Discovering the Basic Components of the Brain: Neurons

Discussion Question

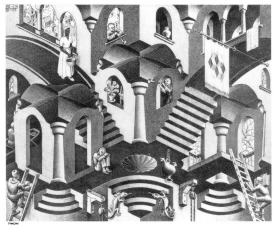
What do you see looking at the picture to the right?

A. Just a mess of lines on a page
B. A couple sitting at a table with wine
C. A skull
D. I see both B and C

Duchamp's Ill-Fated Lovers

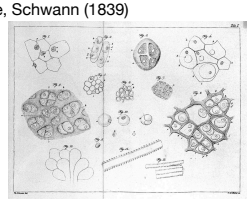
Theory-Laden Perception

- Observations are generally taken as the foundations on which science is built
  - Hypotheses (of laws or mechanisms) advanced to explain them
- But what one sees is influenced by what one knows
  - by the words/concepts one has
- Seeing something new is very difficult



What Constitutes Living Tissues?

- What do physiological tissues consist of?
- Microscopy played a major role in discovering cells, but it was not sufficient
  - The optical elements of 16th and 17th century microscopes produced distortions
    - How would you know that the image your device is generating are products of distortion—artifacts?
- With a (somewhat) improved microscope, Schwann (1839) observed a diversity of structures within tissues
  - What justifies treating them as all the same kind of thing—cells?
    - for Schwann, they all could be seen to form by a process like crystallization!



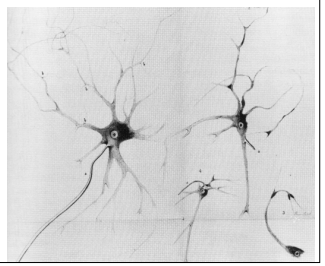
Discussion Question

Schwann argued that cells are the basic living units—the minimal units in which the activities of life occurred. What would justify that claims

A. If one can visually identify cells in all tissues, they must be the units of life
B. Some organisms consist of just one cell, yet they carry out all the basic activities of life
C. Each cell has a complete set of genes for the individual
D. Cells carry out all the basic chemical reactions needed to maintain an organism

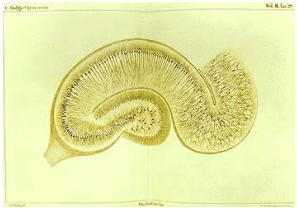
What Constitutes Nerves?

- Microscopists in the mid-19th century began to experiment with adding various substances to their preparations that enhanced the contrast—stains
- These images showed processes projecting from what appeared to be cells
  - Otto Dieters (1865) generated detailed drawings of motor neurons in the spinal cord
  - Identified axons (axis cylinder)
  - Dendrites (protoplasmic processes)



A Remarkable Stain

- Camillo Golgi (1873) developed a stain based on silver nitrate that stained cell processes black
- It only stains some of the neurons, making it easier to see those that are stained

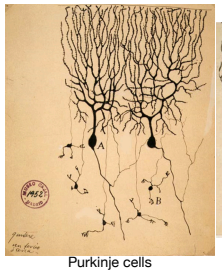


The Challenges of Microscopy

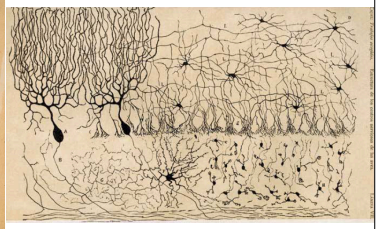
- Golgi comments of the challenges in using his stain:
  - “For microscopic examination the sections are placed in damar varnish . .. or in Canada balsam after they have been dehydrated through the use of absolute alcohol and have been rendered transparent with creosote. Time and light continually spoil the microscopic preparations obtained with my method ….
  - "I must equally declare that I have not yet succeeded in determining with certainty why under the same conditions ... I have obtained very different results"
  - "Permit me to advise, however, that I do not find myself as yet in a position to explain with precision all the necessary procedures for the best results. They are still partly fortuitous"

Improving the Stain

- A few years later (1887) Santiago Ramón y Cajal improved the techniques for using Golgi’s stains, producing highly detailed images of what he took to be neurons



Purkinje cells



Chicken Cerebellum

Do These Drawings Show Cells/ Neurons?

- Golgi: NO!!! They show a reticular network of interconnected processes
- Cajal: YES! Neurons are independent cells that do not connect (even though I cannot see the gap between them)

- How can scientists adjudicate such a disagreement?

