Methods of Neuroscience

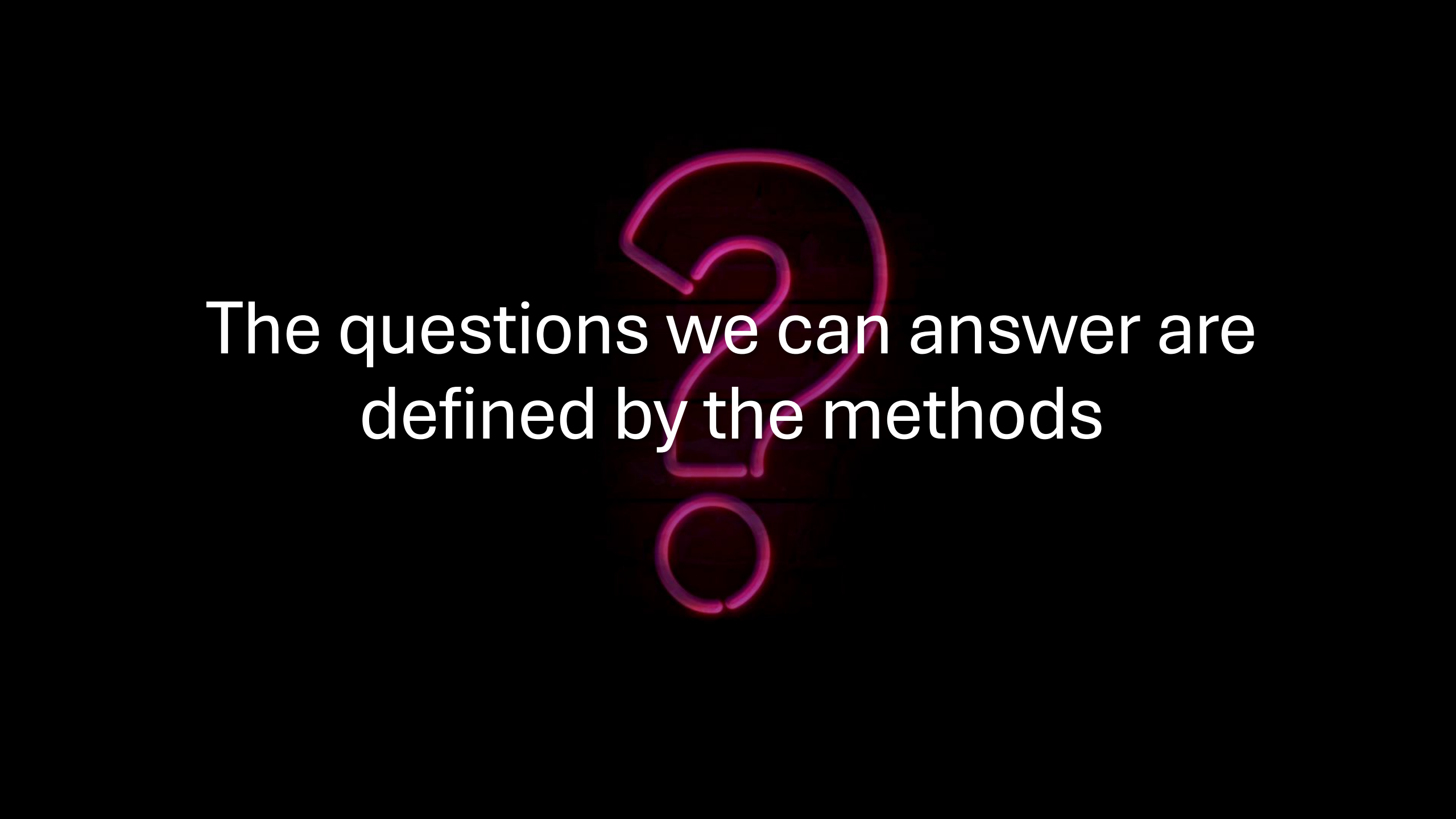
Chapter 6

Introduction to methods

- Neuroscientists use methods borrowed from many disciplines
- Methods are divided into 4 major categories:
  - imaging anatomy
  - imaging function
  - imaging cells
  - manipulating the nervous system

Approach taken by Lim and Sun (co-author)

- Many strategies can be used independently, but some may be used simultaneously
- Each technique can be considered in terms of
  - description of how it works
  - its main advantages
  - its shortcomings or limitations
- Should consider whether use is appropriate for non-humans; some techniques are best for humans


The questions we can answer are
defined by the methods

Resolution

- Resolution can be a concern with imaging techniques
- 2 types of resolution
  - Spatial resolution
    - Ability to differentiate 2 points in space from each other
    - Two signals very close together can be identified as 2 different signals instead of one
    - Usually measured in units of distance or volume
    - Highest spatial resolution of imaging techniques is offered by electron microscopy

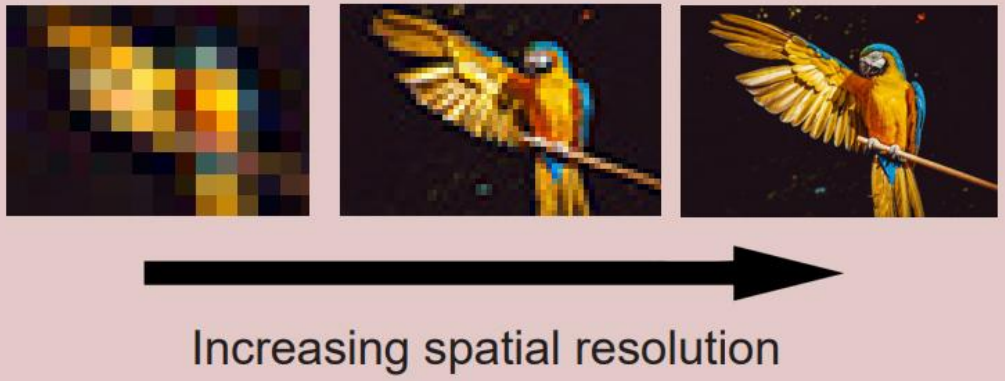


Figure 6.2 Methods with higher spatial resolution are better for identifying the location of different parts of the image. Precisely identifying the parrot's beak in the leftmost image (low resolution) is difficult, but is easy in the rightmost image (high resolution).

Resolution continued

- Temporal resolution
  - Ability to distinguish 2 events in time from one another
  - Measured in units of time
  - High temporal resolution allows differentiation of 2 signals as close together in the hundreds of microseconds range
  - Electrophysiology has high temporal resolution

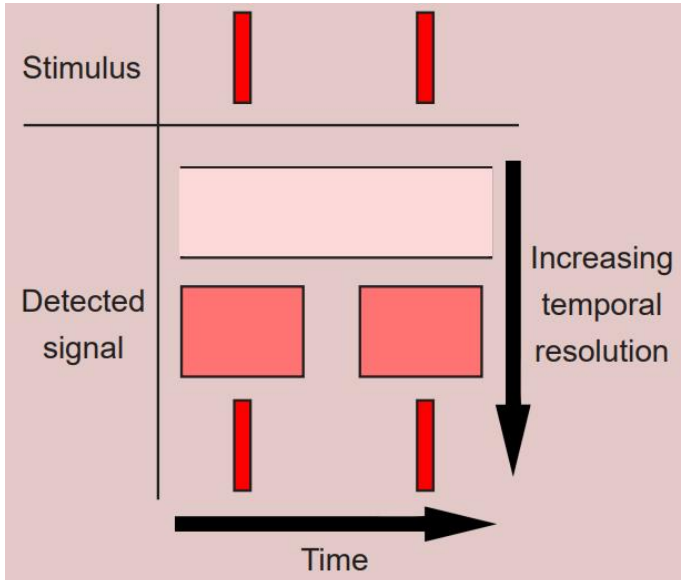


Figure 6.3 Methods with higher temporal resolution are better for precisely detecting when a signal occurs. With low temporal resolution techniques (top of detected signals), two discrete events spaced apart by time appear as one event.

The case for human participants

- Primary end goal of biomedical research is to cure humans
- Humans can be great at following directions without training
- Usually cheaper than studying same question in non-human animal models

Human model organisms

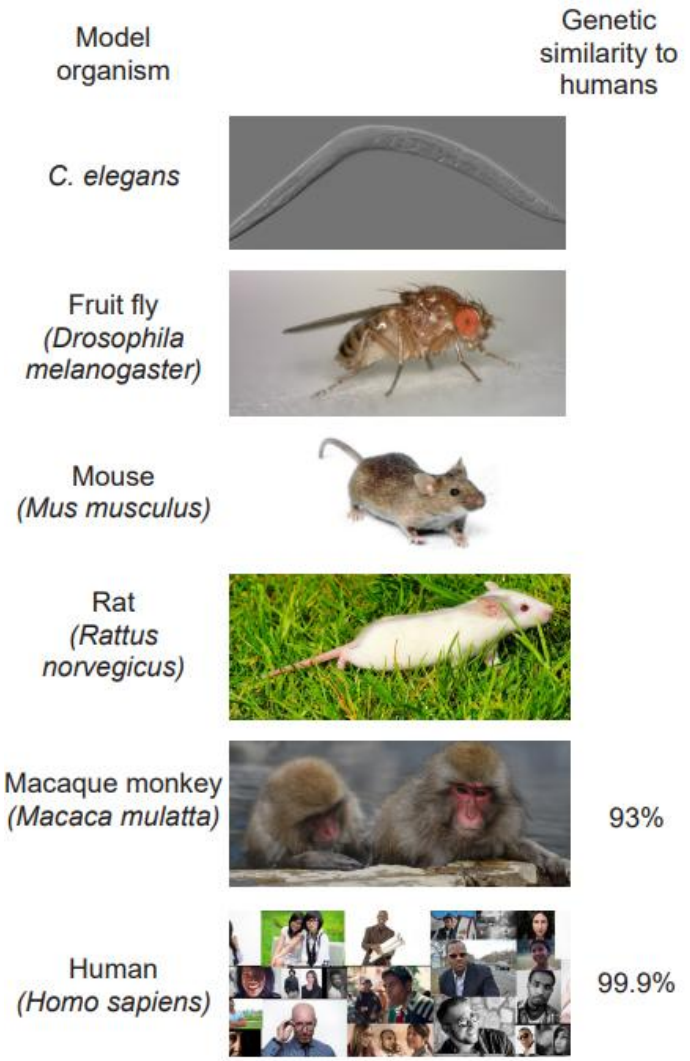


Figure 6.4 A short list of animal models that are often used in neuroscience research and their genetic similarity to humans. Humans are 99.9% genetically identical to other humans.

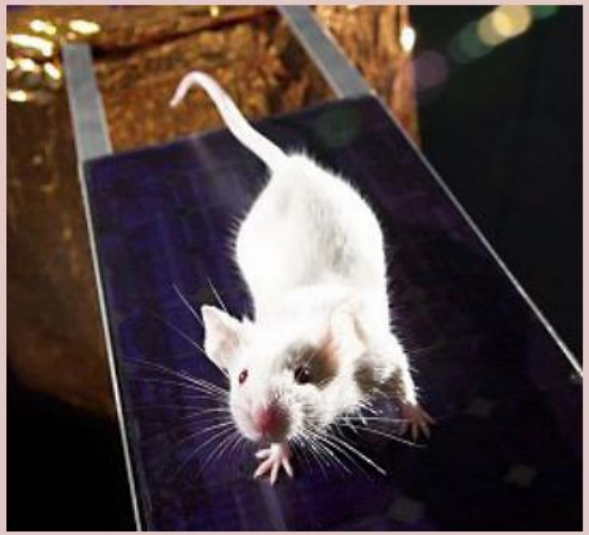
The case for non-human subjects

- Ethical constraints
- Interest in behaviors of non-human animals (behaviors humans do not experience) - ex. Flying or slithering as means of locomotion
- Control of variables that alter behaviors; humans are weird (different backgrounds, with different sets of experiences, flaws, and biases)
- WEIRD (Western, Educated, Industrialized, Rich, and Democratic) - 96% of psychology studies, but 12% of global population – also affects our behaviors

