glycine, lysine and alanine residues at similar positions that interact specifically with Na⁺ ions, and thus constitute a "selectivity filter".

Opening of the "gate" requires a dramatic conformational shift of the linker between Domains III and IV. Note in the text’s diagrams that the conformation of the gate in part b. is more realistic than shown in part c.

In free solutions Na⁺ is associated with 6 waters of hydration. However, the ion binds to the SIC as the tetrahydrate. 2 of the waters present in free solution have been displaced from the ion and are replaced by H-bonds formed to carboxyl groups in the selectivity filter domain of the protein. [M. Noda, et al. (1996) Nature 320, 188.]
The guanidinium group of TTX mimics the Na⁺ ion and tenaciously binds to the channel entrance by interacting with the same carboxyl groups (Asp364 and Glu942) as Na⁺.

![Image of TTX binding site](image_url)

Additional H-bonds between carboxyl groups on the protein and oxygens in TTX further stabilize the binding. These must be very important because a single point mutation of glutamic acid 387 to glutamine (E387Q) results in complete resistance to TTX inhibition. Thus, the TTX molecule forms a cap or plug that blocks the mouth of the sodium channels.

Binding of TTX to the channel is tight (Kd = 10⁻¹⁰ nM). Whereas the hydrated sodium ion binds reversibly on a nanosecond time-scale, tetrodotoxin is bound for tens of seconds (i.e. much longer than the duration of an AP).

Phylogenetic Distribution of TTX Producing Animals

TTX production is widely but sporadically distributed.

The blue-ring ed octopus (*Hapalochlaena maculosa*) has been responsible for numerous human deaths. It employs TTX as both a defense against predators and as a way to immobilize prey.

In the 1930s, Victor C. Twitty, an experimental embryologist at Stanford University, discovered accidentally that three species of West Coast newts of the genus *Taricha* produce TTX. He transplanted eye vesicles from *Taricha torosa* embryos into embryos of another species that does not produce TTX. The embryos died from exposure to TTX produced in the transplanted eyes.

At least one undergraduate student in California died of TTX poisoning after swallowing a newt as part of a fraternity initiation ceremony.

Central American toads of the genus *Atelopus* (often called “harlequin frogs”) possess TTX.

TTX also occurs in a number of marine invertebrates. They represent several, widely divergent phyla, including seastars, several species of xanthid crabs, a horseshoe crab, a number of marine snails, as well as a flatworm, and a South Atlantic “sea squirt.” Two species of ribbonworms and four species of arrow worms contain TTX in their venoms, which they use to subdue their prey. The toxin also has been isolated from a marine red alga.
Bacterial Symbionts as a Source of Animal TTX

The unusual phylogenetic distribution of TTX production poses apparent difficulties for natural selection. It seems unlikely that all of these unrelated organisms independently evolved the enzyme or enzymes required to synthesize the same extraordinary molecule. Nor does it seem likely that TTX biosynthesis could be an ancestral trait, or it would be much more widely distributed in all groups.

In 1981 T. Matsui, reported that pufferfish raised in captivity did not contain TTX. This suggested that TTX may be the product of a microbial symbiont.

Tetrodotoxin is also not detectable in frogs of the genus *Atelopus* if they are raised in captivity.

Daly, J. W. et al. (1997) Toxicon 35, 705

TTX has been identified in pure laboratory cultures of a bacterium isolated from the intestine of the puffer fish. The bacterium was identified as a member of the genus *Vibrio*.


Apparently there are also TTX-producing bacteria that are animal pathogens. A TTX-producing marine bacterium (*Pseudoalteromonas haloplanktis*) was responsible for large-scale die-off of sea urchins in the Caribbean.


There are also reports implicating bacteria in the synthesis of TTX in horseshoe crabs, seastars, octopuses, and arrow worms. I have not seen any evidence that bacteria are responsible for TTX production in California newts, but this would not be surprising.
TTX in Predator-Prey Co-Evolution


Populations of the garter snake *Thamnophis sirtalis* have evolved geographically variable resistance to TTX in a coevolutionary arms race with their toxic prey, newts of the genus *Taricha*. i.e. *T. sirtalis* populations outside the restricted geographic range of the newts demonstrate are TTX-susceptible.

TTX resistance in *Thamnophis* is due to a point mutation in the gene for the SIC. The trade-off is that TTX resistance is associated with slower physiological response.
STUDY QUESTIONS

1. What features distinguish ion channels from ion transporters (pumps)?

2. What are the molecular mechanisms of ion channel gating?

3. Draw the chemical structure of the “guanidino” or “guanidinium” functional group. Which amino acid has this group?

4. Describe the function of the protein inhibited by tetrodotoxin. (You do not need to include a discussion of the cellular or physiological systems this protein is part of. Just describe how the protein itself works.)

5. What other ion-gated channels (in addition to the Na⁺ channel) operate in the conduction of neuronal action potentials?

6. When a sodium channel opens, roughly how long does it remain open? Roughly how many sodium ions cross the channel during this time?

7. Saxitoxin (STX) is a neurotoxin naturally produced by certain species of marine dinoflagellates and cyanobacteria. Ingestion of saxitoxin (usually through shellfish contaminated by toxic algal blooms) is responsible for the human illness known as paralytic shellfish poisoning (PSP).

   Propose a molecular/biochemical mode of STX action.