Clicker Question

What is meant by the neuron doctrine?

A. Neurons are individual cells
B. Neurons are simply parts of a connected reticulum
C. Neurons are far more important than other cells
D. All brain cells are essentially alike

Clicker Question

How does the neuron doctrine relate to localization?

A. They are two names for the same thing—neurons are local units
B. They are direct competitors—a localizationist denies that neurons are distinct units
C. They are mutually supporting views—distinct neurons would support localization of function
D. There is great tension between the views—distinct neurons does not fit well with localization of function

Neurons and the Holist-Localizationist Controversy

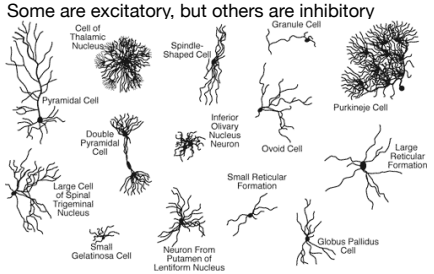
- Cajal's neuron doctrine, according to which each neuron is a distinct entity, fits comfortably with the view that individual operations can be assigned to distinct units in the brain
  - The mechanism works by each part performing its operation
  - Even if the units for a given activity are not individual neurons but larger units (brain areas), because they are built from distinct components they are themselves distinct units
- Golgi's reticularist view, according to which nerves form a continuous network, fits with a holist perspective in which the relevant unit is the whole system
  - The system operates through the coordinated activity of the whole, not through individual parts performing distinct operations
  - Even if some parts of the network are more active on some occasions than on others, one cannot assign distinct operations to separate parts

Resolving the Golgi/Cajal Controversy

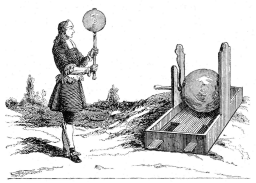
- The vast majority of investigators came to accept the neuron doctrine
  - Sherrington labeled the still hypothesized gap between neurons the synapse
- In the early 20th century numerous researchers began to theorize that the gap between neurons was mediated by chemicals/ neurotransmitters
  - but others argued that electrical conduction carried across the synapse
    - Resulting in "the war between the soups and the sparks"
- Only in the 1940s was the gap between neurons visualized with the electron microscope
  - Electron micrographs also revealed gap junctions between neurons
    - providing small vindication to Golgi

Diversity of Neurons

- While the pyramidal cells has been the prototype of a neuron, there is actually a huge variety of types of neurons
  - Some are excitatory, but others are inhibitory



The Novelty of Electricity in the 18th Century

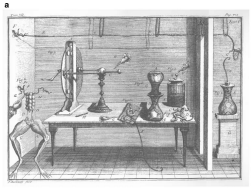


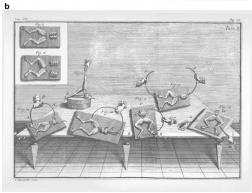
Otto von Guericke's electrostatic generator: sulphur globe and iron rod

Leyden Jar (condenser or capacitor)

Luigi Galvani

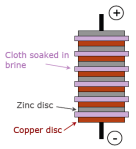
- In 1780 Galvani began to investigate the effects of electric discharges on muscle prepared with wires attached to the inside and outside of the muscle
  - Muscle contracted even when the spark was across the room
  - Or when there was lightening
  - Or even when the contacts, made of different metals, contacted each other
- What to make of this?





Galvani and Animal Electricity

- Within muscle a current flowed from the interior to the exterior
- Galvani interpreted the various experimental protocols he had employed as simply detecting this current



- How might you accept Galvani's findings but reject his claim of animal electricity?
- 1794: Allessandro Volta objected that frogs respond to electricity resulting originating from the two different metals Galvani employ
  - they do not generate it: "It is the difference of metals that does it."
- Based on this interpretation, Volta went on to invent the Voltaic pile/battery

Galvani: *Contrazione senza metallo*

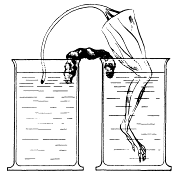


FIG. 3. The 1792 experiment of Galvani. When the circuit is closed with a piece of muscle or with wet paper, the leg contracts. (From Marc Sirol, Galvani et le Galvanisme—L'électricité animale. Paris: Vigòt Frères; 1939: Fig. 5.)

