THE HISTORY, ROLE, AND FUTURE OF ANIMAL EXPERIMENTATION IN NEUROBIOLOGY RESEARCH

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1 Central question: How do we create neuroscientific knowledge?

- Learning a lot in this class, textbooks, and published papers about the brain. But what percent of it is likely to stand the test of time and be useful?
- What does it mean when knowledge is "useful" within the context of neuroscience?
- In order to determine the answer to this question, we need to understand how is it that we create knowledge in the neurosciences, the knowledge that is published in papers, textbooks, and ultimately lectures like the ones you experienced in this class.
  - The natural sciences are different from other fields of inquiry because they are based not just on subjective observation of nature but actually pragmatically testing predictions about it
  - Definitions of empiricism, logical positivism
    - **Empiricism:** From Greek *empeiria* (experience). The idea that all rationally acceptable beliefs or propositions are justifiable or knowable only through experience (Fumerton, 2015)
    - **Logical positivism:** Philosophical movement (also called logical empiricism) started by the "Vienna Circle" (1924-1936) that held that the only meaningful problems in philosophy are those that can be solved by logical analysis, including, when applicable, empirical verification (Creath, 2011; Oxford)
- Based loosely on this foundation, one might then argue that knowledge that is "useful" in neuroscience:
    - Enables accurate predictions about the natural world
    - Enables the development of effective therapies and technologies for enhancing the human condition
- How does animal research fit into this paradigm?
  - We have no strong evidence that any animal model is perfectly predictive of a human being (Shanks et al., 2009)
  - However it does, we sure use it a lot: British statistics (Home Office Science Group, 2014)
- This lecture is not a discussion of ethics; it is, rather, a discussion of utility. Not asking whether animal research is ethical! Asking whether animal research is useful.

2 History of animal experimentation in neuroscience research

- **1700 B.C.-322 B.C. Heart as seat of mind (***Egyptians, Aristotle***):** Dissected animals (human dissection forbidden). Distinction between mind and physical manifestation of life (e.g., motion), as Hippocratic doctors were reportedly aware of motor consequences of head injuries (Taylor and Gross, 2003).
- **130-200 A.D. Mechanism of fluid energies (***Galen***):** Examined non-human animal bodies (monkeys, apes, pigs) to observe functions of heart, lungs, and nerves (von Staden, 1989) and advocated the use of human cadavers, but whether he carried out own advice is unclear (Boylan, 2014)
- **16th c. Balloonist theory (***Descartes***):** Describes animal anatomy in writings; advocates direct experience of animal bodies to unveil their nature as machines (Skirry, 2014).
- **18th c. Disproving balloonist theory (***Swammerdam***):** Experiments on nerve-muscle preparations from frogs (Cobb, 2002)
- **18th c. Animal electricity (***Alexander von Humboldt, Luigi Galvani***):** More frog experiments (Finger et al., 2013)
- **18th c. Phrenology (***Franz Joseph Gall, Johann Spurzheim***):** Pseudoscience. Anecdotal evidence collected from observation of living humans as well as human and animal skulls (Weidman,

2005)

