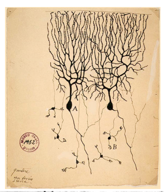
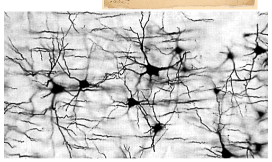
19th and 20th Century Roots of Foundational Neuroscience

Cell theory and neuron Doctrine

- Cells were only recognized as distinct entities with improved microscopes in the middle decades of the 19th century
  - Theodor Schwann (1838) claimed cells were the basic livingunit in the organs and tissues of animals
    - He claimed that all are the same because they originate through a process analogous to crystal formation
      - New material is gradually absorbed around the nucleolus to create first a nucleus and then the cytoplasm of cells
- Among the early cells to be identified were various types of neurons--Purkinje cells discovered by Purkinje in the cerebellum

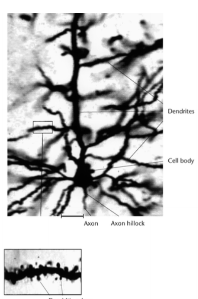


- But not everyone thought they were cells
  - Camillo Golgi developed a stain using silvernitrate that sharply stained some neurons
    - Which he took to constitute a large reticulated network--not separate cells

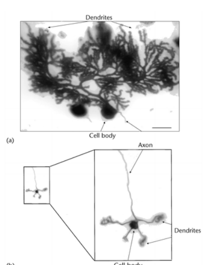


Visualizing Neurons

Large cortical neuron stained with silver nitrate



Cells from two adjacent layers of the cerebellum:
- 2 Purkinje cells
- granule cell



Figures from Matus, A. (2001). Neurons. eLS: John Wiley & Sons, Ltd.

Clicker Question

What is meant by the neuron doctrine?

- Neurons are individual cells
- Neurons are simply parts of a connected reticulum
- Neurons are far more important than other cells
- All brain cells are essentially alike

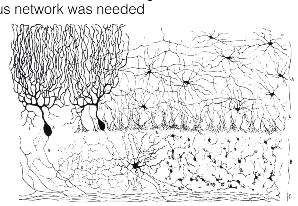
Clicker Question

What features made it difficult to determine whether neurons really are cells?

- They don't have an obvious nucleus
- The nerve fibers appear to run between neurons linking them into a network
- They are much smaller than normal cells and so harder to see with a microscope
- They are so much larger than typical cells such as blood cells or liver cells

Cell theory and the Neuron Doctrine

- Golgi's staining technique was adopted and modified by Santiago Ramón y Cajal
- Cajal soon took issue with Golgi's interpretation of what was to be seen
  - Cajal maintained that neurons were separate cells--he saw them as separate
- How could Golgi and Cajal look at the same thing but see something different?
  - For Golgi, what mattered was communication through nerves—for which purposes a continuous network was needed
  - For Cajal, neurons were basic units that functioned independently and out of which a system could be built
  - Charles Scott Sherrington introduced the concept of a synapse for the gap between neurons



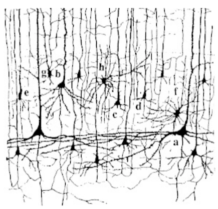
Clicker Question

How does the neuron doctrine relate to localization?

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

Producing Evidence

- Traditional philosophical accounts have treated observations with the senses as unproblematic data
- But what is observed is often the product of extensive manipulations
  - Typically not fully understood
- The results are often variable
- Then why believe them?
  - The results look good
  - Fit with other types of data
  - Fit with what we expect given our theoretical knowledge



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 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

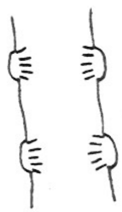
Discussion Question

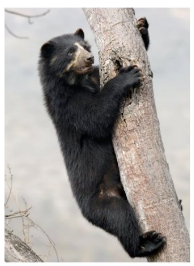
What do you see looking at the picture to the right?

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

Duchamp's Ill-Fated Lovers

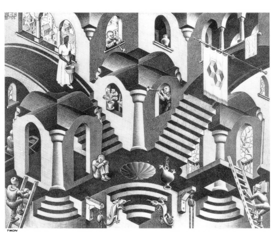
What do you see in this image?





