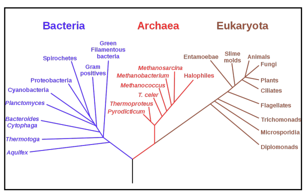
Studying the Brain 3 Model Organisms: Bacteria, worms, and flies

Evolutionary Conservation

- Once solutions to problems are developed in organisms, they tend to be maintained in their descendants
  - although with new variants
    - as genes are duplicated and differentiate
    - as new connections are formed between existing components



Model Organisms without Neurons

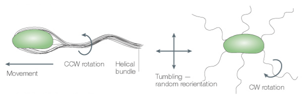
- Neurons are unique to animals
- But other organisms need to control their physiology and behavior
  - they need to select appropriate responses to different conditions
- They rely on chemical reactions that change
  - what proteins are synthesized
  - what alteration are made to them

  which alter both physiology and behavior

- Bacteria
- Archaea
- Plants

Selecting Behavior without Neurons

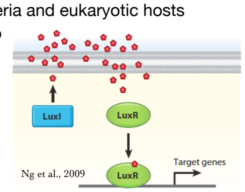
- Bacteria make numerous decisions based on information they procure about their internal state and their environments
  - Chemotaxis
    - based on registering their internal conditions and detecting how gradients of many different molecules are changing in their environment, *E. coli* select between alternative actions: move forward or tumble



  - Forming biofilms
    - when conditions are unfavorable AND enough other bacteria are present, *E. coli* form biofilms—a complete change of life style

Quorum Sensing

- To act cooperatives—form a biofilm, produce bioluminescence, secrete virulence factor, synthesize antibiotics, sporulate
  - bacteria (and other organisms) need to detect what other organisms are present
- Quorum sensing is widespread both within and between bacterial species and between bacteria and eukaryotic hosts
  - Allowing organisms to detect who is in their environment and adjust their behavior accordingly
- Different species produce and detect different molecules, thus keeping their conversations private

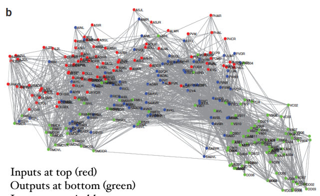


Invertebrate Models

- Invertebrate nervous systems range from quite simple to highly complex
  - Relatively simple invertebrates have often provided useful insights into the basic principles of nervous systems
    - Jellyfish and nerve nets
    - Leech and decision making
    - Crustaceans—lobster
    - *C. elegans*—nematode worm

C. elegans Connectome

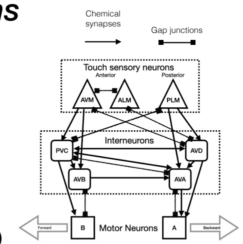
- Using serial electron microscopy, in the mid 1980s White identified a nearly complete connectome for the worm *C. elegans*
  - 302 neurons
  - standardized wiring plan
  - differentiation of sensory, inter, and motor neurons



Inputs at top (red)
Outputs at bottom (green)
Interneurons in blue

Bargmann & Marder, 2013

Local Decision-Making Systems in *C. elegans*



- Chalfie et al. (1985) developed a feedforward account of how, in response to sensory stimuli, *C. elegans* selected forward or backward movement
- Worms learn
  - If you keep touching them and nothing untoward happens, they stop responding (habituate)
- If trials are spaced out, the memory is retained long-term (spaced learning)
- Effect has been traced to a gene expressed in the glutamate receptors of the interneurons

Clicker Question

How important, according to Bargmann, is knowing how neurons are connected for understanding how a circuit of neurons will behave

A. Just as with a wiring diagram, from knowing how neurons are connected one can infer the behavior of the circuit
B. It is of almost no use. Connectivity tells us nothing about what will happen in the circuit
C. It is useful but far from sufficient since activity in the circuit can be altered by neuromodulators
D. Other

Different Types of Synapses

- Gap junctions (electrical synapses): direct cell to cell contact (as Golgi assumed)
- Chemical synapses: neurotransmitters are synthesized in axons and released at the terminals, where they
  - Either bind receptors in postsynaptic cells or are degraded and retaken up
- Neuromodulators: biogenic amines (serotonin, dopamine, etc.) or neuropeptides (>100 in *C. elegans*)
  - diffuse widely, even in the circulatory system
  - released by other cells and not just at synapses
  - alter neuron function
  - modulate the motivational and emotional state, arousal and sleep, pain sensitivity, etc., of the organism

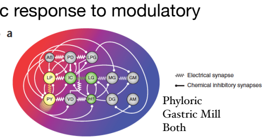
Clicker Question

What is distinctive about a neuromodulator?