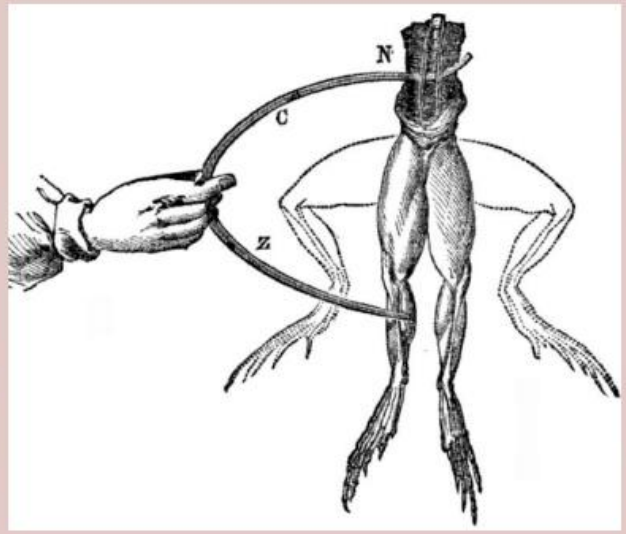
More on ethics of animal research

- "3R" tenet of "Replacement, Reduction and Refinement"
- https://ccac.ca/
- https://www.stfx.ca/programs-courses/science/dean-science/animal-plant-care/animal-care-committee

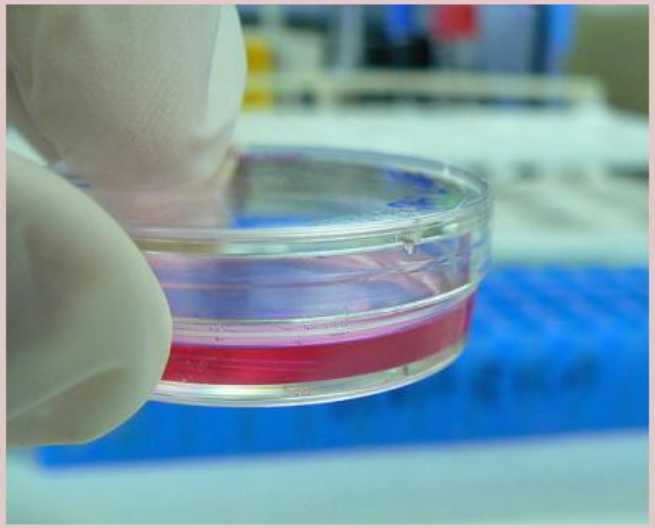
Experimental Studies



in vivo



ex vivo



in vitro

Increasing control over variables

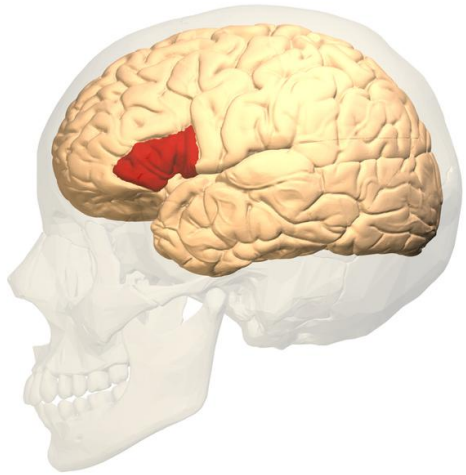
Decreasing ability to predict therapeutic potential

Decreasing strictness of ethical regulations

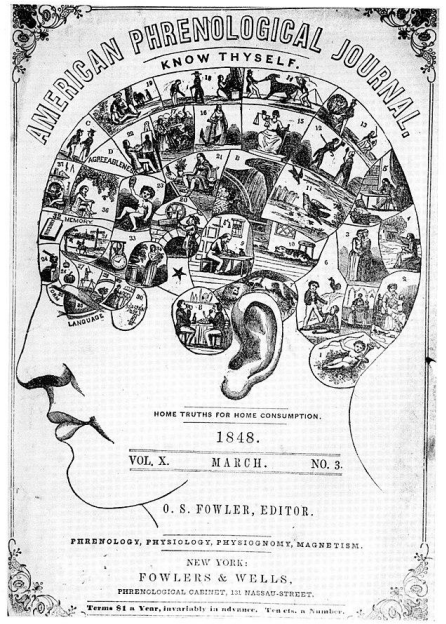
Contents

History of brain “imaging”

- Manual dissection of brain post-mortem
  - 1860s – Paul Broca & Patient Tan, who had severe language deficits
  - Broca performed autopsy and observed localized injury – likely the cause of language deficits
- Same time – Franz Gall, German neuroanatomist, published a treatise describing phrenology, the belief that shape of person's head and bumps on outside of skull could predict personality traits
  - An unsupported fad
- Both support localization of function; today, we think about connections between areas as being important to healthy brain activity



Polygon data were generated by Database Center for Life Science(DBCLS)[2]. License: CC-BY-SA-2.1-jp



Cover of American Phrenological Journal from March 1848, volume 10, number 3. Public Domain.

Computerized tomography scan (AKA CT scan or CAT scan)

- Example questions answered:
  - "Does the patient have a brain tumor, and where is the brain tumor located?"
  - "Are the meninges intact?"
- Essentially a 3D x-ray that revolves around person as they move through scanner
- Computer, not radiographic film, is used to detect passage of x-rays; computer generates 3D reconstruction based on series of 2D images
- Spatial resolution of ~0.5 mm
- Generally used to assess diagnostic changes over several days so temporal resolution is not a consideration
- Can identify tissues of different density, so useful for identifying and diagnosing particular conditions (tumors)
- Hydrocephalus can be quickly identified
- Meningitis may present as increased contrast

CT scan advantages and limitations

- Advantages:
  - Non-invasive (diagnose cause and hope to intervene while person is still alive)
  - Quick (takes only minutes)
- Limitations:
  - Highly mutagenic so information to be gained must outweigh risks of exposure

CT scanner depicted

Figure 6.8 The patient lies on a table that moves through the middle of the CT scanner.

Class discussion

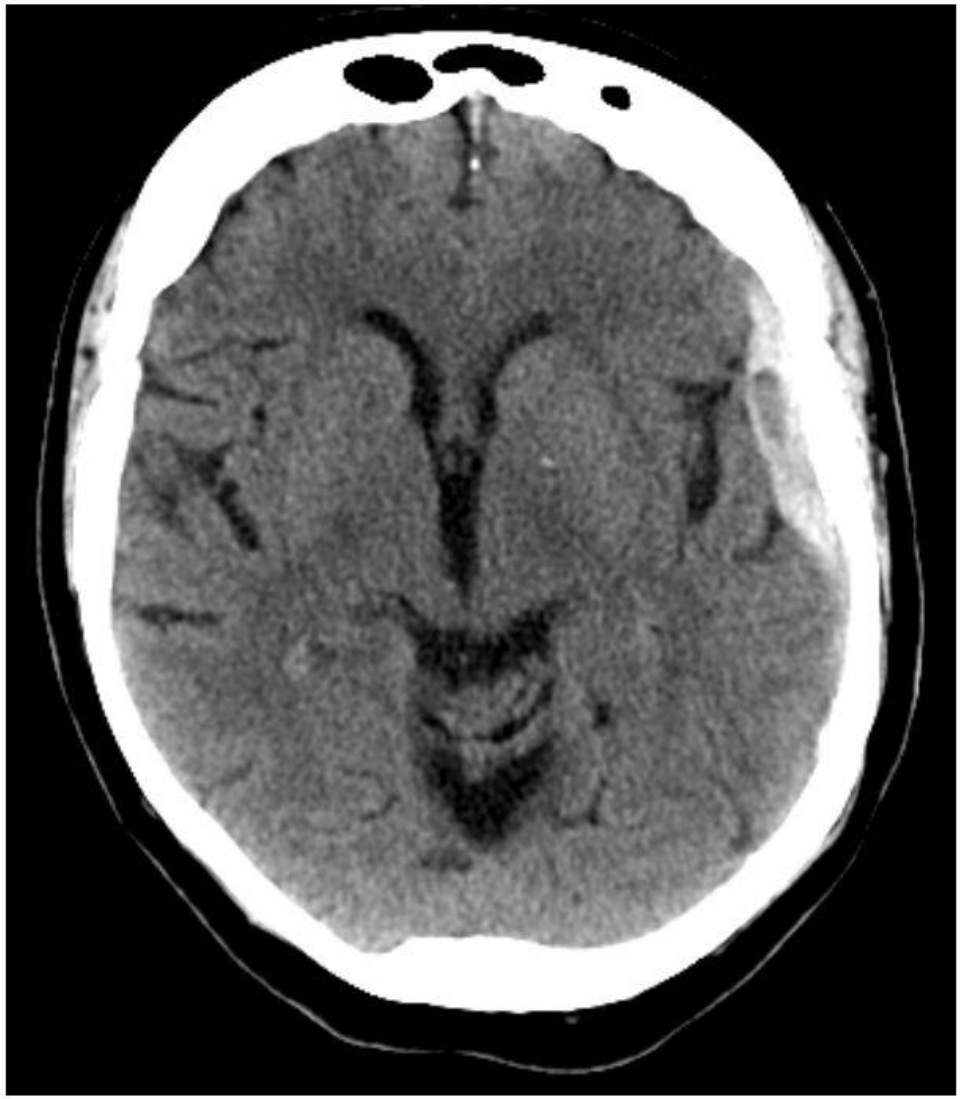


Figure 6.9 What do you notice that is abnormal in this CT scan?

MRI

CT scanner vs. MRI scanner

Some differences

- Magnetic Resonance Imaging
- Magnets are powerful; typical hospital MRI magnets (15,000 gauss; 1.5 Tesla); more powerful machines (100,000 gauss; 10 Tesla)
- Stronger magnets = better spatial resolution (currently in millimeters)
  - But also cause issues
- Radio emission device (“coil”) sends and receives signals
- MRI detects changes in protons response to magnets and radio waves (changes in energy states)

The physics behind MRI

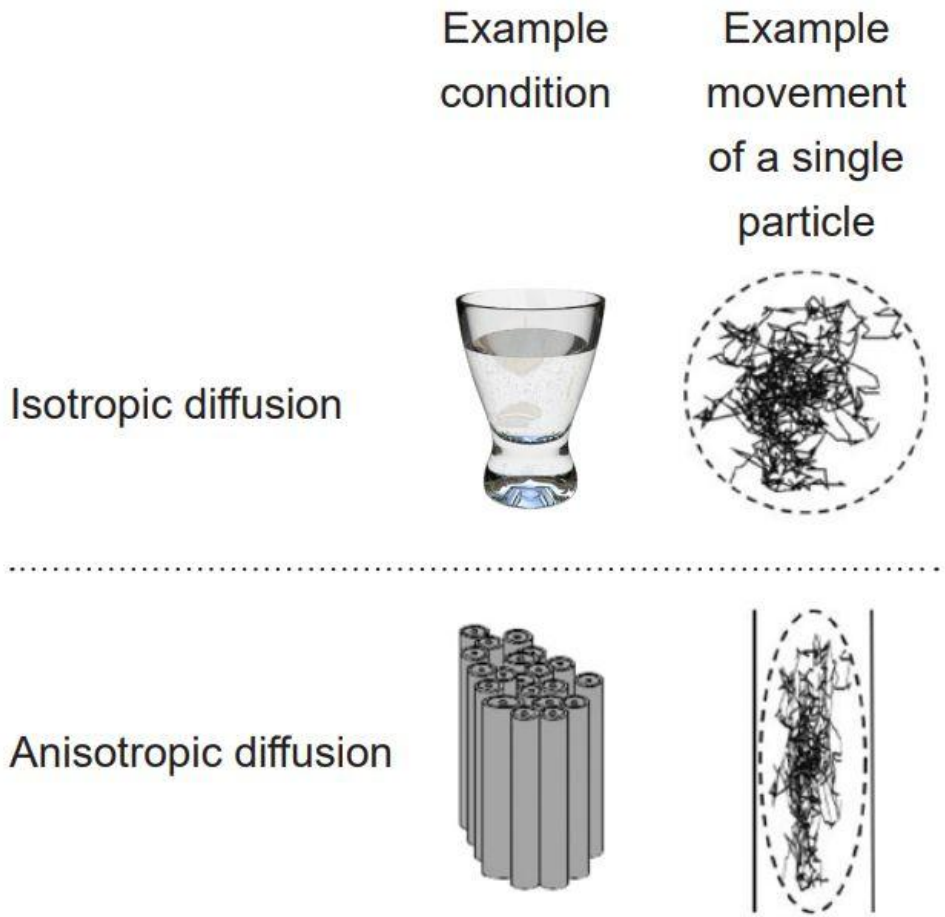
How MRI machine works

Diffusion tensor imaging (DTI)

- Example questions answered:
  - "Are the properties of the medial longitudinal fasciculus related to normal language processing?"
  - Are the properties of white matter different within the normal-appearing white matter of people with multiple sclerosis compared to healthy controls?
  - Are the properties of white matter different before and after learning to juggle?
- Good for subtle anatomical changes **especially related to white matter**
- Uses MRI technology to detect the movement of water molecules, which is different in white matter (anisotropic diffusion) than in gray matter
- Spatial resolution can be on order of millimeters (very good)
- Low temporal resolution; not generally used for tracking over short time periods

Isotropic diffusion vs anisotropic diffusion

Figure 6.11 In isotropic diffusion (top), a single particle is able to move in any direction randomly. However, in anisotropic diffusion (bottom), a single particle is more likely to move along a certain pathway, aligning with the cellular architecture.



