Neurotoxin case for first year medical students – Team Based Learning Format - instructor

I. **Title:** Neurotoxicity

II. **Purpose:** By the conclusion of this TBL module, students will be able to describe the basic divisions of the autonomic nervous system and list the primary target tissues of each division. Students should also have an understanding of muscle anatomy and physiology, and be able to recognize the signs and symptoms of neurotoxicity.

III. **The Learning Objectives:**
   a. Learn to work in small groups effectively to solve a clinical problem.
   b. Describe basic skeletal muscle anatomy and physiology, including sarcomere structure and the contractile process.
   c. Describe the structure and function of motor units and neural junctions.
   d. Describe the basic neurophysiology of neurotransmitter release in a neuromuscular junction.
   e. List the divisions of the autonomic nervous system and their primary target tissues, target receptors, neurotransmitters, and general actions of each division.
   f. Identify cell structures on neuromuscular histological images

IV. **Advanced Preparation Assignment:** This TBL module was designed to be used early in the first year of medical school after the basic cell biology/histology and neuroscience courses have been introduced. The materials for this module can be found in *Rhodes and Tanner; Medical Physiology, 3rd edition*, in chapters 1, 3, 5, 6 and 8. It is intended to assess and overall understanding of basic cell biology and neuromuscular physiology.

V. **Readiness Assurance Questions:** IRAT and GRAT

1) Which of the following statements concerning neuron and neuronal structure is correct?
   *) Microtubule and neurofilaments are found in soma, dendrite and axon.
   B) Dendrites conduct impulse from the cell body towards the effector organ.
   C) Some neurons have two axons.
   D) There are basophilic granular structures within axon called Nissl bodies.
   E) Bipolar neurons do not have axons.

2) Which of the following statements concerning peripheral nerves is correct?
   A) The connective tissue coat that covers each individual peripheral nerve is called perineurium.
   B) Peripheral nerve is made of bundles of tracts.
   C) Peripheral nerve can be found in brain cortex.
   D) Unmyelinated nerve fibers can not be found in peripheral nerve.
   *) One peripheral nerve could contain both sensory and motor nerve fibers.

3) Increased activity of the neurotransmitter dopamine at synapses in the mesolimbic dopamine system is known to cause euphoria. Therefore a good strategy for the development of new euphoriant is to develop drugs that:
   *) Inhibit the sodium cotransporter for dopamine in the nerve terminal
   B) Inhibit the transporter that pumps dopamine into synaptic vesicles
   C) Inhibit tyrosine hydroxylase
   D) Induce enhanced expression of catechol-O-methyl-transferase (COMT)
E) Block voltage-gated calcium channels in the nerve terminals

4) The structure below is

\[
\begin{align*}
\text{HO} & \\
\text{HO} & \\
\text{NH}_3^+ & \\
- & \\
\text{COO}^- & \\
\end{align*}
\]

A) a hormone formed from norepinephrine  
B) an indoleamine from the pineal gland  
C) a biologically inactive breakdown product of catecholamines  
D) the neurotransmitter GABA (γ-aminobutyric acid)  
*) the immediate precursor of dopamine

5) The peak of the action potential approaches the equilibrium potential for what ion in a typical neuron?  
A) calcium  
B) chloride  
C) potassium  
*) sodium  
E) magnesium

6) A 63 year old patient presents with marked muscle weakness of the lower limbs. He also recently has had increasing trouble swallowing food and climbing stairs. However, he says that his symptoms often improve as the day progresses. You suspect which of the following?  
*) an autoimmune disorder that targets pre-synaptic voltage gated calcium channels  
B) an autoimmune disorder that targets nicotinic acetylcholine receptors  
C) an autoimmune disorder that targets muscarinic acetylcholine receptors

7) Which of the following chemicals would reduce neurotransmitter release at the neuromuscular junction synapse?  
*) botulinum toxin  
B) muscarine  
C) atropine  
D) curare  
E) pyridostigmine