- **19th c. Doctrine of specific nerve energies (*Muller*):** Reinvigorated the frog as animal model basis for assertion that different sensations are coded under the same modalities (electricity) but different nerve properties. (Also later collected hundreds of marine animals in quest to categorize on basis of anatomy) (Otis, 2007)
- **19th c. Increased focus on neuron (*Jan Evangelista Purkinje*)**: Purkinje Effect, Purkinje cells, Purkinje fibers. Directly observed animal tissues and cells; emphasized importance of experimentation in physiology education; set up world's first physiology laboratory at Breslau (Hykes, 1936).
- **19th c. Aggregate field theory (*Flourens*):** Functional audiology experiments in pigeons; developed theory of brain function via many lesion studies in pigs and pigeons (Yildirim and Sarikcioglu, 2007).
- **19-20th c. Localization theory (*Pierre Paul Broca, Carl Wernicke, Gustav Fritsch, Hitzig, Brodmann*):** Broca and Wernicke most noted for their lesion studies in humans. Meanwhile, Fritsch and Hitzig were strapping craniectomized dogs to tables and stimulating (without anesthesia) select regions of the cortex to produce movement, showing that it was a functional organ of the brain and not simply "rind" as had been believed for scholars over hundreds of years, as well as that it might be topographically mapped into localized specializations. Built upon previous work with rabbits and human heads as well as observations of patients (Gross, 2007).
- **Franz Nissl (*1860-1919*):** Experimented on dogs and moles to develop extranuclear staining techniques (RNA or DNA) (DeFelipe, 2011)
- **20th c. Topographic organization theory (*Hughlings Jackson*):** Clinical observation; no animal experiments (Balcells, 1999; Franz and Gillett, 2011).
- **20th c. Neuron doctrine (*Golgi, Cahal*):** Cahal used over 2,000 animals of several dozen invertebrate and vertebrate species to prepare his staining slides using Golgi's method (Lopez et al., 2010); shared Nobel Prize in physiology for first time in 1906 (De Carlos and Borrell, 2007).
- **20th c. Holism versus localization (*Lashley v. Penfield*):** Lashley lesion experiments in rats (no one seat of memory function) (Beach, 1961); Penfield mostly clinical experience
- **20-21st c. Connectionism (*modern consensus*):** [statistic on number of animals used per year in United States alone]

We still lack a unified theory of the brain

3 Why is it that can we use other animals to understand ourselves?

Surprising similarities among our nervous systems and those of other organisms

- Genetic
  - Various ways of classifying genetic similarity (ex. *Mus musculus*), but we tend to share a high percentage of important regions with other organisms
    - Can line chromosomes end-to-end to compare sequence divergence in all base pairs.
      - Bonobo autosomal sequences ~98.7% (Prufer et al., 2012) ; genome-wide chimpanzee nucleotide divergence 1.23% (CSAC, 2005)
      - For example, 69.1% of the mouse genome has been shown to be identical to human base-pair sequences (MGSC, 2002).
      - But the grand majority of DNA is in non-protein-coding sequences (35.2% introns plus 62.6% inter-genic DNA in humans; Taft et al., 2007)
    - Can also compare base pairs of protein-coding genes segments only: 85% sequence identity between human and mouse (MGSC, 2002).
    - Or can compare genes on basis of appearance from common ancestor (orthology): mouse 80% of genes orthologous to human (MGSC, 2002); rat 90% of genes orthologous to human (RGSPC, 2004); 70% of human genes have orthologue in zebrafish (Howe et al., 2013)
    - Or can compare orthology of a subset of genes. Human genes 15% orthology to *Drosophila* (Shih et al., 2015); disease protein genes: 77% visibly related to *Drosophila* (Reiter et al., 2001)
- Proteins

- Almost all major human neurotransmitter receptors have analogues in mice (116/141) and rats spanning most known neurotransmitter receptor classes (121/141):
  Adenosine
  Adrenergic
  Cholinergic (muscarinic, nicotonic)
  Dopamine
  GABA (A, B)
  Glutamate (metabotropic, ionotropic)
  Glycine
  Histamine
  Opioid
  Purinergic
  5HT (metabotropic, ionotropic)
  Peptides (bombesin, galanin, somatostatin, cholecystekinin, neuropeptide Y, VIP, neurotensin, TRH, GRH, gastrin releasing peptide, GHRH, CRH, angiotensin, calcitonin, bradykinin, secretin, tachykinin, neuromedin U, glucagon) (Iwama and Gojobori, 2002)
- One mass spectrometry paper showed 70% agreement in postsynaptic density protein types
  - Many differences were in non-functional protein groups (Bayes et al., 2012)
- Muscarinic and nicotinic acetylcholine receptors as well as AChE in *Paramecium primaurelia* (Roschina, 2010)

- Neurotransmitters
  - Catecholamines (dopamine, epinephrine, norepinephrine), serotonin, histamine are used as signal molecules in a variety of creatures from single-celled organisms to plants and fungus to non-human animals (Roschina, 2010)
    - For example, catecholamines can promote growth in *E. Coli* that is blocked by adrenergic and dopaminergic receptor antagonists (Roschina, 2010)
  - Acetylcholine has been shown to regulate stomata function in some plants (*Vicia faba* and *Pisum* savitum); moreover, research has shown that dopamine concentrations in some plants may increase with stress conditions (acid treatment, drought, UV light) (Swiedrych et al., 2004). For these similarities, plant systems (germination, autofluoresence in unicellular microspores) have been proposed as viable models of drug testing as biosensors of neurotransmission modulation. (Roschina, 2010)
- Circuits:
  - Generally speaking, many cortical regions have gross analogues in other mammalian and even non-mammalian species (Buckner and Krienan, 2013)