Diffusion tensor imaging depicted

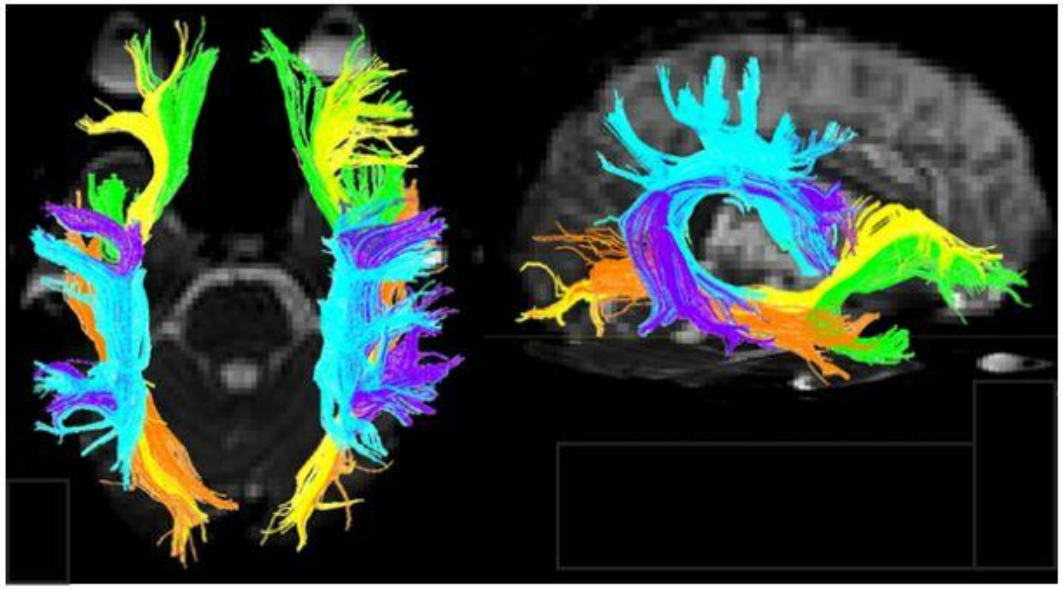


Figure 6.10 Diffusion tensor imaging can be used to visualize tracts of white matter, indicated by different colors.

DTI advantages and limitations

- Advantage: spatial resolution in order of mm
- Limitation: cannot give information about directionality of axonal projections

CLARITY

- Example questions answered:
  - "Do neurons in layer 5 of motor cortex send axonal projections into the spinal cord?"
- Gives microscopic level analysis; able to visualize connectivity in the brain, giving spatial resolution at the order of microns
- Published in 2013 at Stanford by lab of Dr. Karl Deisseroth
- To stop lipids and cellular particles from preventing light to pass through nervous tissue, brain is flushed with chemicals to form gel matrix surrounding every cellular component & washed in detergent to wash away lipids and leave gel matrix
- Light-deflecting lipids being washed away allows a visual of where connections were – causing the brain to appear transparent

CLARITY advantages and limitations

- Advantages:
  - Connections are made visible
  - Able to see at the order of microns (spatial resolution)
- Limitations:
  - Cannot be applied in an intact living organism
  - Destroys tissue; destructive
  - Gel mold is just an image, all function is destroyed

CLARITY depicted

Video

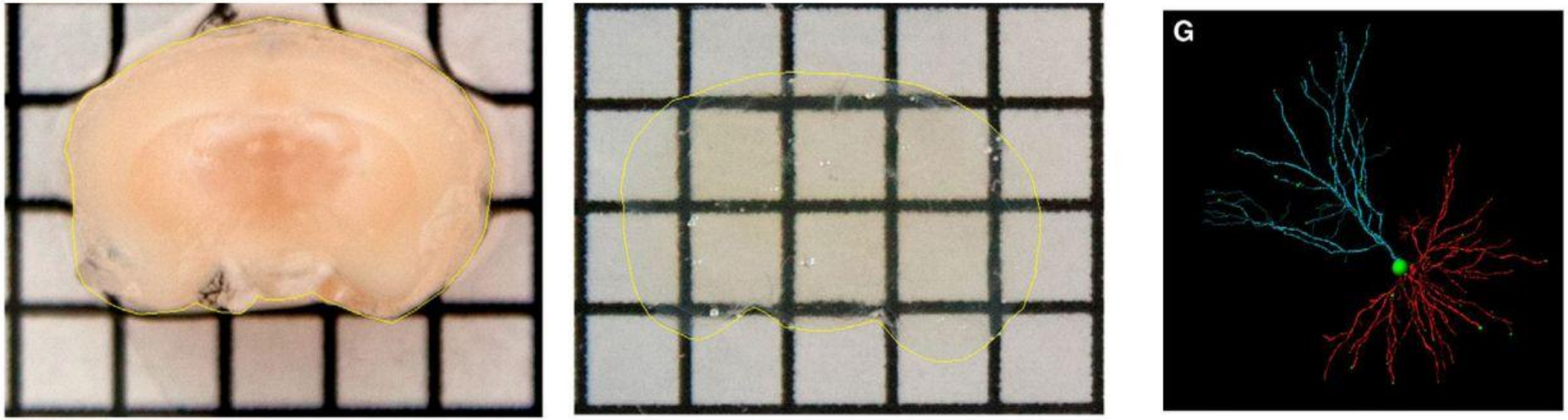


Figure 6.12 With CLARITY, a whole animal brain (left) can be made transparent (middle), allowing for visualization of individual cellular structures (right).

Rationale for imaging brain function

- Brain of a living and a deceased person might look identical anatomically, but they would exhibit significantly different function

Electroencephalography (EEG)

- Example questions answered:
  - "At what times of the night does the sleeping brain exhibit synchronized neuronal activity?"
  - "How does brain activity change when a person is having a seizure?"
- Can observe electrical activity of the brain; when firing simultaneously, currents produced by neurons can be so large they are detected on surface of head
- First used in 1920s by Hans Berger
- Sodium chloride gel is applied to scalp – conductor to allow currents to be picked up by electrodes (20-128 electrodes)
- Each electrode can detect voltage deflections as small as 10 microvolts
- Computer determines which cortical areas are exhibiting patterns of activity

EEG depicted

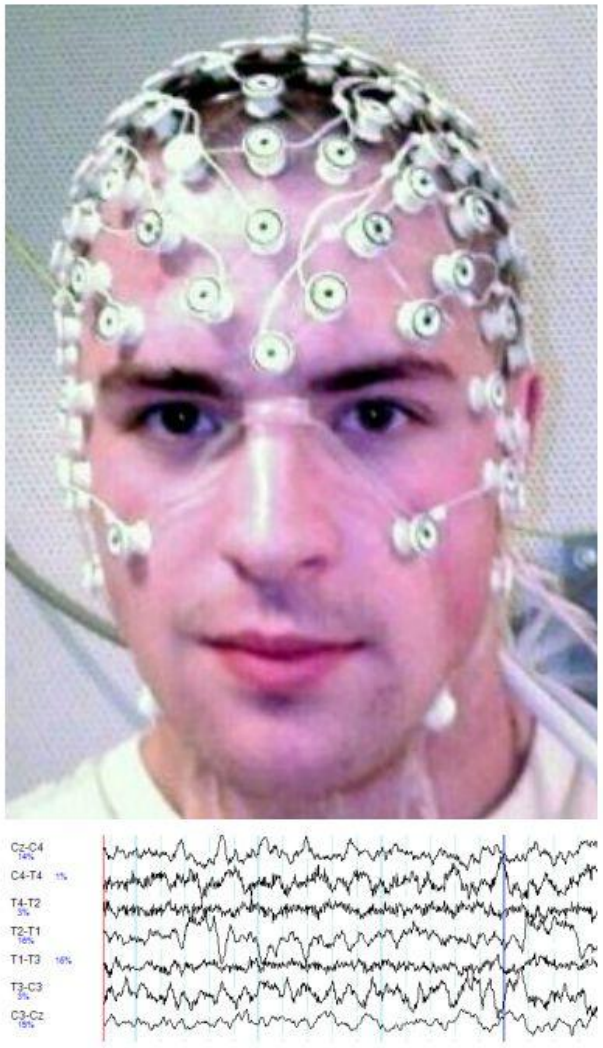
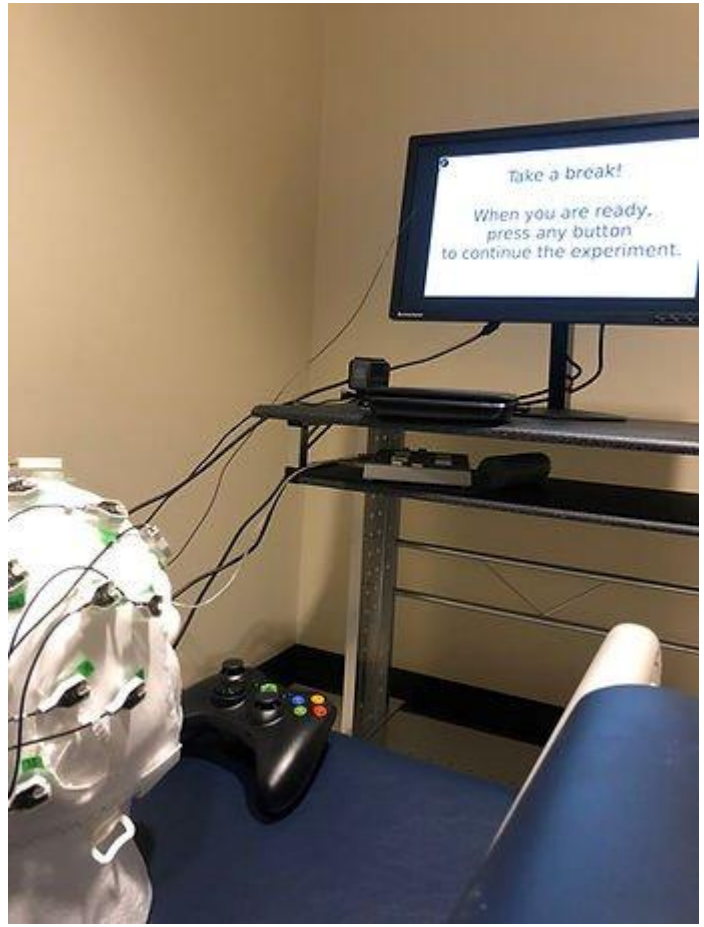


Figure 6.13 The electroencephalogram (EEG) is a noninvasive measure of neuronal activity (top). The information gathered (bottom) reflects changes in electrical potential measured at the scalp.



https://anchorlab.wixsite.com/anchorlab

EEG advantages and limitations

- Advantages:
  - Sensitive enough to examine rapidly-fluctuating potentials and dissect components: beta waves, delta waves, and all frequencies in between
  - Non-invasive; no permanent change or damage
  - Standard part of polysomnogram; in fact, best and most reliable characterization of sleep phases
  - Useful when person is under anesthesia to evaluate level of unconsciousness
  - Relatively cheap – compared to other methods
  - Mobile; heavy equipment is not required
  - Useful in diagnosis of disorders resulting in aberrant cortical neural activity (ex. Epilepsy~~, migraine, Alzheimer's, depression, and possibly ADHD~~)
  - Great temporal resolution (capable of sampling in range of 10,000 Hz) which helps with precise assessments of brain activity
- Limitations:
  - Poor spatial resolution (increased with number of electrodes, but even with 128 electrodes, spatial resolution is ~7 cm^3)

Can EEG diagnose migraine, Alzheimer's, depression, and possibly ADHD?

Search for diagnostic criteria of the disorder or disease

Search for reviews on diagnosis

J Alzheimers Dis Rep. 2024 Aug 20;8(1):1153-1169. doi: 10.3233/ADR-230159. eCollection 2024.