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 observation (perception) is influenced
  - top-down by our previous experience
  - bottom-up by what is in front of our eyes



Scientific "Seeing"

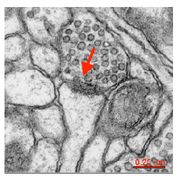
Scientific observations are even more affected by theories

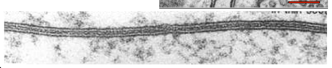
- Scientists do not just open their eyes and look at the world
- Often they create the preparations that they then observe
  - Golgi comments of the challenges in using his stain:
    - "For microscopic examination the sections are placed in damar varnish . .. or in Canada balsam after they have been 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"

Establishment of the Neuron Doctrine

In subsequent decades, researchers succeeded in making the idea of neurons as separate cells fit their observations

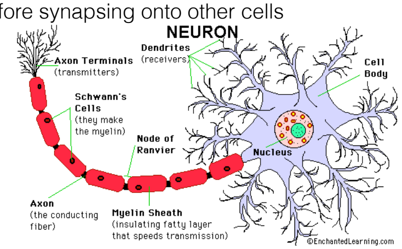
- New and improved staining techniques presented images that supported the individual cell account
  - The introduction of the electron microscope in the 1950s provided the final visual evidence, but by then there were few who needed convincing
  - But it also brought evidence that there are some points of contact that are so close that electric currents are directly transmitted from one cell to another—gap junctions
    - Reintroducing an important claim of the reticular account





Prototypical Neuron

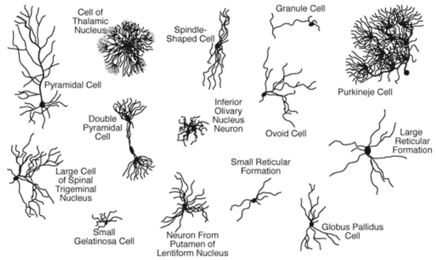
Pyramidal cells are large, with a thousand or more dendritic spines that receive input from other cells, a cell body onto which they project, and a long axon that can span large distances in the brain before synapsing onto other cells



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



Characterizing the Action Potential

Building on Galvani's work showing the role of electricity in contraction of frog muscles, numerous researchers investigated electrical activity in nerves

1840s and 1850s

- Metteucci detected an outward current in a sliced frog muscle (injury current)
- de Bois-Reymond showed that when stimulated the current decreased (negative variation)
- Helmholtz measured the conduction speed in nerve/muscle at about 30m/sec

The Action Potential

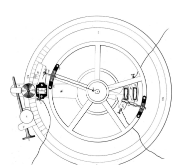
Julius Bernstein in 1868 developed a device to measure the nerve conduction precisely—differential rheotome

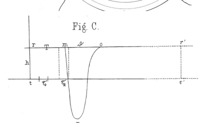
- Not only measured the speed of transmission, but also the amount and shape of the deflection
- Observed that the negative variation (n) exceeds the injury current (h)

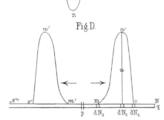
In 1902 Bernstein offered an explanation of the action potential

- Attributed the injury current to a gradient in potassium ions (K^+)
- With stimulation, the membrane becomes more permeable to K^+, resulting in a current

Bernstein *forgot* about the overshoot he had previously identified







Measuring and Modeling the Action Potential

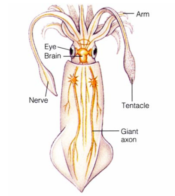
From frogs to squid

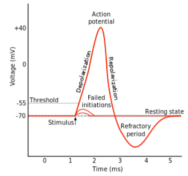
- Squid have a very large axon controlling the muscle that expels water for locomotion
- The size of the axon makes it much easier to insert electrodes

Carrying out experiments on the squid axon, Hodgkin and Huxley rediscovered the overshoot to a positive voltage

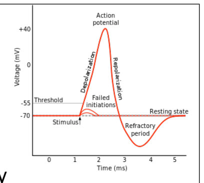
To measure the change precisely they develop the voltage clamp

- Discovered that the depolarization resulted from entrance of Na^+ into the cell





Discussion Question



What made it so challenging to explain the action potential?

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

Clicker Question

To fill out their account of how the experimentally derived pattern for the action potential was generated, Huxley resorted to what?

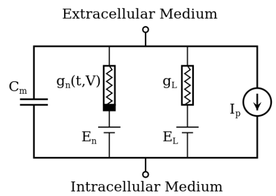
- Very careful and minute observations
- Developing new technology that could identify the ion channels in the axon membrane
- Developing hypothetical equations and seeing if the model generated the right pattern
- Carefully varying the concentrations of sodium and potassium in the cell to determine the effects

Modeling the Action Potential

To try to account for the precise pattern of current change, Hodgkin and Huxley resorted to computational modeling

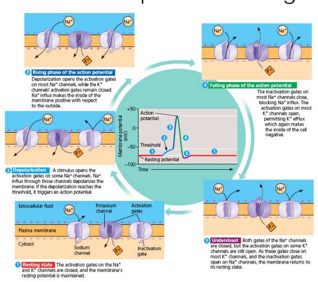
- A long series of runs of the simulations eventually resulted in an equation that accurately described the current in terms of conductances (g), membrane capacitance (C) and membrane potential (V)

$$I = C_m \frac{dV_m}{dt} + g_K(V_m - V_K) + g_{Na}(V_m - V_{Na}) + g_l(V_m - V_l)$$



