

Use of *Renilla* bioluminescence to illustrate nervous function.

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Simmons College

Workshop contents

- Nervous function
- Cnidarians & *Renilla*
- *Renilla* bioluminescence

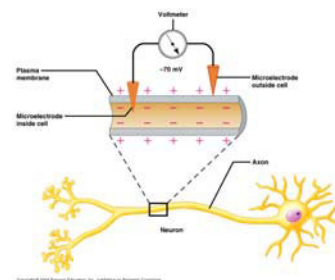


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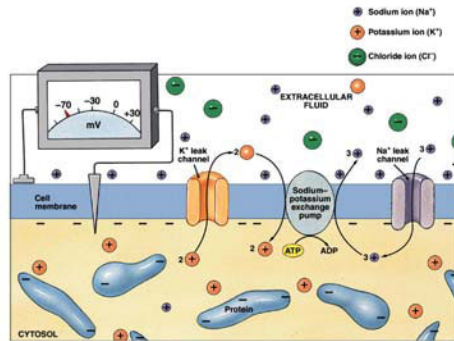
Nervous system basics

- Nerve activity involves changes in membrane potential
 - Differing ion concentrations on either side of a membrane = voltage
 - When ions move across membrane, voltage changes
 - Nerve activity = electrical activity

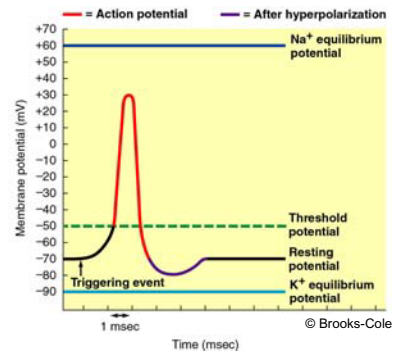


Nervous system basics

- Ion channels open in response to mechanical, chemical or electrical stimuli

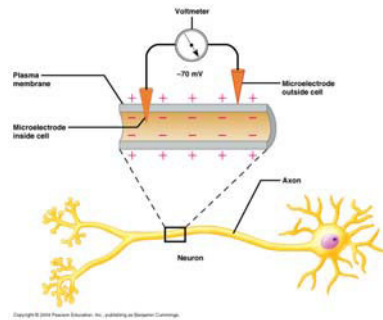


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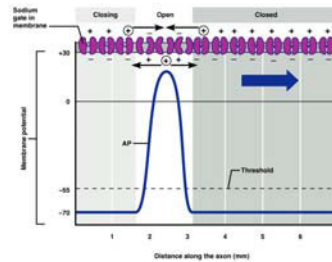


© Brooks-Cole

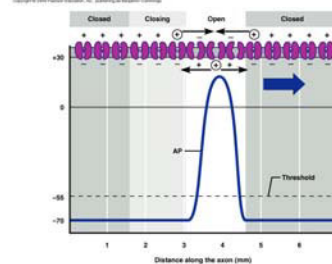
Action potential propagation



Copyright © 2004 Pearson Education, Inc., publishing as Benjamin Cummings.

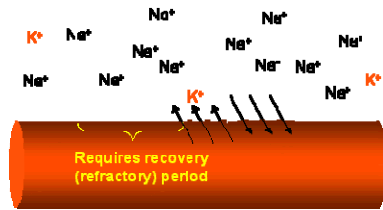


(a) Time = 0 ms

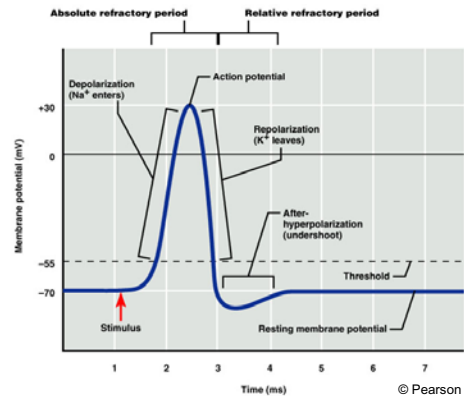


(b) Time = 1 ms

Refractory periods



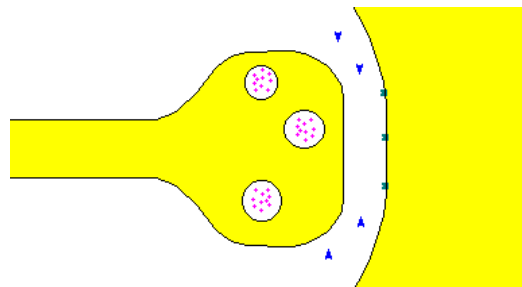
<http://faculty.clintoncc.suny.edu/faculty/Michael.Gregory/files/Bio%20102/Bio%20102%20lectures/Nervous%20System/neurons.htm>