Assessing the Potential of EEG in Early Detection of Alzheimer's Disease: A Systematic Comprehensive Review (2000–2023)

Sharareh Ehteshamzad [1]

Affiliations + expand

PMID: 39247874 PMCID: PMC11380315 DOI: 10.3233/ADR-230159

Abstract

Background: As the prevalence of Alzheimer's disease (AD) grows with an aging population, the need for early diagnosis has led to increased focus on electroencephalography (EEG) as a non-invasive diagnostic tool.

Objective: This review assesses advancements in EEG analysis, including the application of machine learning, for detecting AD from 2000 to 2023.

Methods: Following PRISMA guidelines, a search across major databases resulted in 25 studies that met the inclusion criteria, focusing on EEG's application in AD diagnosis and the use of novel signal processing and machine learning techniques.

Results: Progress in EEG analysis has shown promise for early AD identification, with techniques like Hjorth parameters and signal compressibility enhancing detection capabilities. Machine learning has improved the precision of differential diagnosis between AD and mild cognitive impairment. However, challenges in standardizing EEG methodologies and data privacy remain.

Conclusions: EEG stands out as a valuable tool for early AD detection, with the potential to integrate into multimodal diagnostic approaches. Future research should aim to standardize EEG

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RESEARCH ARTICLE | Open Access

Revised criteria for diagnosis and staging of Alzheimer's disease: Alzheimer's Association Workgroup

Clifford R. Jack Jr., J. Scott Andrews, Thomas G. Beach, Teresa Buracchio, Billy Dunn, Ana Graf, Oskar Hansson, Carole Ho, William Jagust, Eric McDade, Jose Luis Molinuevo ... See all authors

First published: 27 June 2024 | https://doi.org/10.1002/alz.13859 | Citations: 62

Contributing committee members: Eliezer Masliah and Laurie Ryan.
Clifford R. Jack Jr, Billy Dunn, Heather M. Snyder, Eliezer Masliah, and Maria C. Carrillo are the members of the steering committee.

Abstract

The National Institute on Aging and the Alzheimer's Association convened three separate work groups in 2011 and single work groups in 2012 and 2018 to create recommendations for the diagnosis and characterization of Alzheimer's disease (AD). The present document updates the 2018 research framework in response to several recent developments. Defining diseases biologically, rather than based on syndromic presentation, has long been standard in many areas of medicine (e.g., oncology), and is becoming a unifying concept common to all neurodegenerative diseases, not just AD. The present document is consistent with this principle. Our intent is to present objective criteria for diagnosis and staging AD, incorporating recent advances in biomarkers, to serve as a bridge between research and clinical care. These criteria are not intended to provide step-by-step clinical practice guidelines for clinical workflow or specific treatment protocols, but rather serve as general principles to inform diagnosis and staging of AD that reflect current science.

Review > Neurosci Biobehav Rev. 2019 Oct:105:83-93. doi: 10.1016/j.neubiorev.2019.07.021. Epub 2019 Aug 7.

Depression biomarkers using non-invasive EEG: A review

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PMID: 31400570 DOI: 10.1016/j.neubiorev.2019.07.021

Abstract

Depression is a serious neurological disorder characterized by strong loss of interest, possibly leading to suicide. According to the World Health Organization, more than 300 million people worldwide suffer from this disorder, being the leading cause of disability. The advancements in electroencephalography (EEG) make it a powerful tool for non-invasive studies on neurological disorders including depression. Scientific community has used EEG to better understand the mechanisms behind the disorder and find biomarkers, which are characteristics that can be precisely measured in order to identify or diagnose a disorder. This work presents a systematic mapping of recent studies ranging from 2014 to the end of 2018 which use non-invasive EEG to detect depression biomarkers. Our research has analyzed more than 250 articles and we discuss the findings and promising biomarkers of 42 studies, finding that the depressed brain appear to have a more random network structure, also finding promising features for diagnostic, such as, gamma band and signal complexity; among others which may detect specific depression-related symptoms such as suicidal ideation.

Keywords: Biomarkers; Depression; Diagnosis; Non-invasive EEG; Review.

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Revisiting the theoretical and methodological foundations of depression measurement

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Abstract

Depressive disorders are among the leading causes of global disease burden, but there has been limited progress in understanding the causes and treatments for these disorders. In this Perspective, we suggest that such progress crucially depends on our ability to measure depression. We review the many problems with depression measurement, including limited evidence of validity and reliability. These issues raise grave concerns about common uses of depression measures, such as diagnosis or tracking treatment progress. We argue that shortcomings arise because depression measurement rests on shaky methodological and theoretical foundations. Moving forward, we need to break with the field's tradition that has, for decades, divorced theories about depression from how we measure it. Instead, we suggest that epistemic iteration, an iterative exchange between theory and measurement, provides a crucial avenue for depression measurement to progress.

Neuroscience and Biobehavioral Reviews 139 (2022) 104752

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Review article

Can electroencephalography (EEG) identify ADHD subtypes? A systematic review

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ABSTRACT

Attention Deficit/Hyperactivity Disorder (ADHD) has been associated with atypical patterns of neural activity measured by electroencephalography (EEG). However, the identification of EEG diagnostic biomarkers has been complicated by the disorder's heterogeneity. The objective of this review was to synthesize the literature investigating EEG variation in patients diagnosed with ADHD, addressing the following questions: 1) Are the diagnostic ADHD subtypes associated with different EEG characteristics? 2) Are EEG measures correlated with ADHD traits and/or symptom severity? and 3) Do classification techniques using EEG measures reveal different clinical presentations of ADHD? Outcomes highlight the potential for electrophysiological measures to provide meaningful insights into the heterogeneity of ADHD, although direct translation of EEG biomarkers for diagnostic purposes is not yet supported. Key measures that show promise for the discrimination of existing ADHD subtypes and symptomatology include: resting state and task-related modulation of alpha, beta and theta power, and the event-related N2 and P3 components. Prescriptions are discussed for future studies that may help to bridge the gap between research and clinical application.

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ADHD in the DSM-5-TR: What has changed and what has not

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In this article, we critically review the changes made to the DSM-5 Text Revision published in 2022 regarding the diagnostic entity of Attention Deficit/Hyperactivity Disorder (ADHD). We structure our critique around three points. The first discusses the acknowledgment of ADHD as a neurodevelopmental disorder. The second examines the definition of ADHD provided in the updated edition of the manual. The third scrutinizes the changes in the diagnostic criteria for ADHD and assesses whether these changes make the diagnosis more accurate. We conclude that DSM's latest edition does not escape the logical and scientific pitfalls of its predecessor. DSM-5-TR keeps the faith in the neo-Kraepelinian paradigm by explicitly and implicitly cultivating the essentialist medical scientific metaphor of disorder, creating the illusion that it represents scientific progress that validates ADHD as a neurodevelopmental disorder.

KEYWORDS

ADHD, DSM-5-TR, revisions, American Psychiatric Association, diagnosis, diagnostic manual

diagnostics

MDPI

Article

Electroencephalography-Based Depression Detection Using Multiple Machine Learning Techniques

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Abstract: The growth of biomedical engineering has made depression diagnosis via electroencephalography (EEG) a trendy issue. The two significant challenges to this application are EEG signals' complexity and non-stationarity. Additionally, the effects caused by individual variances may hamper the generalization of detection systems. Given the association between EEG signals and particular demographics, such as gender and age, and the influences of these demographic characteristics on the incidence of depression, it would be preferable to include demographic factors during EEG modeling and depression detection. The main objective of this work is to develop an algorithm that can recognize depression patterns by studying EEG data. Following a multiband analysis of such signals, machine learning and deep learning techniques were used to detect depression patients automatically. EEG signal data are collected from the multi-modal open dataset MODMA and employed in studying mental diseases. The EEG dataset contains information from a traditional 128-electrode elastic cap and a cutting-edge wearable 3-electrode EEG collector for widespread applications. In this project, resting EEG readings of 128 channels are considered. According to CNN, training with 25 epoch iterations had a 97% accuracy rate. The patient's status has to be divided into two basic categories: major depressive disorder (MDD) and healthy control. Additional MDD include the following six classes: obsessive-compulsive disorders, addiction disorders, conditions brought on by trauma and stress, mood disorders, schizophrenia, and the anxiety disorders discussed in this paper are a few examples of mental illnesses. According to the study, a natural combination of EEG signals and demographic data is promising for the diagnosis of depression.

Keywords: major depressive disorder (MDD); electroencephalogram (EEG); convolutional neural network; feature extraction; deep learning; depressive disorder

Citation: Ksibi, A.; Zakariah, M.; Menzli, L.J.; Saidani, O.; Almuqren, L.; Hanafieh, R.A.M. Electroencephalography-Based Depression Detection Using Multiple Machine Learning Techniques. *Diagnostics* **2023**, *13*, 1779. https://doi.org/10.3390/diagnostics13101779

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diagnostics

MDPI

Review

Migraine Aura—Catch Me If You Can with EEG and MRI—A Narrative Review

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with an increased risk for stroke, epilepsy, and with anxiety disorder. Diagnosis of migraine with aura sometimes requires exclusion of secondary causes if neurological deficits present for the first time or are atypical. It was the aim of this review to summarize EEG an MRI findings during

systematic literature search. During visual auras, EEG showed no consistent abnormalities related to aura, although transient focal slowing in occipital regions has been observed in quantitative studies. In contrast, in familial hemiplegic migraine (FHM) and migraine with brain stem aura, significant EEG abnormalities have been described consistently, including slowing over the affected hemisphere or bilaterally or suppression of EEG activity. Epileptiform potentials in FHM are most likely attributable to associated epilepsy. The initial perfusion change during migraine aura is probably a short lasting hyperperfusion. Subsequently, perfusion MRI has consistently demonstrated cerebral hypoperfusion usually not restricted to one vascular territory, sometimes associated with vasoconstriction of peripheral arteries, particularly in pediatric patients, and rebound hyperperfusion in later phases. An emerging potential MRI signature of migraine aura is the appearance of dilated veins in susceptibility-weighted imaging, which may point towards the cortical regions related to aura symptoms ("index vein"). Conclusions: Cortical spreading depression (CSD) cannot be directly visualized but there are probable consequences thereof that can be captured Non-invasive detection of CSD is probably very challenging in migraine. Future perspectives will be elaborated based on the studies summarized.

Keywords: migraine aura; MRI; EEG; cortical spreading depolarization

Citation: Riederer, F.; Beiersdorf, J.; Scutelnic, A.; Schankin, C.J. Migraine Aura—Catch Me If You Can with EEG and MRI—A Narrative Review. *Diagnostics* **2023**, *13*, 2844. https://doi.org/10.3390/diagnostics13172844

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Positron emission tomography (PET scan)

- Example questions answered:
  - "Which areas of the brain decrease in activity when a person experiences mild cognitive impairment?"
  - "Do drug-dependent people have a high density of opioid receptors?"
- Application of nuclear medicine in which a tracer is injection into the bloodstream; tracer is unstable and emits positrons; emits gamma particles when positrons interact with electrons of nearby molecules – detected by machine
- Fluorodeoxyglucose-F18 (FDG) is a radioactive analog of glucose, a source of cellular energy; highly metabolically active areas take FDG into cells
- Change in energetic demand can be detected by increased glucose movement
- PET scanner looks similar to CT scanner

How PET works

Example tracer

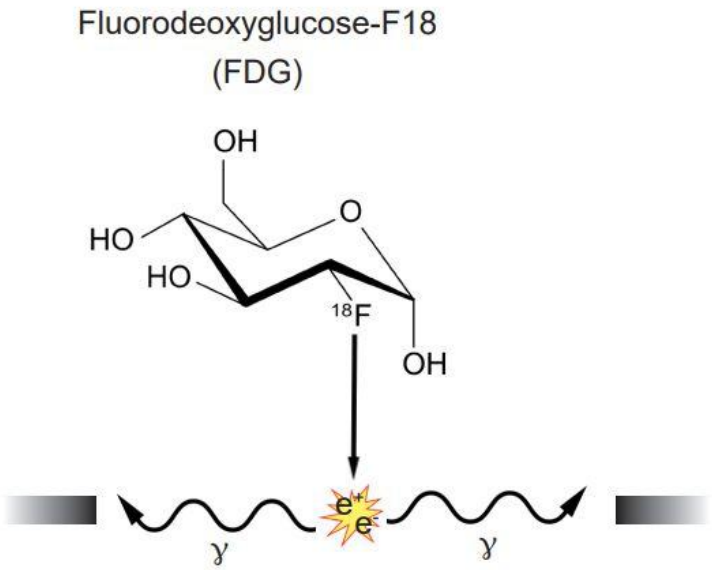


Figure 6.14 When a positron (e^+) emitted by the tracer compound (FDG) interacts with nearby electrons, gamma rays (γ) are emitted at perpendicular angles which are detected by the PET scanner (gray boxes).