8) Extrafusal fiber is innervated by  
A) Sensory fibers  
*) Somatic motor fibers  
C) Sympathetic nerve fibers  
D) Parasympathetic nerve fibers  
E) Both sensory and somatic motor fibers
9) A 13-year-old boy presents with episodes of extreme proximal muscle weakness lasting an hour or more. The episodes are precipitated by rest following strenuous exercise. His father and paternal grandmother have suffered similar symptoms with onset at adolescence. An electromyogram, performed during an attack, reveals normal skeletal muscle action potentials in response to effort. It is not surprising that the disease is associated with
A) a mutation in the voltage sensitive K+ channel of skeletal muscle.
B) elevated PIP2 in skeletal muscle.
C) depressed PIP2 and elevated IP3 in gamma-motoneurons of the peripheral nerves.
D) a mutation in acetylcholine esterase.
*) a mutation in the voltage sensitive Ca2+ channel of skeletal muscle.

VI. **Group Application Exercise:** A Mysterious Case of Poisoning

Pedro, an immigrant field worker from Mexico, woke up to a sharp pain in his leg at 11pm. There was a red welt forming where he felt the pain, which made him look around the sheets for an insect that may have bitten him, but he found nothing, so he lay back down to sleep. During the next hour Pedro began sweating and experiencing abdominal pain with diarrhea. It was 45 minutes later when Pedro felt dizziness, muscle weakness, and began vomiting, then he called 9-1-1. On his way to the emergency room, the paramedics asked Pedro a few questions. They discovered several interesting things that might help explain his symptoms.

First, Pedro’s friends had taken him to a Sushi restaurant to celebrate his birthday that evening, during which time he ate a large variety of sushi and drank Saki. Secondly, Pedro worked each day in a field picking produce. The paramedics asked whether pesticides were used in the fields where he worked, and Pedro replied “yes, and I even mix and spray them myself sometimes”. Pedro told them the names of the pesticides he mixed, at which time the paramedics identified one as a neurotoxin. And thirdly, Pedro had a suspicious looking red welt on his leg which the paramedics noted as a possible insect bite.

1. It seems as though Pedro has been exposed to some kind of toxin. Which of the toxin-actions below would most likely account for his symptoms?
   A) Blockage of the tetrodotoxin sensitive sodium channels.
   B) Blockage of ligand-gated sodium channels.
   C) Decreased activity of acetylcholinesterase.
   D) Increased activity of the choline transporters.
   E) Decreased activity of voltage-gated calcium channels.
2. On the images below, where would this toxin most likely be acting and why?

Answer Choice Explanations:

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**Choice A: blockage of the tetrodotoxin sensitive sodium channels:** Tetrodotoxin blocks fast acting voltage gated sodium channels expressed in neurons and human muscle cells. Though this could explain the patient’s muscle weakness, it would not account for his vomiting, abdominal, sweating, or diarrhea. Further, tetrodotoxin poisoning would produce mild to severe paralysis, particularly in the facial muscles, and not just general muscle weakness.

**Choice B: blockage of ligand gated sodium channels:** The autonomic effects observed in this patient (i.e. sweating, diarrhea, vomiting) must be due to some interaction with post-synaptic receptors associated with the autonomic nervous system, such as adrenergic or muscarinic receptors. These receptors are G protein coupled receptors, not ligand gated sodium channels.
Choice C: decreased activity of acetylcholinesterase: Insecticides cause reversible carbamylation of the acetylcholinesterase enzyme, allowing accumulation of acetylcholine (Ach) at parasympathetic neuroeffector junctions. This would prolong the activity of acetylcholine at both the neuromuscular junction, explaining the patient’s muscle weakness, as well as in the parasympathetic wing of the autonomic nervous system – which could explain the other signs and symptoms (sweating, abdominal pain, diarrhea, and vomiting) observed in this patient.

Choice D: Increased activity of the choline transporters: after acetylcholine is broken down in the synaptic cleft by acetylcholinesterase, choline is transported back into the pre-synaptic neuron, thus replenishing the pool of available choline for acetylcholine synthesis. This should have no bearing, however, on acetylcholine release from the presynaptic terminal, as neurotransmitter release is calcium dependent, not choline dependent. So, this choice is incorrect because all of the patient’s symptoms can be explained by an increase in acetylcholine in the neural junctions, and an increase in choline transporters will not produce this effect.