- Refractory periods ensure unidirectional impulse movement

Synaptic transmission

1. Action potential reaches nerve end
2. Neurotransmitters are released
3. Neurotransmitters initiate action potential on target cell



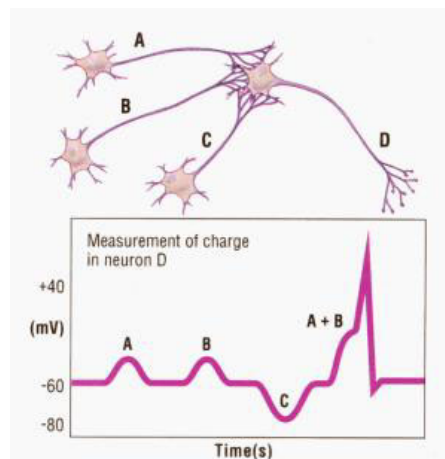
<http://www.biologymad.com/NervousSystem/synapses.htm>

Neurotransmitters

- biogenic amines – vertebrate autonomic nervous system, emotions; many invertebrate functions (dopamine, norepinephrine, epinephrine, serotonin, histamine)
- acetylcholine – released at vertebrate neuromuscular junctions, no activity in cnidarians
- amino acids – in vertebrates & invertebrates (GABA, glutamate)
- peptides – vertebrate & invertebrate; endorphins, antho-RF-amide, antho-RW-amide, etc.

Summation

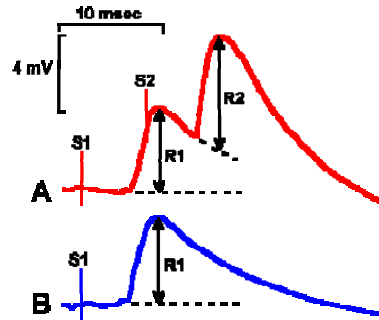
- Neurotransmitters from multiple neurons can act on target cells
- Neurotransmitters may cause stimulation or inhibition
- Multiple stimuli may be needed to reach threshold



<http://www.biologymad.com/NervousSystem/synapses.htm>

Facilitation

- Closely spaced impulses
- Membrane does not fully recover from first impulse
- Resulting effect is greater than simple summation



Intracellular recordings of facilitation of an EPSP. Two identical stimuli, S_1 and S_2 , were delivered to the cortex about 6 msec apart; the resulting EPSP is shown in A. When only S_1 was delivered, the EPSP was as shown in B. Notice the facilitation ($R_2 > R_1$), not summation ($R_2 = R_1$). (Porter R: *J Physiol (Lond)* 207:733-745, 1970)

<http://www.unmc.edu/Physiology/Mann/mann16.html>

Model systems for nervous activity

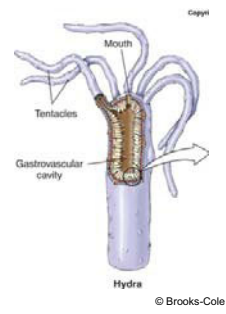
- Which organisms/model systems have you used to illustrate nervous function in laboratory exercises?