PET scan depicted

[How PET works]

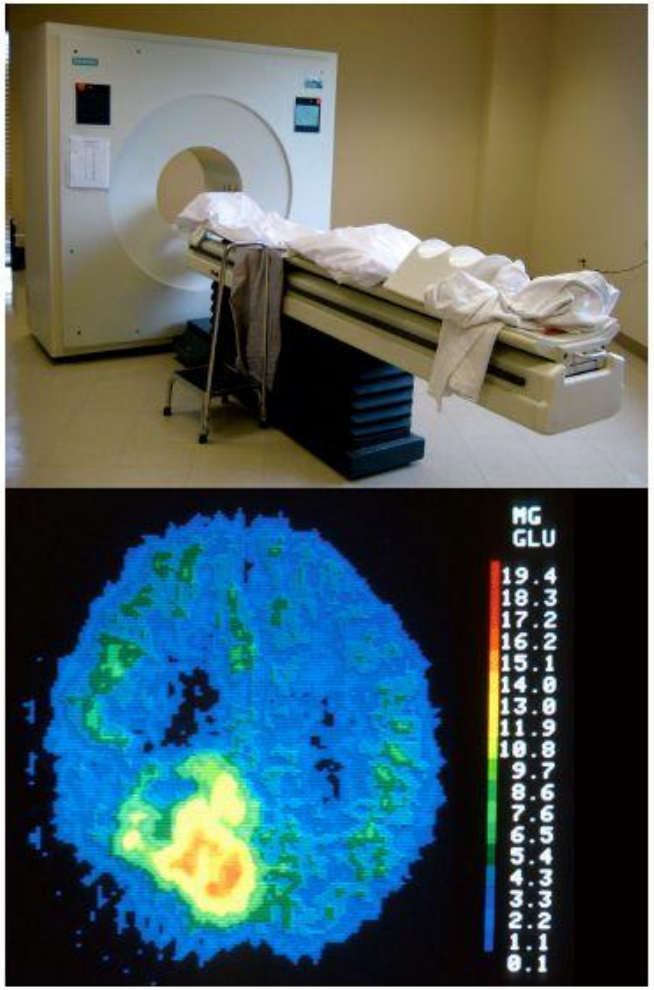


Figure 6.15 The positron emission tomography (PET) scanner (top) is quiet compared to the CT scan or MRI. It can generate data sets (bottom) that visualize the approximate location of highly-energetically demanding tissue (yellow and orange), such as a brain tumor.

PET advantages and limitations

- Advantages:
  - Effective at diagnosing and identifying locations of tumors
  - Provides an overall picture of brain activity, which may help in diagnosing cognitive deficits, like dementia with Alzheimer's or Pick's disease
  - Can visualize levels of receptors in vivo
- Limitations:
  - Exposed to radioactive compounds and gamma wave radiation, which can be mutagenic
  - Difficult to identify boundaries between tissues (so, often performed simultaneously with CT scan)
  - Poor spatial resolution – differences between areas is only noticeable if volume is in range of 5-10 cm^3
  - Poor temporal resolution – takes tens of seconds or minutes before a change in signal can be observed and detected

Proton reactions to magnets and radio emissions

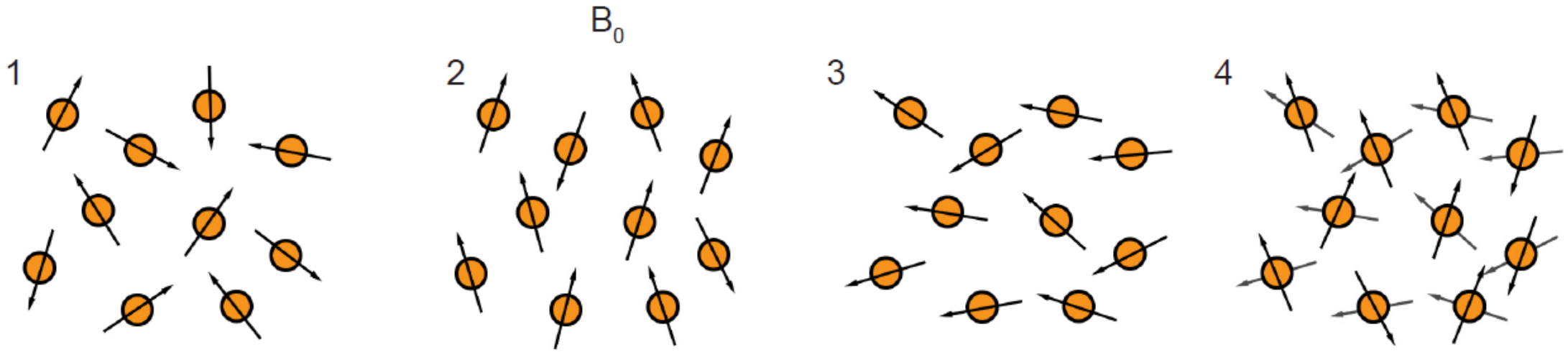


Figure 6.16 (1) Protons spin around an axis, aligning randomly at rest. (2) In the presence of a magnetic field B_0, their spin aligns with or directly opposite of the magnetic field. (3) Exposure to radiowaves knocks the protons out of their alignment into a high energy state. (4) As the protons return back to their alignment with the magnetic field, they release energy.

Functional magnetic resonance imaging (fMRI)

- Example questions answered:
  - "Does blood flow/oxygenation increase in the right hemisphere cingulate gyrus when a person sees their loved ones?"
  - "Which areas of the brain change in activity when a person is planning a motor action?"
- Can be used while person engages in a task; used to correlate behavior with activity patterns in specific parts of the brain

fMRI continued

- Oxygenated hemoglobin has different magnetic properties than deoxygenated hemoglobin
- Blood vessels near neurons needing more oxygen dilate – more oxygenated hemoglobin is delivered to those areas
- Change in blood flow is detected and called blood oxygenation level-dependent signal, or BOLD signal
- Temporal resolution is limited by speed of blood vessel dilation (seconds to tens of seconds)

fMRI advantages and limitations

- Advantages:
  - Able to visualize brain activity in real-time during complex behavioral tasks
- Limitations
  - Scanning tunnel (bore) is small and some people may experience anxiety, panic, or claustrophobia
  - Machine is loud
  - People cannot enter scanner with magnetosensitive implants
  - Data can be difficult to analyze and frequently subjected to false positives
- Assumption of blood flow being related to neural activity, but may not always be the case

fMRI depicted

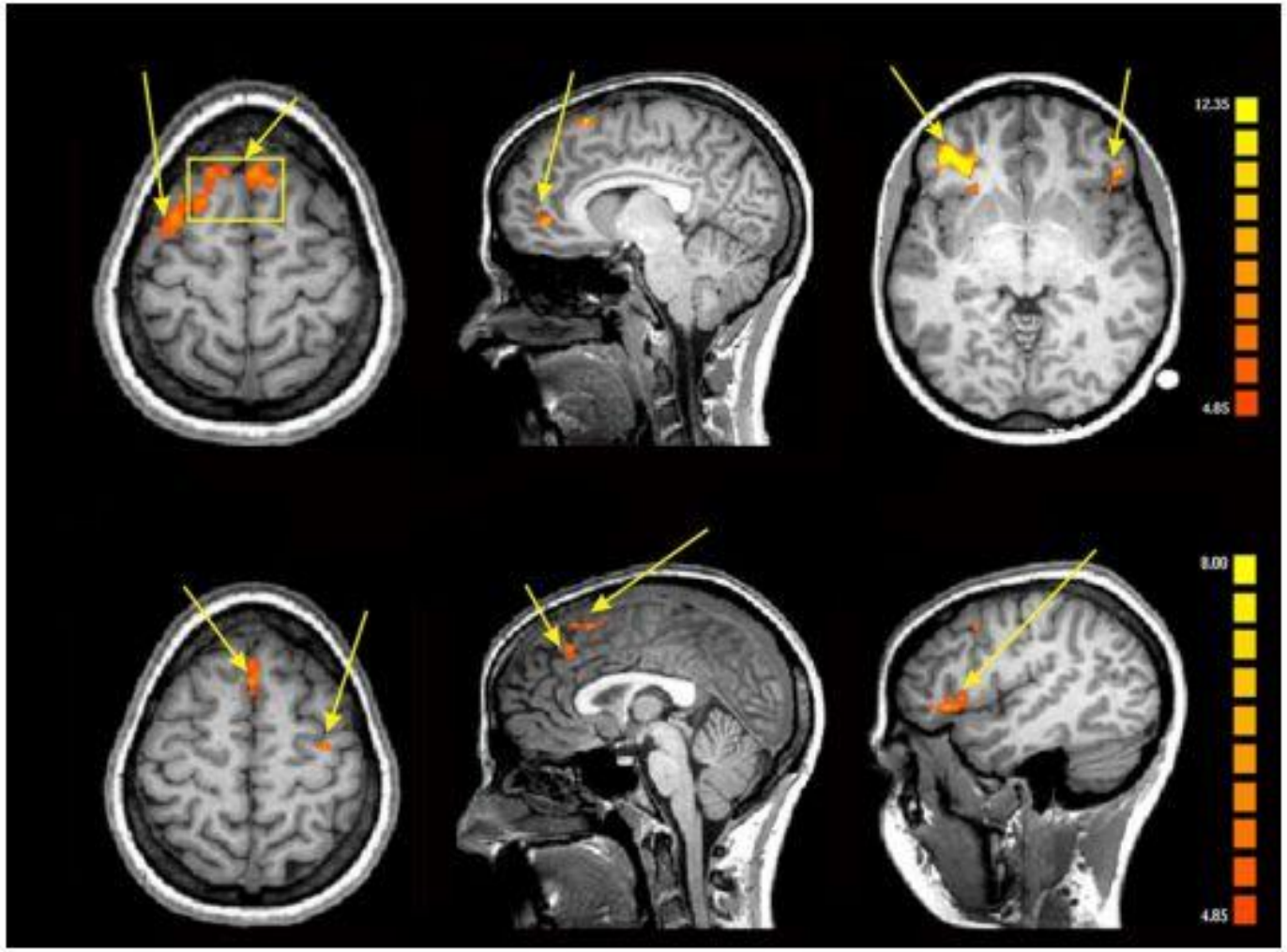


Figure 6.17 The fMRI scan detects the location of blood flow changes as a person pays attention to different visual stimuli (top vs. bottom).

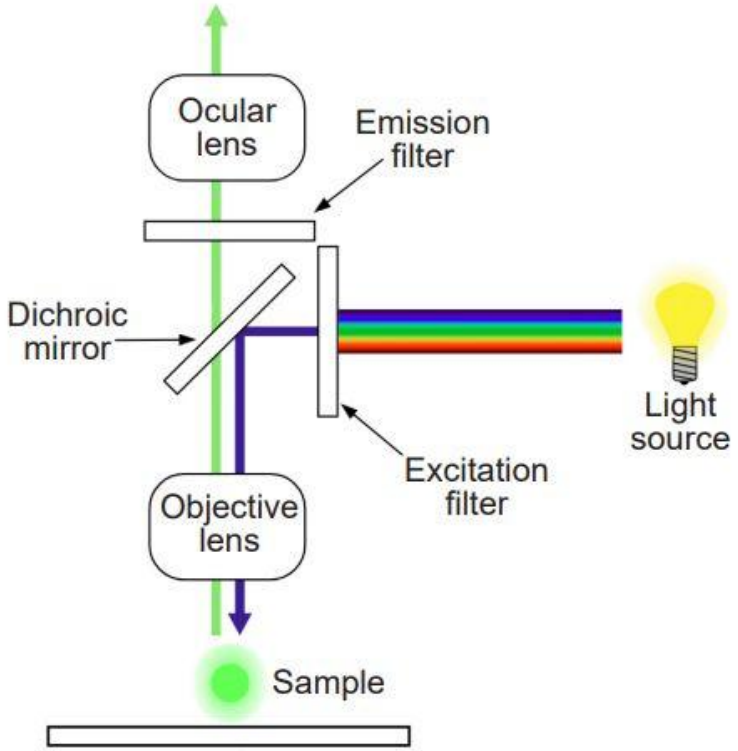
Imaging the cells of the nervous system

- Used to visualize morphology of structures smaller than hundreds of microns

Microscopy

- Example questions answered:
  - "What is the diameter of the soma of a granule cell?"
  - "How are the retinal neurons at the fovea different from the neurons in the periphery?"
- An old technique developed by Dutch scientist Antonie van Leeweunhoek (late 1700s)
- Lenses and a bright source of light were used to magnify tiny water-dwelling animals (~40 times closer than the unaided eye)
- Today, magnification achieved is up to 400 or 1000 times closer
- Types of microscopy
  - Electron microscopes were developed in 1930s; use an electron emission device in conjunction with high-speed detectors to visualize structures on the order of nanometers (up to a million times magnified)
  - Fluorescent microscopes use light sources to emit light at specific wavelengths – wavelengths at which protons activate proteins, which emit light at a different wavelength (which is then detected)
- Most other techniques rely on microscopy

Fluorescent microscopy depicted



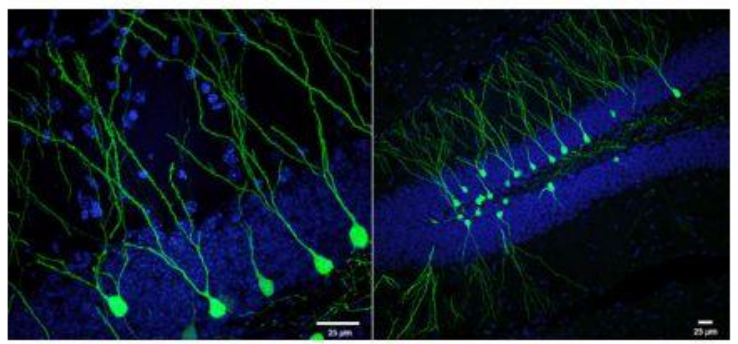


Figure 6.18 The fluorescence microscope (diagram, top) can be used to excite GFP (green) expressed in hippocampal neurons (bottom).