The Full Account: Channels

Hodgkin and Huxley did not know how the ions moved across the membrane: channels that open and close in response to voltage



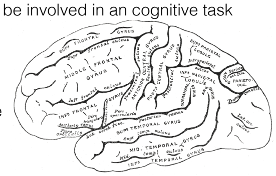
Higher-level Units in the Brain

Despite the success of researchers (during the same period when HH were developing their model) in recording from individual neurons in the performance of cognitive activities, the neuronal level is *generally* regarded as too low a level of organization to make sense of cognition

- Populations of neurons seem to be involved in an cognitive task

Challenge: can one distinguish brain structures at a higher level of organization (that might be related to cognitive activities)

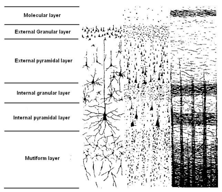
- 19^{th} century: sulci and gyri were hypothesized to be the relevant units of the brain
- With the identification of neurons, researchers began to focus on how different types of neurons are found in different tissues

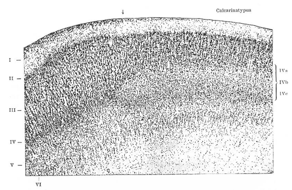


Layers of Cortex

In viewing cortical tissue from many organisms under the microscope, Korbinian Brodmann found a common pattern of six layers distinguished by the types of cells they contained, and hence how they appeared when stained

The thickness of these layers often varied across cortex, and this provided Brodmann's chief tool for distinguishing brain regions





Discussion Question

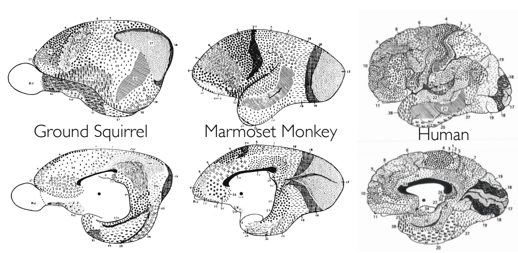
Why would Brodmann find the fact that the thickness of layers differed across the cortex as indicating that the different areas might perform different cognitive functions

- He read Gall and thought he had a better tool than bumps on the skull
- He assumed that different types of neurons which appeared in different layers would carry out different functions
- He assumed that neurons in different layers would do different things and if in one region one layer was thicker, that would explain what the region could do
- Other

Mapping Brain Regions

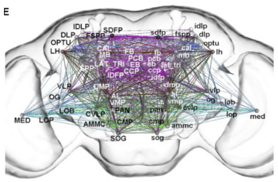
Korbinian Brodmann mapped out brain areas on the basis of the cytoarchitecture

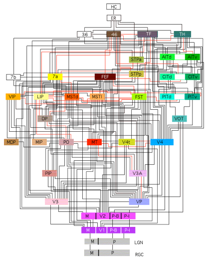
- He numbered areas in the order in which they were encountered
- Identified comparable areas in numerous mammalian species



Mapping BRAIN REGIONS

More recently connectivity and functionality been added as a tool for differentiating brain regions





From Structures to Functions

- Brodmann clearly sought to delineate brain regions that were functionally important
  - But he had no tools to determine what these areas do
- This required other techniques
  - Lesions
  - Stimulation
  - Recording
    - Single- and multi-cell
    - Electroencephalograms
    - PET and fMRI

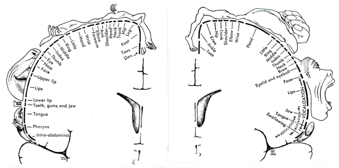
Lesion Studies

- Brain damage such as Leborgne experienced is one source of lesions in humans
  - Surgery is another (HM)
- Challenges in interpreting lesion studies
  - Lesions do not typically align with natural boundaries in the brain
  - From deficits it is not straight forward to predict what the removed area does when not damaged
    - It is assumed to contribute to the capacity that is lost, but that capacity may depend on many other areas as well

Stimulation Studies

Just as eliminating a functioning component should change behavior in a determinate manner, so should enhancing its activity by additional stimulation

- Challenge 1: What part of the brain is altered by the added stimulation?
- Challenge 2: Just what does the stimulated component contribute to the mechanism?