4 But how different are we, really? Important caveats in generalizing from animal data

- Genetic
  - Small mutations can give rise to big effects
    - Point mutations in gene for cyclin D2 protein that prevents its regulatory glycogen synthase kinase 3beta phosophorylation manifests as a whole syndrome of megacephaly and polydactyly (Mirzaa et al., 2014)
  - Same gene in different epigenetic situations can react completely differently
    - For example, seizure-causing mutations in P/Q-type calcium channelopathy Cacna1a potassium channel protein almost completely rescued by knocking out Kcna1 gene for potassium channel (Glasscock et al., 2007)
- Proteins
  - Subtle differences in the structure of a single protein can have drastic consequences for phenotype.
  - "A recent study suggests that in the rhodopsin family of G protein coupled receptors (GPCR), which includes most of the pharmaceutically interesting targets, only about 58% of the rat genes have an orthologue with human genes" (Gloriam et al., 2007)
  - Binding affinities can vary widely between rodent and human. Examples: D1, D4, 5HT-2A,

5-HT7, muscarinic M4. Among 45 drugs tested, D1 receptor affinities varied an average of 7 times between rats and humans (Geerts, 2009)
    - Mutations widespread among orthologous proteins between humans and rodents
        - 85% sequence identity between human and mouse (MGSC, 2002) still corresponds to several million base pair differences
    - Neurotransmitter receptor distributions different between humans and other animals. For example, GABA-A epsilon subunit found in CA3 and locus ceruleus of primates but not rodents, and GABA-A theta subunit is found in cerebral cortex and hippocampus of primates but not rats (Iwama and Gojobori, 2002). Table from (Geerts, 2009)
    - Among 30% of proteins found to be differentially enriched in human and mouse PSD, mice had more ion channels and translation enzymes and humans had more cytoskeletal components, enzymes like reductase, transferase, and dehydrogenase, and kinases (Bayes et al., 2012)
    - Despite similar protein structures, expression patterns can differ widely across species, such as genes expressed in the cortices of humans and chimpanzees (Oldham et al., 2006)
    - General conservation of neurotransmitter receptor classes, but important differences in particular receptors. For example, human lacks functional 5HT-5B receptor found in rodents (Grailhe et al., 2001)
- Neurotransmitters
    - TH interneurons abundant in human cortical layers V-VI but absent in great apes (chimp, gorilla, bonobo, orangutan) and found mostly in layers II-III in rat (DeFelipe, 2011)
- Neuronal morphology and circuitry
    - Despite regional analogues relative size and circuitry can differ greatly. Human versus chimpanzee (Carroll, 2003); human versus macaque (Patel and Iverson, 2014)
    - Studies since have found that cortical neural density decreases with increasing brain size across species, varying up to 3-fold across even primates (Herculano-Houzel et al., 2008)
    - Mouse versus human cortex
        - Cortex of mice is about half as thick (2622 microm to 1210 microm)
        - Has more than twice as many neurons (158 to 364) (DeFelipe, 2011)
        - Generally fewer synapses per cortical neuron (DeFelipe, 2011)
        - Human dendritic spines are 100% more voluminous and about 30% longer than those in mice (DeFelipe, 2011)
        - Different layers are not as distinct (Jones, 2009)
        - 25-30% of cortical neurons are (mostly GABAergic) interneurons in primates versus 15-20% in mouse (Jones, 2009)
            - Double bouquet GABAergic interneurons do not appear to exist in rodents (DeFelipe, 2011).
    - Rockel et al., 1980 found that numbers of laminar neurons across cortex (except visual cortex) was conserved across mouse, rat, cat, monkey, and human, though replication has been spotty (Rakic, 2008). Still an important debate.

5 Examples of animal experiment successes in neurobiology research

Note: Examples are from neurobiology research only. Great strides in other fields like oncology and endocrinology have been made on the backs of many animals as well.