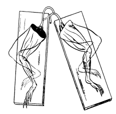


FIG. 5. The 1797 experiment of Galvani. When the surface of the sectioned right sciatic nerve touches the intact surface of the left sciatic nerve, both legs contract. (From Marc Sirol, Galvani et le Galvanisme—L'électricité animale. Paris, Vigòt Frères; 1939: Fig. 7.)

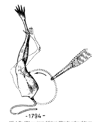
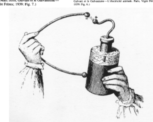


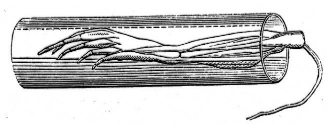
FIG. 4. The 1794 experiment of Galvani. When the surface of the sectioned nerve touches the muscle, the leg contracts. (From Marc Sirol, Galvani et le Galvanisme—L'électricité animale. Paris, Vigòt Frères; 1939: Fig. 6.)



- In response to Volta, Galvani prepared frog leg with sciatic nerve attached and showed contraction
  - When circuit completed with moistened paper
  - When nerve from one preparation touches another
  - When two are joined and one stimulated
- Galvani's conclusion: Animal have within them their own source of electricity
  - Analogy: muscle is like a Leyden Jar

Dual Role of Frog Leg

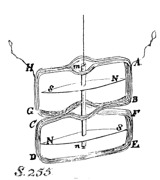
- Muscle as source of electricity
  - This was the phenomenon Galvani claimed to discover
    - whose subsequent development we will trace
- Instrument (*frog electroscope*/*rheoscopic frog*) for detecting electrical current
  - Contraction shows there is a current

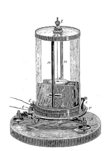


- Are there limitations to the use of frog leg's as detectors of electrical currents? What more would one desire in an instrument to measure animal electricity

Galvanometer: Instrument for Measuring Current

- Drawing upon Hans Christian Örsted's discovery (1819) that an electric current in a wire would deflect a magnetic needle, Johann Schweigger (182 created a *multiplikator* (galvanometer) by coiling the wire around the needle
- Nobili developed a much more sensitive galvanometer by increasing the number of times the wire is wound and used two coils to compensate for earth's magnetic field
- du Bois-Reymond built an even more sensitive galvanometer
  - 3,280 feet, coiled 4,650 times, to measure muscle current
  - 3.17 miles, coiled 24,160 times, to measure nerve current



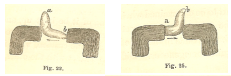


Du Bois-Reymond: Muscle and Nerve Current

- **Muscle Current**—present in each muscle or part there of
  - "the law of the muscular current may be expressed as follows: *Any point of the natural or artificial longitudinal section of the muscle is positive in relation to any point of the natural or artificial transverse section*" (du Bois-Reymond, 1843)

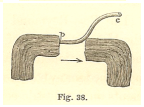
When two transverse sections (natural or artificial) placed between galvanometer pads, no current

When one longitudinal section and one traverse section placed between pads, current from the transverse to the longitudinal



- **Nerve Current**—present in each nerve

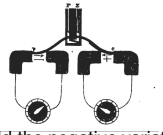
When one transverse section and one longitudinal section placed on pads, current from the transverse to the longitudinal



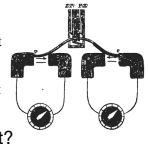
An additional phenomenon: Negative Variation

- Before du Bois-Reymond began his investigations, Matteucci had found that when he repeatedly stimulating a muscle without allowing it to relax so that it seized up, the current was reduced
- du Bois-Reymond investigated this phenomenon systematically in muscle and nerve, finding that it traveled along a nerve

Current added to nerve: Current in opposite direction than the nervous current



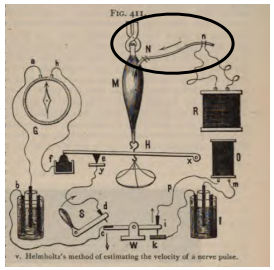
In tetanus: Induced current eliminated; only the nervous current remains



- What could the negative variation represent?