Staining

- Example questions answered:
  - "Where is the white matter located in the brain stem?"
  - "What is the morphology of dendritic arbor of a cerebellar Purkinje cell?"
- Often used in conjunction with microscopy
- Chemicals used to stain cells have different affinities for different parts of cells
- Process involves steps
  - Fixation with a chemical like paraformaldehyde (PFA)
    - Perfusion - hijack the endogenous circulatory system by flushing fixative through arteries
  - Slicing of brain into sections with microtome or cryostat (~10 microns)

Staining depicted

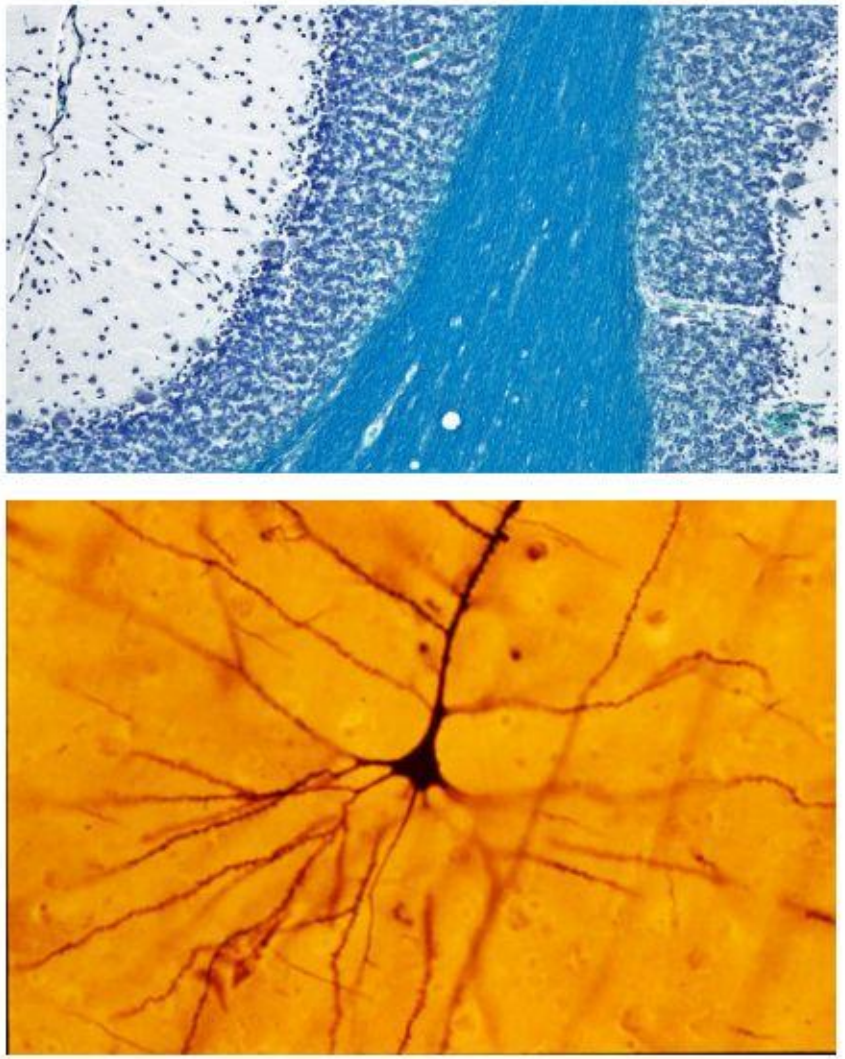


Figure 6.19 A slice of cerebellum, with the myelin stained with Luxol Fast Blue and the neurons stained with cresyl violet (top; 100x magnified). A pyramidal cell treated with a Golgi stain (bottom).

Your Neuron Smears from Lab

Slide

- H&E Stain: hematoxylin and eosin stain

Slicing devices

Figure 6.21 A microtome (left) or a cryostat (right) are devices for taking brain sections. The microtome fits on a tabletop, and cuts accurately down to 100 uM thin. The cryostat is larger, and can cut 10 uM thin slices.

Tract tracing

- Tract tracing - certain staining methods can be used to determine projections – anterograde trace (from soma to axon terminal) and retrograde trace (from axon terminal to soma)

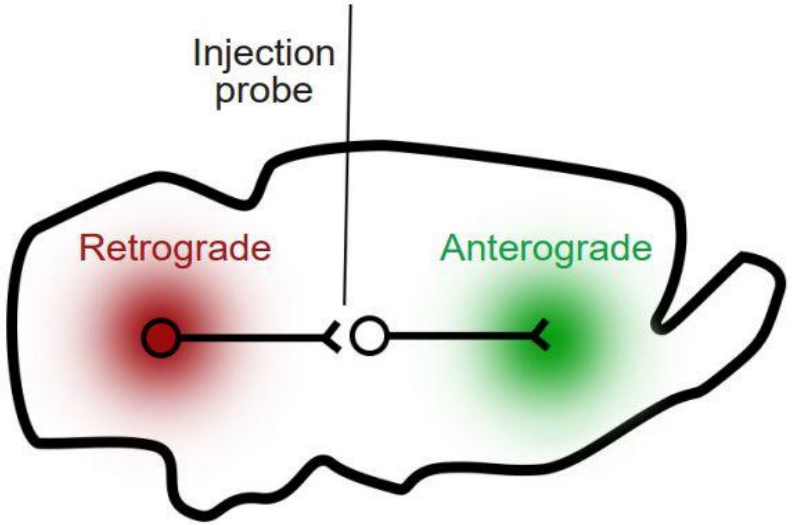


Figure 6.22 In tract tracing, injection of different staining dyes can help us identify the connectivity of neurons. If an injection site contains somata, the dye is transported in an anterograde direction towards the area where the axon terminals are located (green). If the injection site contains axon terminals, the dye is taken up and transported in a retrograde direction towards the cell bodies (red).

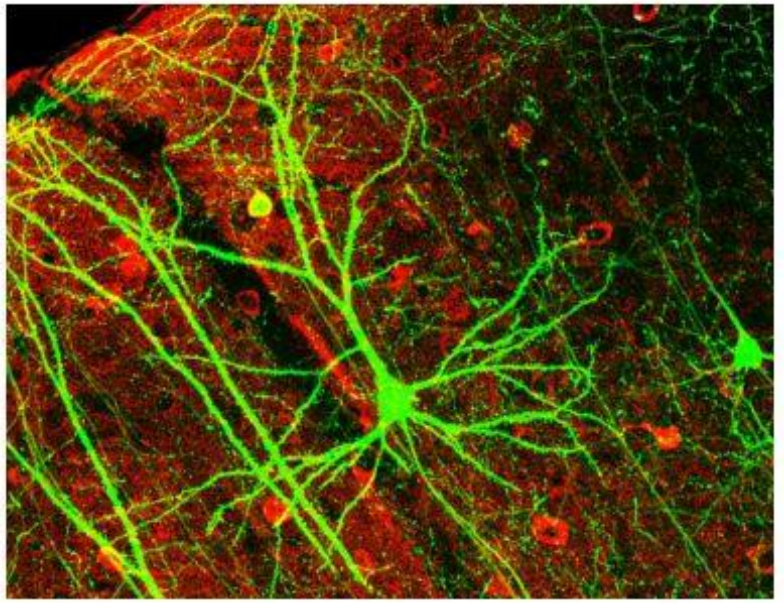
Staining: limitation

- Limitation: Cannot be used with living tissue; only fixed tissue

Immunohistochemistry (IHC) staining

- Example questions answered:
  - "Where is the protein actin located in a neuron?"
  - "Is the protein PSD-95 expressed in the same areas as the NMDA receptor protein?"
- Modification of staining with increased specificity
- Primary antibody is selected because it binds to protein of interest
- Secondary antibody, conjugated with a fluorophore (light-producing molecule), is used
- Activation of fluorophore is used to identify location of primary antibody

IHC example



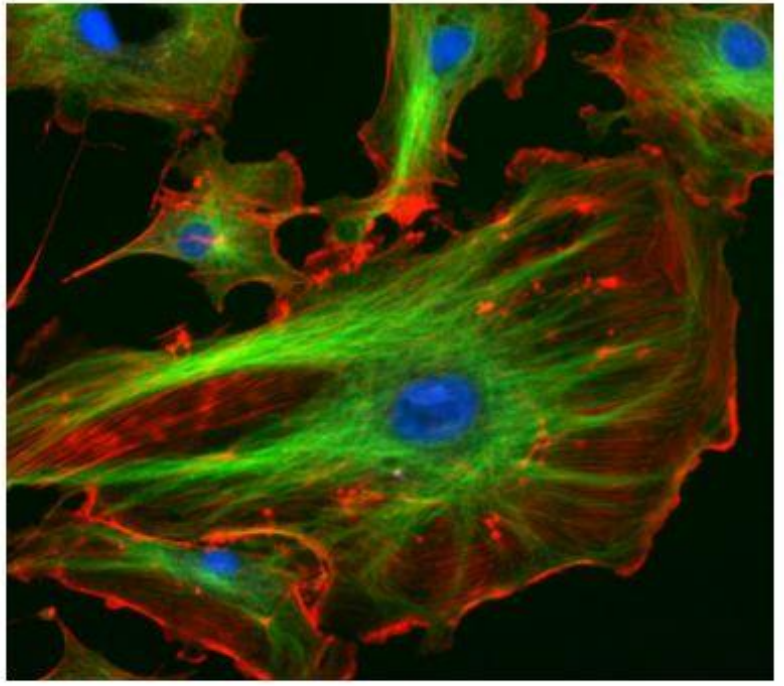


Figure 6.23 At low magnification (top), immunohistochemistry in conjunction with fluorescence microscopy can help us identify the morphology and location of different populations of cells. At high magnification (bottom), we can identify the location of different proteins within a cell, such as tubulin (green), which makes up the microtubules.

IHC: Underlying mechanism

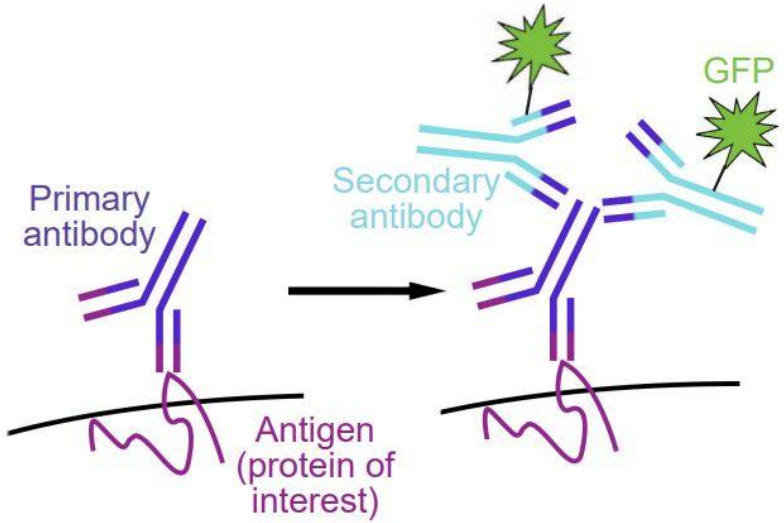


Figure 6.24 The primary antibody (purple) is chosen because it binds to a specific protein of interest (magenta). In a second step, the secondary antibody (light blue), conjugated with a fluorophore (in this case GFP), binds to the primary antibody.