- **1** All but one Nobel prize in physiology or medicine related directly to neural function or behavior depended on animal experimentation (chart)
- **2 *Caenorhabtidis elegans*:** Complete wiring diagram of 302 neurons has been anatomically mapped and the sensory-motor circuit responsible for swimming well characterized (Sengupta and Samuel, 2009); hundreds of genes responsible for axon regrowth have been identified following neural lesioning (Chen et al., 2011)
- **3 *Drosophila melanogaster*:** Several genes later implicated in key signaling pathways of neural development (*notch, hedgehog, achate, wingless, decapentaplegic/tumor growth factor-beta, roundabout, semaphorins*), circadian rhythm (*period, timeless, and clock*), multi-responsive ion channels (*trp*), potassium channels (*shaker, eag*) were initially discovered upon their mutation in the fruit fly,

leading to discovery of at least two diseases:

- Cerebral autosomal dominant ateriopathy with subcortical infarcts and leukoencephalography/CADASIL (mutations in *Notch* gene)
- Familial advanced sleep phase syndrome (mutations in two *Clock* genes)

Functions of several proteins have also been examined in vivo. Advantages include ease of altering many genes at once, inserting single copies of desired DNA sequence into genome, and knocking out specific genes in designated area (Bellen et al., 2010). Example is of holoprosencephaly following knockout of a hedgehog gene (Motoyama, 2006).

- **3 Mouse:** Discovery of anti-orexogenic leptin starting with chance mutation of ob/ob mouse (Ingalls et al., 1950) followed by cloning of protein product (leptin) in 1994 (Li, 2011) and development of drug Metreleptin for lipodystrophy patients (Nainggolan, 2014) (started out as victory for neuroscience that spilled into endocrinology)
- **4 Rat:** Place cell discovery in CA1 by John O'Keefe and grid cell discovery in medial entorhinal hippocampus by May-Britt and Edward Moser weighed in on a long-running debate to show that cognition could not be boiled down to simple sensorimotor function (Kiehn and Forssberg, 2014)
- **5 Lagomorphs:** Tissue plasminogen activator stroke treatment discovered in 1990 following tests on rabbits (Bednar et al., 1990)
- **6 Non-human primates:** More than 1/3 of the citations used by the paper reporting ten-degree brain-controlled motion in upper limb prosthesis in a human quadriplegic for the first time were based on data in non-human primates (Wodlinger et al., 2015).

6 Examples of animal experiment failures in neurobiology research

- "Currently, nine out of ten experimental drugs fail in clinical studies because we cannot accurately predict how they will behave in people based on laboratory and animal studies." – Mike Leavitt, U.S. Secretary of Health and Human Services, 2007 (Shanks et al., 2009)
- **Mice and Alzheimer's:** Not a single treatment has been yielded of over 200 that have passed preclinical trials for amelioration of cognitive deficit in APP mice (Zahs and Ashe, 2010)
  - Mutations in APP and presenilins (part of gamma-secretase complex) cause memory loss, plaques, neurofibrillary tauopathy, and neuronal death, but mice develop only plaques and memory loss but not tauopathy or neuronal loss (Zahs and Azhe, 2010)
  - Humans have several APOE proteins, of which variants in APOE4 are shown to increase Alzheimer's risk; mice have only one, which behaves like human APOE3 (Geers, 2009).
  - In humans and transgenic mice with mutant human tau, amyloid-beta pathology has been shown to induce tauopathy, a finding that we have been unable to replicate in transgenic mice with wild-type human tau (Zahs and Azhe, 2010)
- **Preclinical models of stroke:** 500 pharmaceuticals have passed preclinical trials in animals and yet we have only two therapies deemed effective enough for the clinic (van der Worp et al., 2010): aspirin and early intravenous thrombolysis with tissue plasminogen activator
- **Animal models of TBI:** Thirty years of promising pre-clinical trials have been unable to produce a single neuroprotective drug that has passed clinical trials in TBI patients (Xiong et al., 2013)

Tentative conclusion: "Animal models are sometimes able to identify possible and innovative mechanisms, such as metabotropic glutamate receptor 2 agonism as a possible treatment for schizophrenia; however, they are much less useful for the actual drug discovery process" (Geerts, 2009)

7 What if animal experimentation were outlawed tomorrow? Alternatives

A. In vitro
   i. In vitro blood-brain barrier for drug discovery (Wong et al., 2013)
   ii. 3D in vitro neural model of Alzheimer's (Choi et al., 2014)

B. In silico
   i. Blue Brain project, a European collaboration centered at the L'Ecole Polytechnique Federale de Lausanne that aims to simulate a rat and then human brain, has already produced 65 publications in electrical signal processing, cortical organization, neural

communication, and processing efficiency (The Blue Brain Project, 2015).