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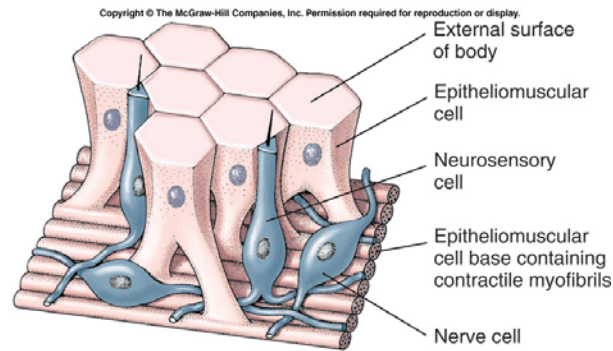
Cnidarians

- Hydra, corals, anemones, jellyfish, etc.
- Aquatic organisms with tissues, radial symmetry
- Have nervous system, water vascular system, muscular activity



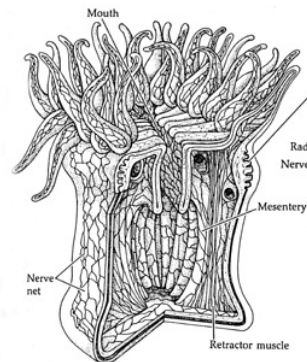
Cnidarian nervous function

- Sensory cells, ganglion cells, effector cells (muscles, glands, etc.)



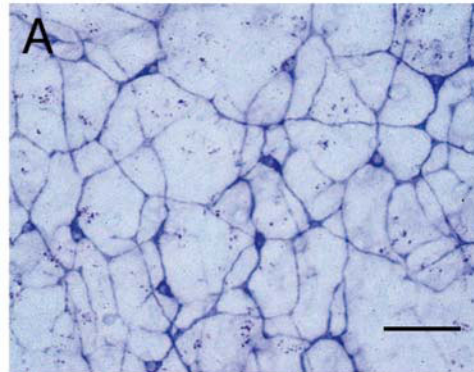
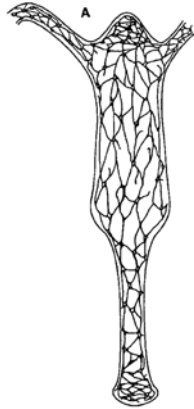
Nerve nets

- Neurons are non-myelinated
- Conduction can be unidirectional or bidirectional (nonpolar)
- Neurotransmitters are released at synapses
- Multiple behaviors/ activities coordinated by net



http://www.lifesci.ucsb.edu/~mcdougal/neurobehavior/modules_homework/jellies.html

Hydra nerve net



Koizumi (2002) *Can J Zool* 80:1678-89

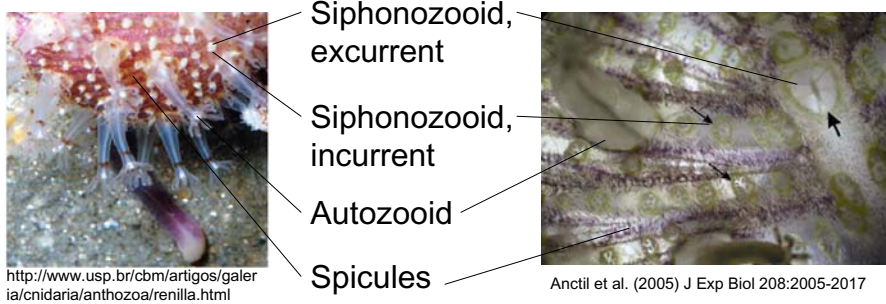
Shimizu et al (2004) *J Comp Physiol A* 190:623-630

Cnidarian nerve net

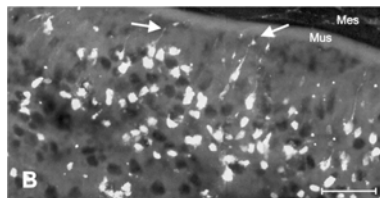
DVD: *Shape of Life, vol. 2: Cnidarians*

Renilla anatomy

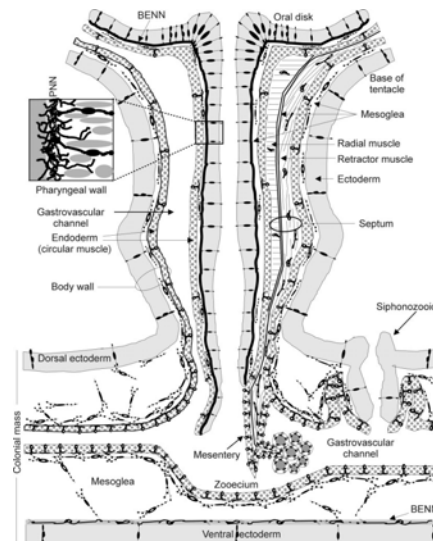
- Autozooids - feeding polyps
- Siphonozooids - water flow polyps