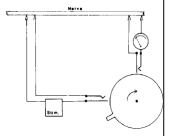
The Negative Variation and the Nerve Impulse

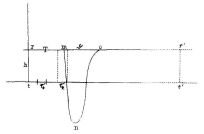
- How could one demonstrate that the negative variation was in fact the nerve impulse known to travel along nerves?
- Big hint: Hermann Helmholtz had measured the speed with which the nerve impulse travels
  - When switch S is closed, galvanometer records current until muscle contracts
  - Galvanometer responds longer when stimulus is n than N
  - Additional time divided by additional distance reveals the speed between n and N
  - Approx. 27 meters/second
- Much lower than electrical transmission in wires
- So what do you need to measure with respect to the negative variation



The "Good Student"—Julius Benstein

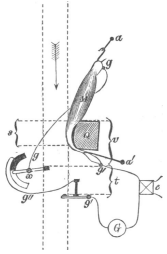
- du Bois-Reymond's strategy: show that the negative potential travels at the same rate as the nerve pulse
- He handed this task over to his student Julius Bernstein, who decided the differential rheotome for this task
  - Measured velocity at 28.718, close to Helmholtz's rate for the nerve pulse
- Bernstein also plotted the time course for the negative variation
  - observed that it overshot 0 current





The "Bad Student"—Ludimar Hermann

- Dared to question whether the muscle current even existed, claiming it only results when the muscle was injured
  - and set about trying to demonstrate this right in du Bois-Reymond's laboratory
- Challenge: how to demonstrate that the current is due to injury when exposing the inside of the muscle is required to measure the current?
- Hermann's strategy: show that the current increased in the time after injury
  - devised the fall rheotome in which it was possible to vary the time between exposing the muscle and measuring the current
  - found that it increased as predicted
- Renamed the current the "injury current"



What is the "Good Student" to Do?

- While du Bois-Reymond dismissed Hermann's findings, Bernstein recognized that they brought into question the muscle and nerve currents
  - If not a current, what is the negative variation a reduction in?

Recasting the Phenomenon of the Muscle Current as the Membrane Potential

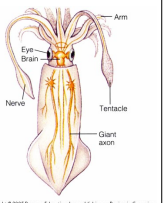
- Independent line of research in the late 19th century on electrolytes separated by membrane
  - Osmotic pressure resulted from dissociation of salts into ions
    - When ions are maintained at different concentrations on different sides of the membrane, an electrical potential is generated
    - Nernst developed an equation to characterize the resulting potential:

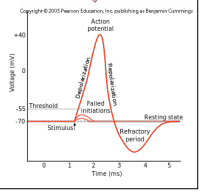
$$E = \frac{RT}{F} \ln \frac{[K^+]_{out}}{[K^+]_{in}}$$

- What preexists, according to Bernstein (1902), is a potential across the membrane, not a current
  - Which Bernstein characterized solely in terms of potassium ions
- With only one ion, there is no possibility of the current reversing—it goes to 0 when concentrations are equally balanced
  - So what about the overshoot?

Rediscovering the Overshoot

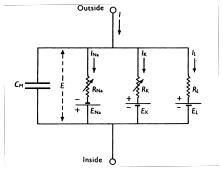
- Bernstein simply "forgot" about the overshoot and so did everyone else until
  - Hodgkin and Huxley, using new techniques, set about measuring the current changes during the action potential in the giant axon of the squid (this axon permitted inserting electrodes into the axon)
- Hodgkin and Huxley rediscovered the overshoot to a positive voltage
- To measure the change precisely they develop the voltage clamp
  - Discovered that the depolarization resulted from entrance of Na^+ into the cell





Modeling the Action Potential

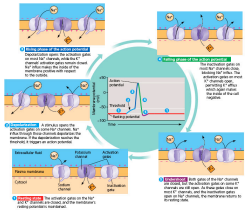
- To try to account for the precise pattern of current change, Hodgkin and Huxley resorted to computational modeling
  - A long series of runs of the simulations eventually resulted in an equation that described the current in terms of conductances (g), membrane capacitance (C) and membrane potential (V)



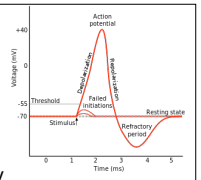
$$I = C_m \frac{dV_m}{dt} + \bar{g}_K n^4 (V_m - V_K) + \bar{g}_{Na} m^3 h (V_m - V_{Na}) + \bar{g}_l (V_m - V_l),$$

From An Equation to a Full Account of the Mechanism

- To fit the data, Hodgkin and Huxley employ parameters: n^4 and m^3, but they had no account of what these represented
- Much later they were shown to characterize voltage-gated ion channels



Discussion Question



What made it so challenging to explain the action potential?

A. The fact that when neurons were depolarized beyond threshold, they would overshoot into positive voltage
B. The fact that repolarization goes beyond the resting state and only gradually returns to it
C. The fact that axons are so small that it is hard to observe what is going on it them
D. The fact that the resting potential is approx. -70 mV rather than 0