C. Human research
   i. Behavioral studies
   ii. Clinical trials
   iii. Patient case studies: A search of "patient H.M." brings more than 250 papers in PubMed. His damage, restricted to medial temporal lobe, as well as constellation of symptoms, taught the field four main principles: Medial temporal lobe structures are not necessary for:
      1. motor skills (learned motor skills);
      2. attention or working memory (had normal working memory),
      3. perception or intellect (above-average I.Q.),
      4. long-term memory storage (memories for events before surgery intact). (Squire and Wixted, 2011)
   iv. Epidemiological studies: Framingham heart study milestones in (Framingham Heart Study, 2015)

8 Animal testing without compromising basic ethical standards: Current consensus

- A hundred years ago, no regulation of animal testing. Tested on animals trapped in wild (Humboldt), pets (Fritsch and Hitzig)
- IACUC and Three R's
  - Replacement (When you can use methods other than animal testing to explore the same hypothesis, do it)
  - Refinement (When a design using fewer animals can explore the same hypothesis, do it)
  - Reduction (When you can take measures to alleviate pain and stress in your animal subjects, do it)

(Pankevich et al., 2012)

- Choosing the right tool for the job
  - Nerve conduction studies using voltage clamping of squid giant axon
  - Genetic basis of vocal learning using zebra finch
    - Only cetaceans, birds, and bats have shown evidence of vocal learning. Chimpanzees are "more related" to us but would not be a good model organism for this research. (Brainard and Doupe, 2002)
    - Dynamic learning of song sequences: Birds raised in isolation will produce abnormal songs, but those raised with unrelated tutors will develop songs similar to those of the tutor. (Brainard and Doupe, 2002)
    - 100% sequence identity with humans in DNA-binding region of transciption factor FoxP2 (forkhead box P2!) (White et al., 2006)
- Choosing test conditions with the most translational validity
  - MWM: Single biggest predictor of performance has been found to be retinal atrophy, not memory; also considered to be ethologically irrelevant for mice (Garner, 2014)
  - Barbering in mice used as model for OCD when barbering in humans is an entirely different disorder (trichotillomania) and exclusionary for OCD (Garner, 2014)
  - Biomarkers over behavioral phenotyping (Garner, 2014)
- Strong knowledge of ethology and species-specific physiology necessary for correct interpretation of experimental results

REFERENCES

Annese, J., N.M. Schenker-Ahmed, H. Bartsch, P. Maechler, C. Sheh, N. Thomas, J. Kayano, A. Ghatan, N. Bresler, M.P. Frosch, R. Klaming, S. Corkin. (2014) Postmortem examination of patient H.M.'s brain based on histological sectioning and digital 3D reconstruction. *Nature Communications* 5: 3122.

Balcells Riba M1. (1999). [Abstract] Contribution of John Hughlings Jackson to the understanding of epilepsy. *Neurologia* 14(1): 23-8.

Bayes, A., M.O. Collins, M.D.R. Croning, L.N. can de Lagemaat, J.S. Choudhary, S.G.N. Grant. (2012) Comparative study of human and mouse postynaptic proteomes finds high compositional conservation and abundance differences for key synaptic proteins. *PLOSOne* 7(10): e46683.

Beach, F.A. (1961). *Karl Spencer Lashley*. Washington, D.C.: National Academy of Sciences.

Bednar, M.M., T. McAuliffe, S. Raymond, and C.E. Gross. (1990) Tissue plasminogen activator reduces brain injury in a rabbit model of thromboembolic stroke. *Stroke* 21(12):1705-9.