Choice E: Decreased activity of voltage gated calcium channels: This would result in reduced neurotransmitter release – reduced release of acetylcholine could result in muscle weakness, such as that observed in Lambert Eaton syndrome, but would not explain the autonomic symptoms, such as increased sweating and diarrhea, which would be due to increased activity in the parasympathetic nervous system.

2. On the images below, where would this toxin most likely be acting and why?

Choice A: The skeletal muscle fiber
Choice B: synaptic cleft, which is where acetylcholinesterase is localized.
Choice C: vesicles within the motor end plate
Choice D: pre-synaptic membrane
Choice E: post-synaptic membrane

**Additional information about spider bites (black widow):**

**Pathophysiology:** The venom of the black widow is a neurotoxin. It primarily causes systemic symptoms with little local damage at the bite site and no local necrosis. The venom mediates its effects through an initial release of massive amounts of acetylcholine at neuromuscular junctions. Latrotoxin is specific to nerve terminals; no direct release of transmitters from the adrenal medulla has been shown. As depolarization of the membrane occurs, a Ca\(^{+}\) dependent release of neurotransmitters down the concentration gradient ensues. Reuptake of the neurotransmitters appears to be blocked as well.

**History:** Initially, a severe pain in local muscle groups occurs, which then spreads to regional muscle groups. Severe cramps and contraction of musculature may extend throughout the body. The abdominal pains are frequently most severe, mimicking appendicitis, colic, or food poisoning. Other symptoms include headache, restlessness, anxiety, fatigue, and insomnia.

**Physical:** Signs of latrodectism include salivation, lacrimation, diaphoresis, tremors, tachycardia, bradycardia, hypertension, shock, and coma. Slight erythema, piloerection locally, mild edema or urtication, local perspiration, and lymphangiitis are the primary local features that may be present.

**Additional information about Tetrototoxin poisoning:**

The first symptom of intoxication is a slight numbness of the lips and tongue, appearing between 20 minutes to three hours after eating poisonous pufferfish. The next symptom is increasing paraesthesia in the face and extremities, which may be followed by sensations of lightness or floating. Headache, epigastric pain, nausea, diarrhea, and/or vomiting may occur. Occasionally, some reeling or difficulty in walking may occur. The second stage of the intoxication is increasing paralysis. Many victims are unable to move; even sitting may be difficult. There is increasing respiratory distress. Speech is affected, and the victim usually exhibits dyspnea, cyanosis, and hypotension. Paralysis increases and convulsions, mental impairment, and cardiac arrhythmia may occur. The victim, although completely paralyzed, may be conscious and in some cases completely lucid until shortly before death. Death usually occurs within 4 to 6 hours, with a known range of about 20 minutes to 8 hours.

**Additional information about Insecticide Poisoning (N-Methyl Carbamate):**

N-Methyl carbamate insecticides are widely used in homes, gardens, and agriculture. They share with organophosphates the capacity to inhibit cholinesterase enzymes and therefore share similar symptomatology during acute and chronic exposures. Likewise, exposure can occur by several routes in the same individual due to multiple uses, and there is likely to be additive toxicity with simultaneous exposure to organophosphates. However, due to the somewhat different affinity for cholinesterases, as compared to organophosphates, these poisonings are often somewhat easier to treat, as discussed later in this chapter.

**Signs and Symptoms:**
- Malaise, muscle weakness, dizziness, sweating,
- Headache, salivation, nausea, vomiting, abdominal pain, diarrhea
- CNS depression, pulmonary edema in serious cases
**Toxicology:**
The N-methyl carbamate esters cause reversible carbamylation of the acetylcholinesterase enzyme, allowing accumulation of acetylcholine, the neuromediator substance, at parasympathetic neuroeffector junctions (muscarinic effects), at skeletal muscle myoneural junctions and autonomic ganglia (nicotinic effects), and in the brain (CNS effects). The carbamyl-acetylcholinesterase combination dissociates more readily than the phosphoryl-acetylcholinesterase complex produced by organophosphate compounds. This lability has several important consequences: (1) it tends to limit the duration of N-methyl carbamate poisonings, (2) it accounts for the greater span between symptom producing and lethal doses than in most organophosphate compounds, and (3) it frequently invalidates the measurement of blood cholinesterase activity as a diagnostic index of poisoning.