A. It is produced outside the nervous system
B. It has minimal effects on other neurons
C. It can alter how other neurons behave
D. It is transmitted directly from one cell body to another

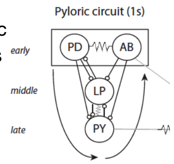
Stomatogastric Ganglion of the Crab

- Eve Marder and colleagues have spent decades analyzing the pattern generating circuit controlling stomach actions in the crab
- Two connected networks generates two rhythmic patterns of stomach movements
  - Pyloric rhythm—continuously active triphasic motor patterns
  - Gastric mill rhythm—episodic response to modulatory inputs from sensory neurons
  - Two circuits are highly interactive—several neurons are involved in both



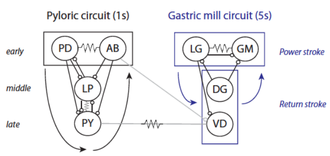
Pyloric Circuit

- Simple pyloric circuit producing three-phases of response:
  - AB is a pacemaker with rhythm of 1 sec
  - Electrical synapse of AB to PD neurons drive one muscle group
  - AB and PD together release inhibitory transmitters onto LP and PY neurons that drive other muscle groups
  - But dopamine switches LP to PY connections from depolarizing to hyperpolarizing



Interactions of Pyloric and Gastric Mill Circuits

- Neurons can switch circuits depending on circumstances
  - Neuro-modulators can lead VD to join pyloric circuit
  - LG can exhibit pyloric rhythm if gastric mill circuit receives no input
  - VD and LG can reset the phase of both circuits

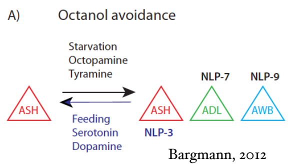


Lessons from the Crab Stomatogastric Network

- Even relatively simple circuits can generate complex (dynamic) behavior
- But unlike electrical circuits, these circuits function differently under different inputs
  - and can change behavior as a result of neuromodulators

Different Circuits for the Same Behavior

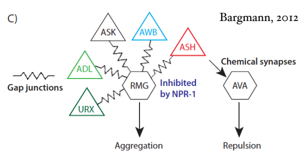
- In different circumstances, either one or three nociceptive sensory neurons elicit octanol avoidance (each producing a different neuropeptide).
  - Switch induced by either feeding/starvation or neural amines



Bargmann, 2012

Different Behaviors from Same Neuromodulator

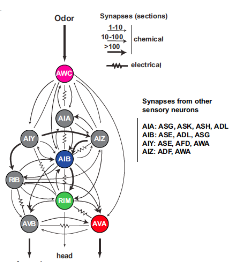
- Both aggregation and retreat from noxious stimuli regulated by nociceptor ASH, but in different ways
  - Chemical synapse to AVA
  - Gap junction to the hub neuron RMG, which integrates signals from many other sensors (e.g., URX, an oxygen sensor)
    - if RMG is inhibited, aggregation is prevented
    - but repulsion is maintained



Bargmann, 2012

Dynamical Decision-Making in *C. elegans*

- The decision-making circuits are not as feedforward as Chalfie's model suggests
  - Gap junctions convey signals in both directions
  - Many chemical synapses project back towards sensory neurons
- Recurrent projections create the potential for complex dynamics
  - in which the decision is influenced by the dynamical state of the network



(Gordus et al. 2015)

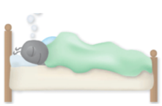
Lessons from *C. elegans*

- With just 302 neurons, *C. elegans* seemed to provide an easily tractable nervous system
  - Thirty years should have been plenty to figure out how the whole system works
- Part of the problem is that each neuron is connected to many others, creating what is known as a *small world*
- But a bigger problem is posed by the fact that the parts don't always do the same thing

Discussion Question

What is the function of sleep?