Bjarnadóttir, T.K., D.E. Gloriam, S.H. Hellstrand, H. Kristiansson, R. Fredriksson, H.B. Schiöth. (2006) Comprehensive repertoire and phylogenetic analysis of the G protein-coupled receptors in human and mouse. *Genomics* 88: 263–273.

Boylan, M. 2014. Galen (130-200 C.E.) Internet Encyclopedia of Philosophy. Available online at http://www.iep.utm.edu/galen/.

Brainard, M.S. and A.J. Doupe. (2002) What songbirds teach us about learning. *Nature* 417: 351-358.

Buckner, R.L. and F. M. Krienan. (2013) The evolution of distributed association networks in the human brain. *Trends in Cognitive Sciences* 17(12): 648-685.

Choi SH, Kim YH, Hebisch M, Sliwinski C, Lee S, D'Avanzo C, Chen H, Hooli B, Asselin C, Muffat J, Klee JB, Zhang C, Wainger BJ, Peitz M, Kovacs DM, Woolf CJ, Wagner SL, Tanzi RE, Kim DY. (2014) A three-dimensional human neural cell culture model of Alzheimer's disease. *Nature* 515:274–278.

Cobb M. (2002). Timeline: exorcizing the animal spirits: Jan Swammerdam on nerve function. *Nat Rev Neurosci.* 3(5): 395-400.

Creath, R. (2011). Logical empiricism. Available online at http://plato.stanford.edu/entries/logical-empiricism/.
DeFelipe, J. (2011) The evolution of the brain, the human nature of cortical circuits, and intellectual creativity. *Frontiers in Neuroanatomy* 5(29).

Elizabeth A. Franz, Grant Gillett. (2011) John Hughlings Jackson's evolutionary neurology: a unifying framework for cognitive neuroscience. *Brain: A Journal of Neurology* 3114-3120.

Finger, S., M. Piccolino, and F.W. Stahnisch. (2013). Alexander von Humboldt: Galvanism, Animal Electricity, and Self-Experimentation Part 1: Formative Years, Naturphilosophie, and Galvanism. *Journal of the History of the Neurosciences: Basic and Clinical Perspectives* 22(3): 225-260.

Framingham Heart Study. (2015) Framingham Heart Study Bibliography. Available online at https://www.framinghamheartstudy.org/fhs-bibliography/index.php.

Fumerton, R. (2015) Philosophy: Empiricism. Encyclopedia Britannica. Available online at http://global.britannica.com/EBchecked/topic/186146/empiricism.

Garner, J.P. (2014) The Significance of Meaning: Why Do Over 90% of Behavioral Neuroscience Results Fail to Translate to Humans, and What Can We Do to Fix It? *ILAR Journal* 55(3): 438-456.

Geerts, H. (2009) Of mice and men: Bridging the translational disconnect in drug discovery. *CNS Drugs* 23 (11): 915-926.

Glasscock E1, Qian J, Yoo JW, Noebels JL (2007). Masking epilepsy by combining two epilepsy genes. *Nature Neuroscience* 10(12):1554-8.

Gloriam DE, Fredriksson R, Schio¨th HB. (2007) The G proteincoupled receptor subset of the rat genome. *BMC Genomics* 8: 338-45.

Grailhe, R. G.W. Grabtree, R. Hen. (2001) Human 5-HT receptors: the 5-HT receptor is functional but the 5-HT 55B receptor was lost during mammalian evolution. *European Journal of Pharmacology* 4(18): 157–167.

Gross, Charles G. (2007) 'The Discovery of Motor Cortex and its Background', *Journal of the History of the Neurosciences* 16(3): 320 – 331.

Hay, M., D.W. Thomas, J.L. Craighead, C. Economides, J. Rosenthal. (2014) Clinical development success rates for Herculano-Houzel S, C.E. Collins, P.Y. Wong, J.H. Kaas, and R. Lent. The basic nonuniformity of the cerebral cortex. *PNAS* 105(34): 12593-12598.

Home Office Science Group. *Annual Statistics of Scientific Procedures on Living Animals Great Britain 2013*. London: Williams Lea Group 2014.

Howe, K. et al. (2013) The zebrafish reference genome sequence and its relationship to the human genome. *Nature* 496(7446): 498-503.