Renilla nervous system



Antho-RF-amide-positive neurons (a subset of the total neuronal population)



Pernet et al. (2004) J. Comp. Neurol 472:208-220

Renilla habitat

- U.S.: Gulf, Atlantic & Pacific coasts
- Sand or mud in intertidal region
- Anchors into sand, expands & contracts with tide
- Can be found up to ~20 m depth



<http://californiadiveboats.com/Truth/2005.05.28-30/>

Renilla care

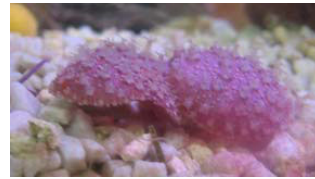
- Will survive in aquarium for several months, possibly up to one year
- Water: room temperature, well-aerated & filtered seawater
- Food: ground seafood, "Cyclop-eeze", etc.
- Light: 12 hr day/12 hr night cycle



<http://www.freezerbar.com/>

Renilla nervous function

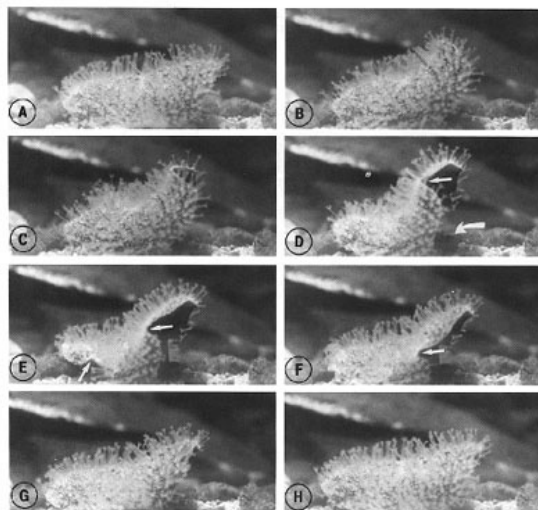
- Nervous-regulated behaviors
 - Feeding responses
 - Peristalsis
 - Bioluminescence
- Neurotransmitters:
 - Epinephrine (adrenaline)
 - Serotonin
 - Melatonin
 - Antho-RF-amide



Renilla peristalsis

Functions:

- Shake off sand
- Move
- Escape from predators



<http://abonnes.collegebdeb.qc.ca/profs/ggermain/redaction/exemple2.html>

Nervous control of muscle activity

- Peristalsis

Video clip

- Polyp retraction

Video clip

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Mechanisms of bioluminescence

- Luciferin + oxygen + calcium + luciferase = blue light (490 nm) + CO₂
- Blue light + green fluorescent protein = green light (509 nm)
- Bioluminescent cnidarians utilize similar reaction
- Fireflies, dinoflagellates & bacteria use different reactions

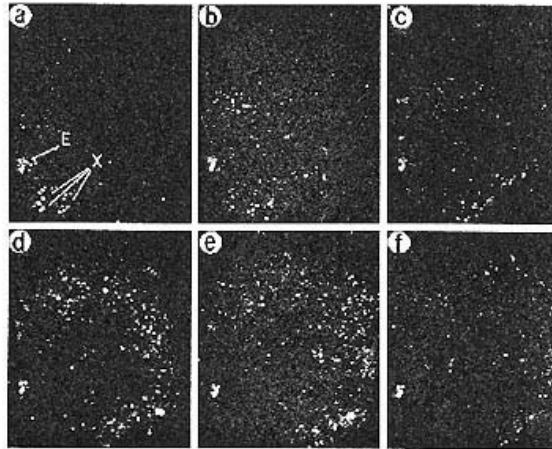


<http://www.steve.gb.com/images/science/aequoria.jpg>

Stimulation of bioluminescence

- Mechanical
 - Touch to rachis or peduncle
- Electrical
 - Threshold 8-60 V
 - Facilitation required
- Chemical
 - Epinephrine, not ACh or serotonin

Pattern of bioluminescence



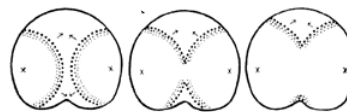
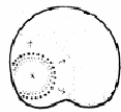
Transmission speed:
6.66-10.15
cm/sec (Buck, 1955)