A. To enable consolidation of long term memories
B. To make organisms take time off from work
C. To allow restoration of neurons
D. To allow muscles to be repaired



Example: Studying Sleep in Fruit Flies

- Understanding sleep (both why it occurs and the mechanisms that generate it) remains a major challenge
  - Sleep, and deficiency in sleep, has important roles in cognitive performance
- In 2000 two groups of researchers
  - Joan Hendricks and colleagues at the University of Pennsylvania
  - Ralph Greenspan and colleagues at the Neurosciences Institute in La Jolla

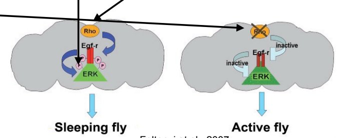
  demonstrated that fruit flies exhibit the behavioral criteria for sleep
  - they are immobile for several hours at night, during which they exhibit heightened thresholds to vibratory, visual, or auditory stimuli
  - prior to these episodes they move to locations away from food sources (sites of social activity)
  - when deprived of sleep, they exhibit rebound effects

Using Flies to Identify Part of the Sleep Mechanism

- Sleep is partly controlled by the circadian clock mechanism
- In mammals, one suspected output of the central circadian clock, transforming growth factor-α (TGF-α), is a known ligand of ErbB-1, a receptor suspected to be involved in sleep regulation
  - But the mammalian mechanism is complicated as there are four members of the ErbB family
  - Such redundancy makes it difficult to determine what operations the individual components perform
- Flies have only one receptor (EGFR)
  - Three of its four ligands are homologs of TGF-α
- Accordingly, flies provide investigators a much simpler version of the mechanism for sleep

Using Flies to Identify Part of the Sleep Mechanism

- Foltenyi et al. (2007) found that over-expression of Rho (a protease that cleaves membrane bound TGF-α ligands) in the pars intercerebralis (homolog of the hypothalamus) activates EGFR
  - Using heat shock to stimulate EGFR, they showed that Rho increased sleep episodes
  - They determined that the likely mechanism involves EGFR phosphorylating extracellular signal-regulated kinase (ERK) in the tritocerebrum
- Blocking Rho blocks generation of pERK and shortens sleep bouts (as in insomnia)
- This model provides a basis for investigating the more elaborated mechanism in us



Foltenyi et al., 2007

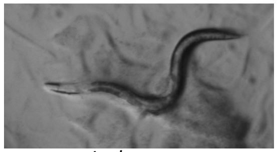
Sleep: Its Spreading

- Since the research finding sleep in fruit flies in 2000, researchers have found it in a variety of organisms:
  - Octopus
  - Nemotodes
  - Jellyfish

Sleep in Nemotodes

- Starting in 2008 David Raizer at Penn identified two sleep states in round worms (C. elegans):
  - lethargus: 2-3 hour bouts during each larval stage
  - stress-induced sleep in adult worms
  - (found some evidence of another state after satiety)

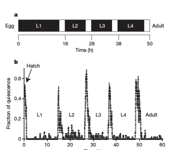

Awake


Lethargus

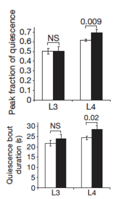

Stress-induced

Establishing that Lethargus is Sleep

- Lethargus involves:
  - Periodic behavioral quiescence in which a worm
    - assumes stereotypical posture
    - exhibits decreased response to sensory stimuli
  - Homeostatic response to enforced wakefulness in which peak quiescence and mean bout duration both increase
    - but with a fixed termination time


Raizen et al., 2008


Response to dish tap



Could the Mechanism of Lethargus be the same as that of sleep?

- Worms do not exhibit circadian rhythms
  - Yet already in 1999 they were shown to have *lin-2* —a homolog of period (*per*)
    - the first discovered circadian gene (in flies)
    - three homologs of *per* are found in mammals
  - LIN-2 (the protein product of this gene)
    - does not oscillate on a circadian basis but in phase with molting time
    - when mutated, generates aberrant timing of molting and lethargus