Ingalls AM, Dickie MM, Snell GD. (1950) Obese, a new mutation in the house mouse. *J Hered* 41(12): 317-8. investigational drugs. *Nature Biotechnology* 32(1): 40-51.

Iwama, H. and T. Gojobori. (2002) Identification of neurotransmitter receptor genes under significantly relaxed selective constraint by orthologous gene comparisons between humans and rodents. *Mol. Biol. Evol.* 19(11):1891–1901.

Jones, E. (2009) The Origins of Cortical Interneurons: Mouse versus Monkey and Human. *Cerebral Cortex* 9(19):1953-1956.

Juan A. De Carlos, José Borrell. (2007). A historical reflection of the contributions of Cajal and Golgi to the foundations of neuroscience. *Brain Research Reviews* 55: 8-16.

Kettenmann and Wade, 2014. A short history of European neuroscience. Federation of European Neuroscience Societies. Available online at http://www.fens.org/Global/Online%20History%20Projects/Short%20History%20of%20European%20Neuroscience.pdf.

Kiehn, O. and H. Forssberg. (2014) Nobel Prize 2014 scientific background: The brain's navigational place and grid cell system. Available online at http://www.nobelprize.org/nobel_prizes/medicine/laureates/2014/advanced-medicineprize2014.pdf.

Lahvis G. P., Alleva E., Scattoni M. L. (2011). Translating mouse vocalizations: prosody and frequency modulation. *Genes Brain Behav.* 10, 4–16.

Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, LaCroix-Fralish ML. (2010) Coding of facial expressions of pain in the laboratory mouse. *Nat Methods* 7:447–449.

Li, MD. (2011) Leptin and beyond: An odyssey to the central control of body weight. *Yale Journal of Biology and Medicine* 84: 1-7.

Logical positivism. Oxford Advanced Learner's Dictionary. Available online at http://www.oxforddictionaries.com/definition/english/logical-positivism.

Mangels, J. (2003). "History of Neuroscience." W1010x : Mind, Brain, and Behavior. Columbia University. Available online at http://www.columbia.edu/cu/psychology/courses/1010/mangels/neuro/history/history.html

Mirzaa GM, Parry DA, Fry AE, Giamanco KA, Schwartzentruber J, Vanstone M, Logan CV, Roberts N, Johnson CA, Singh S, Kholmanskikh SS, Adams C, Hodge RD, Hevner RF, Bonthron DT, Braun KP, Faivre L, Riviere JB, St-Onge J, Gripp KW, Mancini GM, Pang K, Sweeney E, van Esch H, Verbeek N, Wieczorek D, Steinraths M, Majewski J, Boycott KM, Pilz DT, Ross ME, Dobyns WB, Sheridan EG. (2014) De novo CCND2 mutations leading to stabilization of cyclin D2 cause megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome. *Nat Genet* 46:510-515.

Motoyama, J. (2006) Brain Development Mechanism and Diseases Caused by Its Failure. RIKEN BSI News 32. Available online at http://www.brain.riken.go.jp/bsi-news/bsinews32/no32/speciale.html.

Mouse Genome Sequencing Consortium (MGSC). (2002) Initial sequencing and comparative analysis of the mouse

genome. *Nature* 420: 520-562.

Nadine. "Phrenology." New Dictionary of the History of Ideas. 2005. Encyclopedia.com. 17 Apr. 2015 <http://www.encyclopedia.com>.

Nainggolan, L. (2014) Myalept (Metreleptin) Approved for Generalized Lipodystrophy. Medscape. Available online at http://www.medscape.com/viewarticle/821056.

O. V. Hykeš. (1936) Jan Evangelista Purkinje: His Life and Work. *Osiris* 2: 464-471.

Otis, L. *Mueller's Lab.* Oxford: Oxford University Press, 2007.

Oxford Dictionaries. Logical positivim. Available online at http://www.oxforddictionaries.com/definition/english/positivism.

Pablo Garcia-Lopez, Virginia Garcia-Marin, and Miguel Freire. The histological slides and drawings of Cajal. *Frontiers in Neuroanatomy* 4(9): 1-16.

Pankevich, DE, TM Wizemann, AM Mazza, and BM Altevogt. *International Animal Research Regulations: Impact on Neuroscience Research: Workshop Summary.* Washington, D.C.: National Academies Press, 2012.