Immunocytochemistry

- Could use antibodies & fluorescence microscopy to visualize elements of cells in a culture dish (in vitro)
- Called immunocytochemistry (cyto = cell, while histo = tissue)

- Immunolabeling strategy can be applied to whole brain after use of CLARITY

Immuno-chemistry: limitations

- Antibodies often exhibit nonspecific binding, leading to false positives
- Nonspecific binding also increases background noise, making it more difficult to identify a genuine fluorescent signal
  - Rinsing and exposure to blocking agents can help minimize nonspecific binding
- Not every single protein or enzyme has an antibody
- Some proteins are structurally similar to others, and an antibody to differentiate the two has not yet been

Changing nervous system activity

- Interested in controlling some part of nervous system (either increasing or decreasing activity)
- Manipulating neural activity can allow establishment of causation (part of nervous system and function)
- Late 1700s, Italian biologist Luigi Galvani discovered frog muscles would twitch when nerves were electrically stimulated
    - bioelectricity = endogenous electrical activity is important for muscle control, and by extension, the activity of the organism
- Work of Wilder Penfield in 1950s on epilepsy treatments allowed for neural manipulation in humans
    - patients were given local anesthetic, skin above skull was resected, allowing access to brain, and brain could be stimulated without pain since brain has no pain receptors

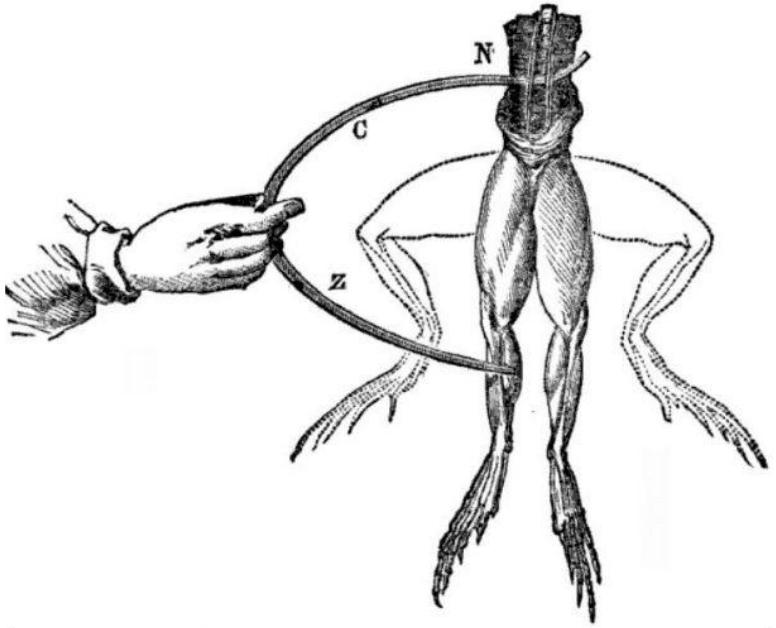


Figure 6.25 Luigi Galvani observed that electrically activating a nerve caused muscular activity in the frog leg. The phrase "galvanized to action" is a reference to Galvani's discovery.

Electrophysiology (Ephys)

- Example questions answered:
  - "How frequently do neurons in the striatum fire action potentials?"
  - "Does increasing cortical activity change the excitability of spinal cord neurons?"
- Use a rig to measure and manipulate electrical properties of nervous system
- Early use with *Loligo* squid
- In 1963, Hodgkin and Huxley won a Nobel prize for work that came out of Ephys techniques

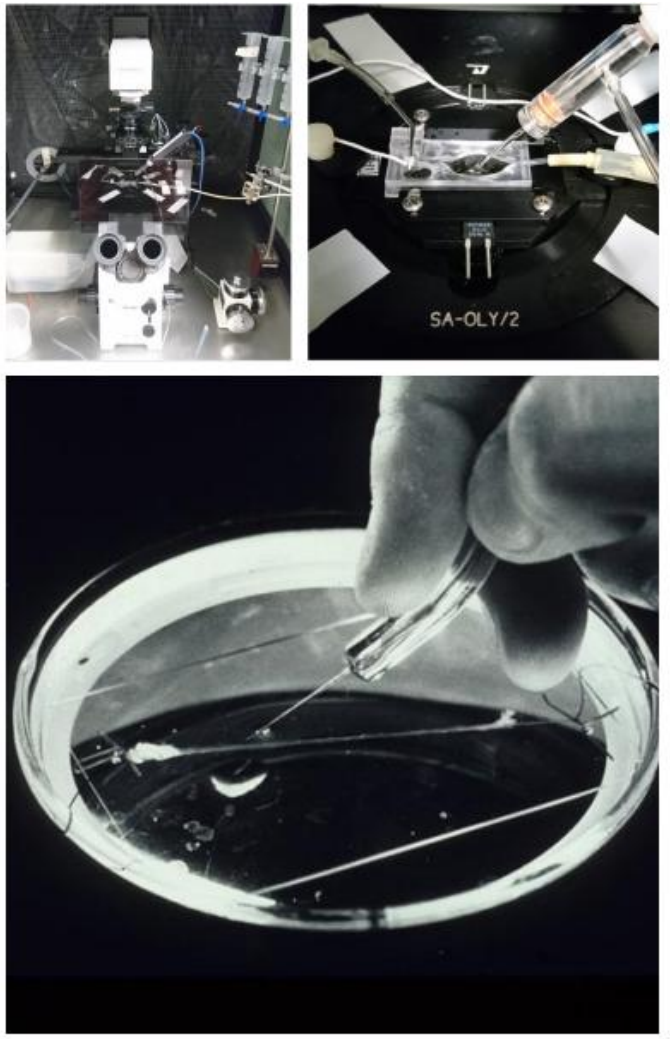


Figure 6.26 An electrophysiology rig is the setup used for recording and controlling the electrical activity of neurons (top). The isolated squid giant axon (bottom) was one of the earliest preparations that informed us about the electrical properties of neurons.

Ephys continued

- Originally used to detect difference between inside and outside of neuron
- Often requires concurrent use of microscope
- Tiny micropipettes filled with an electrolyte solution can be inserted in neuron
- Electrical currents can be detected and recorded
- Currents can be associated with movement of charged ions during an action potential
- Can be used to manipulate activity, too
- Gives direct physical control over electrical properties of neuron

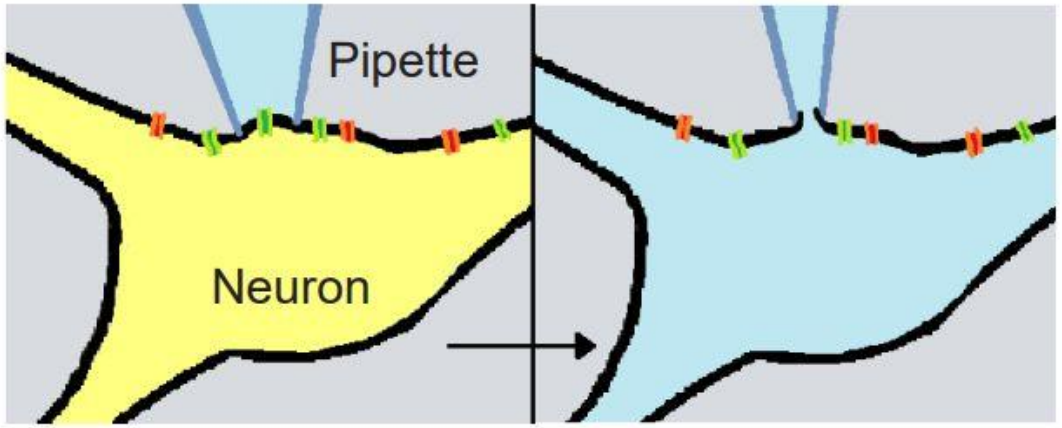


Figure 6.27 Rupturing the cell membrane with a microscopic glass pipette allows the experimenter to monitor and control the electrical conditions of the neuron.

Electrophysiology (Ephys): advantages and limitations

- Very versatile
  - Can ask questions from many levels, from behavior and neuronal circuitry to individual neurons and ion channels
    - Record patterns of neuronal activity as subject performs behavioral task
    - Stimulate neurons to see if behavior can be modified
    - Examine activity of individual ion channels to determine conditions when they open and close
- Weakness: highly experimental nature of technique
  - Many different preparations can be used: intact anesthetized, thin slice of living brain, neuronal culture, etc.
  - Gains in experimental control, loss in ability to generalize about data

Transcranial magnetic stimulation (TMS)

- Example questions answered:
  - "Which part of the motor cortex sends signals to contralateral foot?"
  - "Can activation of parts of the prefrontal cortex decrease the symptoms of depression?"
- **Induction**: movement of magnets can generate electric currents, and electricity can generate magnetic fields
- TMS machine = handheld coil of wires in a loop
- Passing electrical current through loop produces magnetic field
- Magnetic field generated induces an electrical current at some distance away from coil
- Place coil at surface of scalp to activate small areas of brain tissue

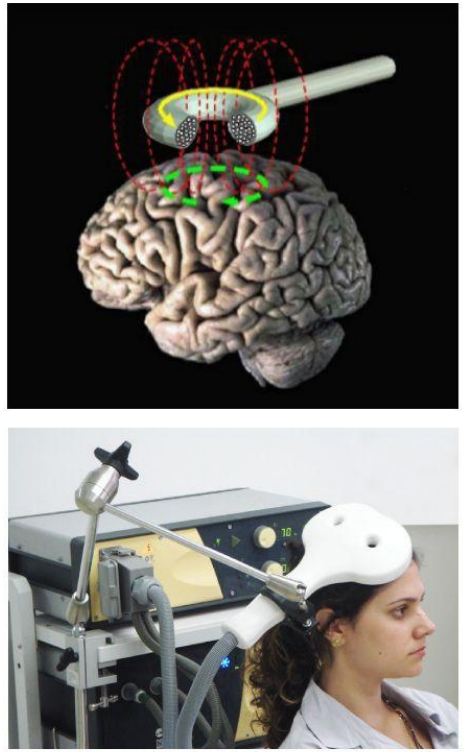


Figure 6.28 Running current through a magenetic coil generates an electric field via induction (top), which can activate or inactivate neural circuits in TMS (bottom).

TMS: advantages and limitations

- Believed to have moderate benefits for several psychiatric conditions (potentially a therapeutic intervention)
  - motor cortex activation for chronic pain conditions, Parkinsonian symptoms, and contralateral motor function following stroke injury
  - prefrontal cortex activation can decrease anxiety, antidepressive symptoms, cigarette craving or consumption, and schizophrenia
  - stimulations of other brain regions can be antiepileptic and may minimize auditory hallucinations or tinnitus
  - Used clinically in NS (https://www.nshealth.ca/patient-education-resources/1975)
- Non-invasive

- Unexpected consequences (ex. temporary headaches, localized pain, changes in hearing, and bizarre changes in somatosensation)
  - Severe side effect: seizures
- Can cause dangerous interactions with an magnetosensitive implants, ex. deep brain stimulation devices, cochlear implants, or aneurysm clips (like MRI)

Genetic modification

- Example questions answered:
  - "Do rats behave differently if they do not synthesize the hormone leptin?"
  - "How do mutated voltage-gated sodium channels change their neuronal action potential firing?"
- Rise in molecular genetics techniques since 1970s & genomes have been mapped out
- Knowledge of and capability to manipulate genomes allowed researchers to create animal models of a variety of human conditions
  - Knock-out – use gene excision to remove a small section of genetic code
  - Knock-in - gene insertion to put in exogenous genes into an animal
  - Knock-downs – cause a moderate decrease in function
  - Upregulations – some increase in function
  - Conditional knock-outs – organism is normal until exposed to certain chemicals

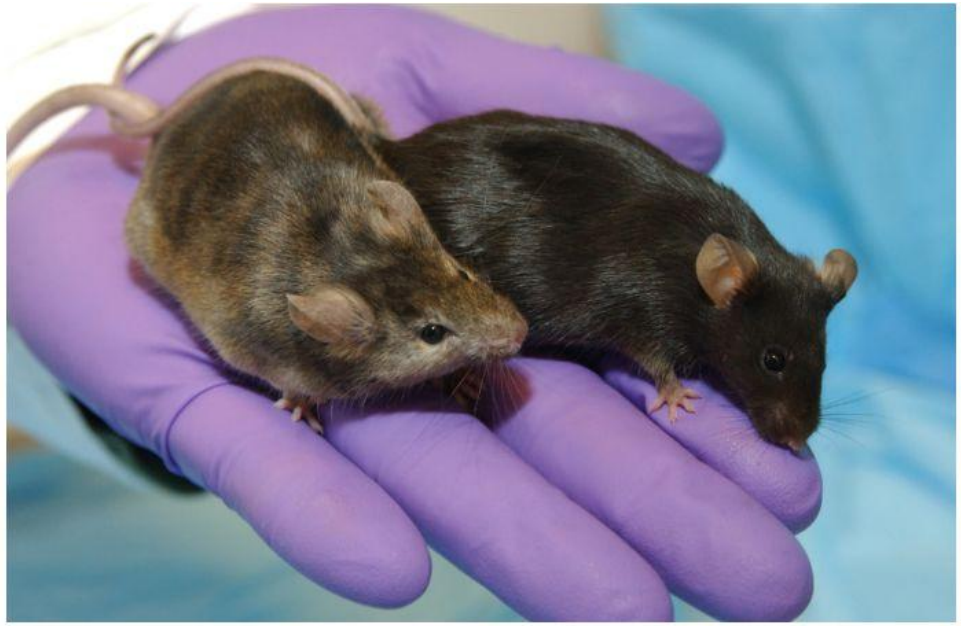


Figure 6.30 A mouse with a hair-related gene knocked out (left) compared to a wild type mouse (right).