N-methyl carbamates are absorbed by inhalation and ingestion and somewhat by skin penetration, although the latter tends to be the less toxic route. For example, carbofuran has a rat oral LD50 of 5 mg/kg, compared to a rat dermal LD50 of 120 mg/kg, which makes the oral route approximately 24 times more toxic when ingested. N-methyl carbamates are hydrolyzed enzymatically by the liver; degradation products are excreted by the kidneys and the liver.

At cholinergic nerve junctions with smooth muscle and gland cells, high acetylcholine concentration causes muscle contraction and secretion, respectively.

At skeletal muscle junctions, excess acetylcholine may be excitatory (cause muscle twitching), but may also weaken or paralyze the cell by depolarizing the end-plate. In the brain, elevated acetylcholine concentrations may cause sensory and behavioral disturbances, incoordination, and depressed motor function (rarely seizures), even though the N-methyl carbamates do not penetrate the central nervous system very efficiently. Respiratory depression combined with pulmonary edema is the usual cause of death from poisoning by N-methyl carbamate compounds.

**VII. Context:** This module is one of 3 modules for the Progressive Academic Education (PAcE) Program at Ross University School of Medicine, an independent study program for first year medical students.

**VIII. Facilitation Schema:** (Explanation of the Team Based Learning (TBL) format)
This case was written to be used in a Team-Based Learning (TBL) format. TBL cases utilize a specific written format and method of facilitation to produce very effective small group and class discussion. Larry A. Michaelsen describes in detail this theory and method in the book “Team-Based Learning, A Transformative Use of Small Groups”. You can aslo learn more about TBL at [http://www.ou.edu/pii/teamlearning/](http://www.ou.edu/pii/teamlearning/).

In short, TBL cases should be written to utilize the “Three S’s” in order to foster team work and group discussion. These are: (1) all students in the class should be working on the same problem or assignment, (2) students should be required to make a specific choice, and (3) group should all simultaneously report their choices.

1. It is important that all groups are working on the same problem because this enables a discussion both within group and between groups. If each of the groups is working on a different problem, then there is no common ground for discussion between groups.
2. The assignment should be written so students have to make a specific choice (i.e. put a multiple choice question at the end of the assignment). If students are asked an open-ended question at the end of the assignment, they make come up with one or two answers then end their discussion. If faced with a choice between five to seven possibilities, they have to discuss each possibility fully in order to accept it or reject it. Thus, more discussion is elicited when students are asked to make a specific choice. The choices should be written rather vaguely to stimulate discussion, with one best choice but other possible correct choices.

3. After group discussion, the group should be instructed to report their answer choice simultaneously. I do this by giving each group an envelope that contains 5 colored note cards lettered A, B, C, D, or E. The groups are asked to raise the note card which corresponds to their answer choice on the count of three. This allows the facilitator to immediately assess the overall performance of the class, and prevents groups from choosing their answer based on what other groups think. It also requires each group to commit to one answer choice and be ready to defend it.

This session requires approximately 80-90 minutes to complete in the TBL format. When students arrive to the class/session, they should sit in their assigned groups and the quizzes should be distributed. The students should be given approximately 10 minutes to complete the IRAT and 15 minutes to complete the GRAT, then allow 20 minutes for whole class discussion following the GRAT.

Next, the clinical application case should be distributed and the groups should discuss the case within their groups and answer the questions. Initially the groups should be allowed 15 minutes for this group discussion, then if more time is required, you can add an additional 5-10 minutes as needed. This discussion period should be closed-book, and no outside resources (internet, handouts, journals, etc.) should be used during the discussion or to answer the questions. After the discussion is finished, the students should simultaneously report their answer for the first question when you instruct them to do so. To facilitate simultaneous reporting, it is useful to hand out colored note cards that have the answer choices (A, B, C, D, E) written on them. Then you can ask the groups to raise the note card which corresponds to their answer choice on the count of three. This allows immediate assessment of the class responses, and makes it easy to facilitate whole class discussion based on which answers the students chose. After the first question is discussed, proceed to the remaining questions in the same format. Allow 20-30 minutes for class discussion of all of the questions.