Patel, A.D. and J.R. Iversen. (2014) The evolutionary neuroscience of musical beat perception: the Action Simulation for Auditory Prediction (ASAP) hypothesis. *Front. Syst. Neurosci.*8:57.

Prüfer K et al. (2012) The bonobo genome compared with the chimpanzee and human genomes. *Nature* 486(7404): 527-31.

Reiter, L.T., L. Potocki, S. Chien, M. Gribs, E. Bier. (2001) A Systematic Analysis of Human Disease-Associated Gene Sequences In Drosophila melanogaster. *Genome Res.* 11(6): 1114–1125.

RGSPC. (2004) Genome sequence of the Brown Norway rat yields insights into mammalian evolution. *Nature* 428(6982): 493-521.

Roshchina, V. Chapter 2: Evolutionary Considerations of Neurotransmitters in Microbial, Plant, and Animal Cells. *Microbial Endocrinology.* Springer, 2010. 17-52.

Shanks, N. R. Greek, J. Greek. (2009) Are animal models predictive for humans? *Philosophy, Ethics, and Humanities in Medicine* 4(2).

Shih, J., R. Hodge, M.A. Andrade-Navarro. (2015) Comparison of inter- and intraspecies variation in humans and fruit flies. *Genomics Data* 3: 49-54.

Skirry, 2014. Descartes (1596-1650). Internet Encyclopedia of Philosophy. Available online at http://www.iep.utm.edu/descarte/.

Squire, L.R. and J.T. Wixted. (2011) The cognitive neuroscience of human memory since H.M. *Annual Reviews in Neuroscience* 34: 259-288.

Taft RJ, Pheasant M, Mattick JS. (2007) The relationship between non-protein-coding DNA and eukaryotic complexity. *Bioessays* 29(3):288-99.

Taylor, C.S. and C.G. Gross. (2003) Twitches versus movements: A story of motor cortex. Neuroscientist 9(5): 332-342.

The Blue Brain Project. (2015) Publications. http://bluebrain.epfl.ch/page-52755-en.html.

The Chimpanzee Sequencing and Analysis Consortium (CSAC). (2005) Initial sequence of the chimpanzee genome and comparison with the human genome. *Nature* 437: 69-87.

University of Utah. (2015) Animal models for addiction research. University of Utah Health Sciences Genetic

Science Learning Center. Available online http://learn.genetics.utah.edu/content/addiction/mice/.

Van der Worp, H.B., D.W. Howells, E.S. Sena, M.J. Porritt, S. Rewell2, V. O'Collins, M.R. Macleod. (2010) Can Animal Models of Disease Reliably Inform Human Studies? *PLOS Medicine* 7(3): e1000245.

Von Staden, H. (1989) *Herophilus: The Art of Medicine in Early Alexandria.* Cambridge University Press.

Weidman, Nadine. "Phrenology." New Dictionary of the History of Ideas. 2005. Encyclopedia.com. 17 Apr. 2015 <http://www.encyclopedia.com>.

Wodlinger B, Downey JE, Tyler-Kabara EC, Schwartz a B, Boninger ML, Collinger JL. (2014) Ten-dimensional anthropomorphic arm control in a human brain-machine interface: difficulties, solutions, and limitations. *J Neural Eng*. 12(1):016011.

Wong, A.D., Y. Mao, A.F. Levy, J.D. Rothstein, D.E. Bergles, and P.C. Searson. (2013) The blood-brain barrier: an engineering perspective. *Frontiers in Neuroengineering* 6(7): 1-22.

Xiong, Y., A.Mahmood & M. Chopp. Animal models of traumatic brain injury. (2013) *Nature Reviews Neuroscience* 14, 128-142.

Yildirim FB, Sarikcioglu L. (2007) Marie Jean Pierre Flourens (1794 1867): an extraordinary scientist of his time. *Journal of Neurology, Neurosurgery, and Psychiatry* 78(8): 852.

Zahs, K.R., K.H. Ashe. (2010) 'Too much good news' – are Alzheimer mouse models trying to tell us how to prevent, not cure, Alzheimer's disease?, *Trends in Neurosciences* 33(8): 381-389.