Genetic modification: advantages and limitations

- CRISPR-Cas9 (recent advance) allows for targeted editing of genome – its simplicity, efficiency, and precision of method + potential therapeutic applications for treating genetic diseases
  - Read more: https://www.technologyreview.com/2023/03/10/1069619/more-than-200-people-treated-with-experimental-crispr-therapies/
- Many scientific advances have come from these research models developed from research with genetically modified mice
- Possible to develop multiple genetic crosses which allows for complex questions to be answered

- Difficulty in generalizing findings beyond genetic strain
- Unexpected side effects as a result of changing genetic code (manipulation may interact with other aspects of animal's physiology)

Video

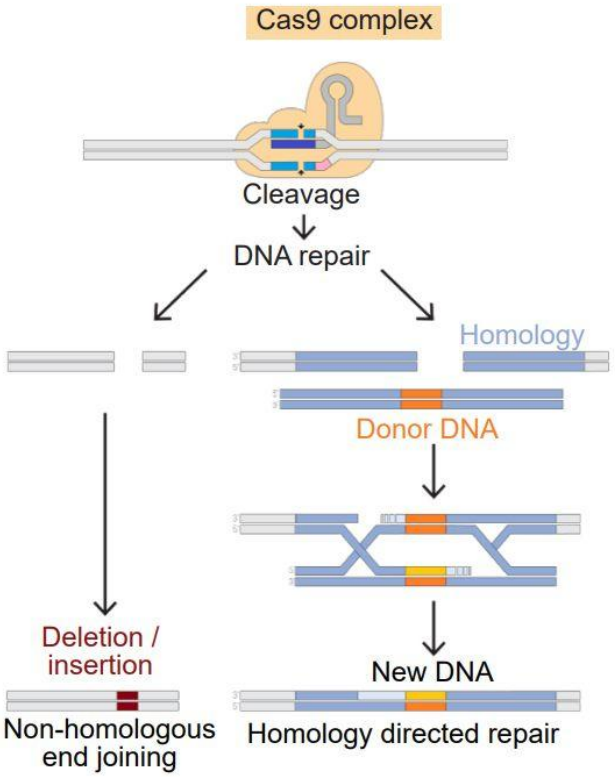


Figure 6.31 The CRISPR-Cas9 system is a powerful method of gene editing.

Optogenetics / Chemogenetics

- Example questions answered:
  - "How does activating cortical glutamatergic drive affect firing rate of neurons in the striatum?"
  - "Does inactivation of GABA-ergic circuits in the amygdala change anxiety behaviors?"
- Problems with early neural stimulation strategies: lack of specificity at area of stimulation + fibers of passage problem
- Both strategies increase the specificity of brain stimulation

Optogenetics

- Developed in 2006 by researchers at Stanford University
- Light sensitive-ion channel proteins called channelrhodopsin, ChR2
- Photon of blue light fits ChR2 molecule, protein changes shape, causing cation channel to open, enabling inward flow of positively charged Na+
- ChR2 allows us to excite neurons with flashes of light

Video

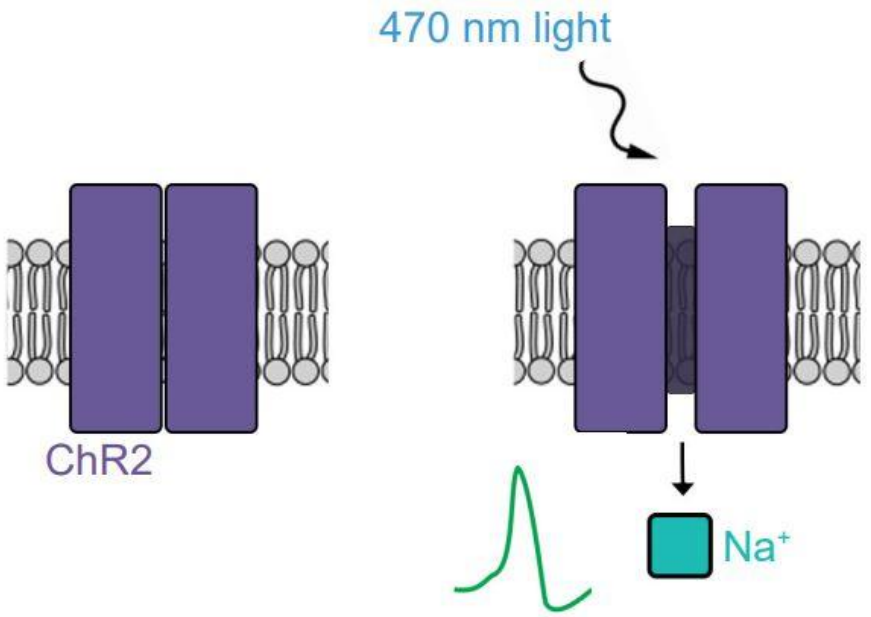


Figure 6.32 ChR2 is a transmembrane protein that is opened by blue light, which can cause action potential firing.

Optogenetics: advantages

- Gives temporal resolution on the order of milliseconds
  - Very fast – brief 0.5 ms flash of blue light is enough to trigger sufficient Na+ current to induce single action potential
    Once light is turned off, channel closes in less than a ms
- ChR2 is not the only optogenetic molecule
  - Halorhodopsin (yellow-green wavelengths to push Cl^- into cell and inhibit neuron)
  - Archaerhodopsin (light-sensitive proton pump that extrudes H^+ ions and inhibits neurons)
- High specificity, since optogenetics is used in conjunction with knock-in genetic modification technology or viral gene delivery to insert ChR2 into unique cell populations
  - Isolate effect of activation of specific cell population within an area of the brain with many types of cells

Chemogenetics

- Use various chemical agonists
- Usually G-protein coupled receptors that are activated only by exogenous drugs, but not endogenous neurotransmitters
- DREADD = designer receptor exclusively activated by designer drugs
  - When exposed to designer drug, activation of intracellular signaling pathway that can either excite or inhibit the neuron, depending on the nature of the DREADD
  - Insertion of DREADD protein causes no changes in neuronal activity at rest
  - Once exposed to exogenous drug, DREADD is activated, changing neuron activity

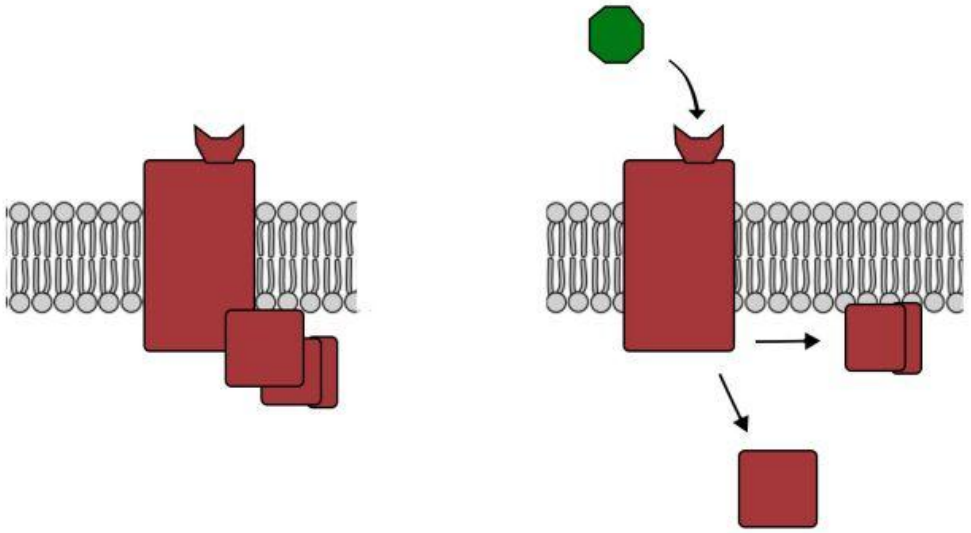


Figure 6.33 A chemogenetic system such as DREADD allows for control over intracellular signaling pathways upon exposure to an exogenous ligand.

Chemogenetics: limitation

- Requires drug to enter system and subsequent GPCR activation before a change in behavior can be observed, DREADD offers significantly less temporal resolution than optogenetics
  - seconds to 10s of seconds in an *ex vivo* preparation
  - minutes in behaving animals

Optogenetics/chemogenetics: advantages and limitations

- Combining optogenetics with Ephys has helped with understanding of connectivity between brain areas
- Behavioral testing with simultaneous optogenetic or chemogenetic stimulations gave insight into roles of specific neural circuits or neurotransmitters
- Quickly advancing understanding of nervous system

Introduction to Computational Neuroscience

- Branch of neuroscience that focuses on explaining neural mechanisms through computational methods
- Draws from other fields (electrical engineering, physics, and computer science)
- Sometimes described as being ***in silico*** preparation
- Provides tools and methods for developing models
  - Descriptive models characterize what nervous systems do
  - Mechanistic models determine how nervous systems relate with known anatomy and physiology
  - Interpretive models lead to understanding of why nervous systems operate in a particular way

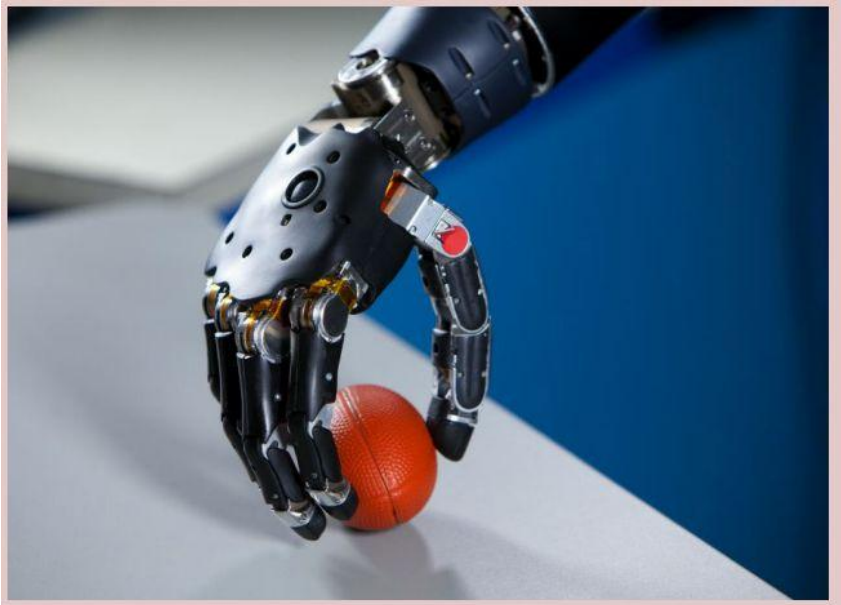


Figure 6.29 Advances in computational neuroscience have led to the development of robotic protheses that read neural signals, and predict the corresponding motor output.