K. Patenaude

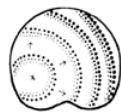
Renilla bioluminescent wave forms

“normal”

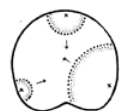


Refractory period

“volley”



“frenzy”



Buck (1973) Biol Bull 144:19-42

Facilitation & adaptation

- *Facilitation*: repeated stimulation increases intensity of response
- *Adaptation*: continuous stimulation eventually eliminates response

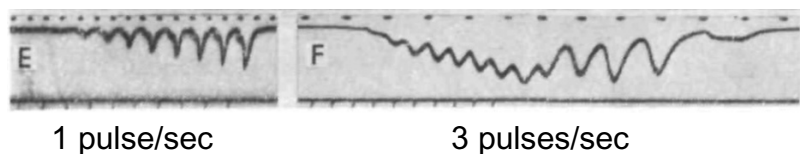


Fig. 5. Comparison of electrical and mechanical stimulation. A: response to a burst of 10 shocks at 1/sec. Dots below represent nervous impulses. The 1st flash appeared on the 2nd pulse. Time scale 14/min. B: responses to mechanical stimulus (dropping a weight on the rachis). Straight line below: duration of mechanical stimulus. Dots below: successive nervous impulses.

Nicol (1955) Exp Biol 32: 619-635

Summation

- If pulses occur $>1/\text{sec}$, light never completely returns to baseline
- Subsequent flashes have greater intensity (recruitment of more photocytes)



Nicol (1955) Exp Biol 32: 619-635

Stimulation notes

- *Renilla* luminesce better if maintained in non-stagnant water
- Keep *Renilla* in dark at least 2 hr prior to experiments
- Mechanical stimulation gives a brighter response than electrical stimulation
- First electrical pulse not sufficient to initiate response; facilitation required

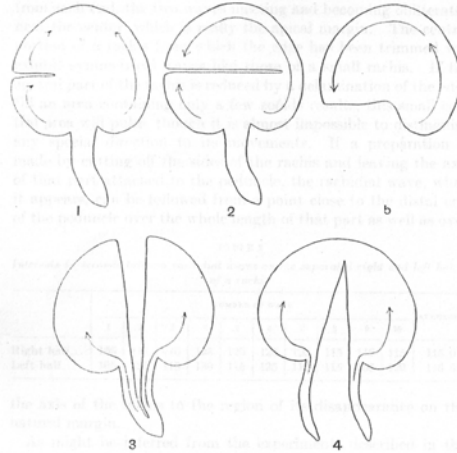
Further studies

- *Renilla* peristalsis
- Surgical/chemical nerve blocks

- Anemone tentacle contraction
- Hydra digestive movements
- Anemone nematocyst discharge

Conduction pathway analysis

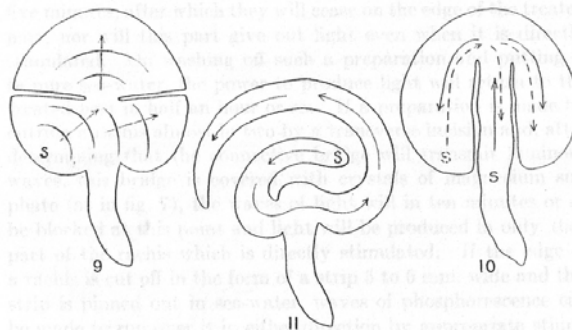
- Pattern of peristalsis can be altered
- Surgical disruption
- $MgSO_4$ crystals



Parker (1920) J Exp Biol 343-67

Conduction pathway analysis

- Surgical or chemical ($MgSO_4$) disruptions

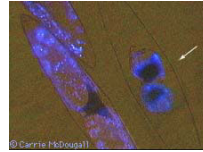


Parker (1920) J Exp Biol 343-67

More lab-friendly bioluminescence

- Dinoflagellates

- 'sea sparkle'
- <http://www.lifesci.ucsb.edu/~biolum/organism/dinohome.html>



- Fireflies

- WARD's kit for in vitro flash
- Harvest & observe
- <http://iris.biosci.ohio-state.edu/projects/FFiles/biolum.html>