The sensory and motor "homunculi" identified by Penfield by using electrical stimulation. From Penfield and Rasmussen, 1950

Delgado's Bull Experiment

Delgado claimed he found a center that inhibited agression

Valenstein: Delgado really activated a pathway that controlled movement

Single-Cell Recording

Once the technique of inserting an electrode next to a neuron and recording its electrical behavior was developed it became the workhorse of sensory (especially visual) neuroscience

Challenge 1: Finding the full set of stimuli that elicit response in a given neuron

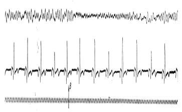
Challenge 2: Determining what that neuron is contributing to the processing of a given stimulus

Challenge 3: Activity of individual cells may not be the relevant brain activity

- Cannot detect what is going on in populations of cells and how timing of the responses in multiple cells might encode information

Electrophysiological Recordings

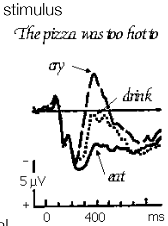
- Electroencephalogram (EEG): Electrodes placed on the skull detect ongoing electrical signal
  - Berger (1930) distinguished large amplitude, slower waves during rest (8-12 Hz alpha rhythms) and lower-frequency, faster waves after stimulation (12-30 Hz beta rhythms)
  - Subsequent discovery of both higher-frequency (>30 Hz gamma rhythms) and lower-frequency (4-7 Hz theta and 0.1-4 Hz delta rhythms) oscillations
    - Much of the focus directed at the lower-frequency rhythms associated with stages of sleep



- These oscillations were interesting even as researches were uncertain as to their origin
- But until recently they did not seem to have much to do with cognitive activities--BUT THAT HAS CHANGED DRAMATICALLY

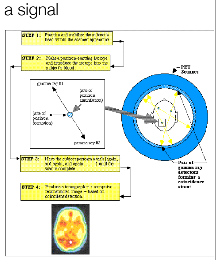
Evoked Response Potentials (ERPs)

- By time-locking the EEG signal to the presentation of a stimulus and averaging over many trials, researchers could extract a detectable signal
  - Thought to reflect the brain's processing of that stimulus
    - N400 (discovered at UCSD by Marta Kutas) thought to reflect violations of semantic expectations
- ERP studies can provide high resolution information about timing of activity
  - But little information about where the signal is coming from
    - As there is no general solution to the inverse problem--inferring from what is recorded at different electrodes to the source of the signal



Neuroimaging: PET

- Positron emission tomography (PET)
  - Employs a radioactive compound to provide a signal
    - 2-deoxyglucose is transported to cells like glucose but not metabolized
      - Builds up in cells as they recruit glucose for energy
    - Water labeled with ^{15}O is carried by the bloodstream
      - Increasing with the increased blood flow as energy is needed
  - The products of the radioactive decay (gamma rays generated as an emitted positron collides with an electron) are detected by a scanner when they arrive simultaneously
    - Computerized tomography is used to generate a three-dimensional image from which slices in any direction can be viewed



Neuroimaging: MRI and fMRI

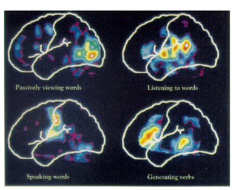
- Magnetic Resonance Imaging (MRI)
  - In a strong magnetic field, hydrogen nuclei align the axes of their spin
    - The energy from a radiowave pulse perturbs this alignment
    - When the pulse ends, nuclei return to the low-energy aligned state
      - And release radiowaves with a specific frequency
- Structural MRI uses the difference in frequency from atoms in grey and white matter to construct an image
- Functional MRI (fMRI) detects changes in deoxyhemoglobin resulting from changes in blood flow that *exceed* oxygen required by neurons
  - Blood oxygen level-dependent (BOLD) signal

The question of why blood flow exceeds that required to provide oxygen to neurons is still a matter of serious dispute

Neuroimaging: Relating Signal to Cognition

- Just as with single-cell recording, what one can infer from the results of a PET or fMRI scan depends on the input stimulus/task
- Researchers must find a means of relating inputs/task to the signal
  - During any task there will be activity throughout the brain (it is not dead when no task is presented)
- One of the most widely used strategies for relating task to detected activity is subtraction
  - An approach first developed by Donders in the 19th century for reaction time studies
    - Compare two different task conditions and subtract the time required for one from that required for the other
  - In neuroimaging, compare two tasks conditions and subtract blood flow produced by one task from that produced by another (baseline) task

Neuroimaging: The Verb-Generate Task



- Four subtraction conditions
  - Passively viewing words - resting
  - Passively listening to words - resting
  - Speaking viewed words - passively viewing words
  - Generating and speaking verb in response to viewed words - speaking viewed words
- Last subtraction resulted in increased activity in the left prefrontal cortex, anterior cingulate, right cerebellum
  - The researchers contended that the left prefrontal cortex reflected semantic processing
  - This was one of the first studies to highlight the anterior cingulate, but they and others assumed it was involved in executive control