Experimental Methods

Literature of Physiology

- Journals
  - General purpose versus narrow field
  - Original articles versus reviews

A Systematic Review of Randomized Trials Comparing Radiofrequency Ablation with Antiarrhythmic Medications in Patients with Atrial Fibrillation

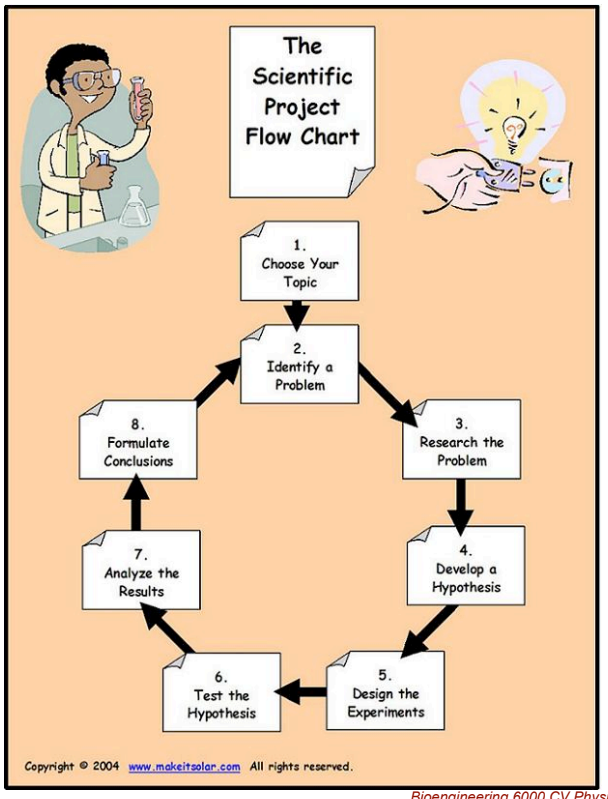
GIRISH M. NAIR, M.B.B.S., PABLO B. NERY, M.D., SYAMKUMAR DIWAKARAMENON, M.B.B.S., JEFFREY S. HEALEY, M.D., STUART J. CONNOLLY, M.D., F.A.C.C., and CARLOS A. MORILLO, M.D., F.A.C.C.

Complex Fractionated Electrograms in the Right Atrial Free Wall and the Superior/Posterior Wall of the Left Atrium Are Affected by Activity of the Autonomic Nervous System

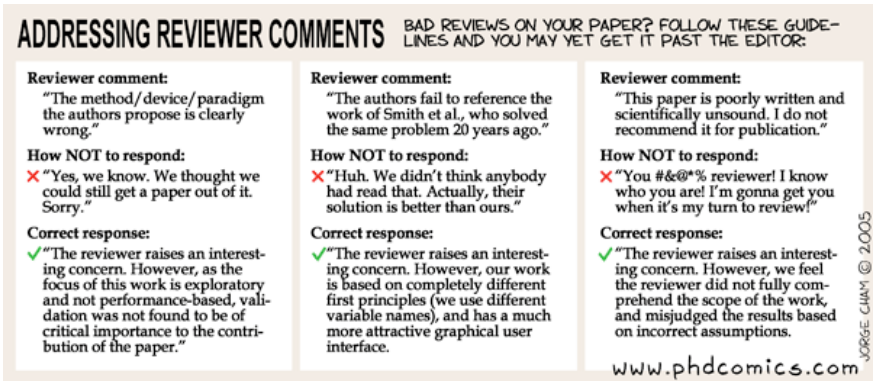
SEVASTI-MARIA CHALDOUPI, M.D.,* ANDRE C. LINNENBANK, PH.D.,†,‡ FRED H. WITTKAMPF, PH.D.,* LEIF H. BOLDT, M.D.,§ HARRY VAN WESSEL,* VINCENT J. VAN DRIEL, M.D.,* PIETER A. DOEVENDANS, M.D., PH.D.,* RICHARD N. HAUER, M.D., PH.D.,* JACQUES M. DE BAKKER, PH.D.,*,†,‡ and PETER LOH, M.D., PH.D.*

Literature of Physiology

- Journals
  - General purpose versus narrow field
  - Original articles versus reviews
- Process
  - Perform experiments
  - Write and submit paper
  - Peer review
  - Iterate ad nauseum

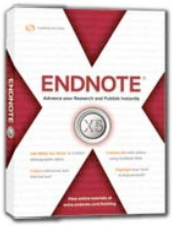


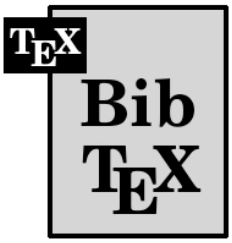
Review Process



Literature of Physiology

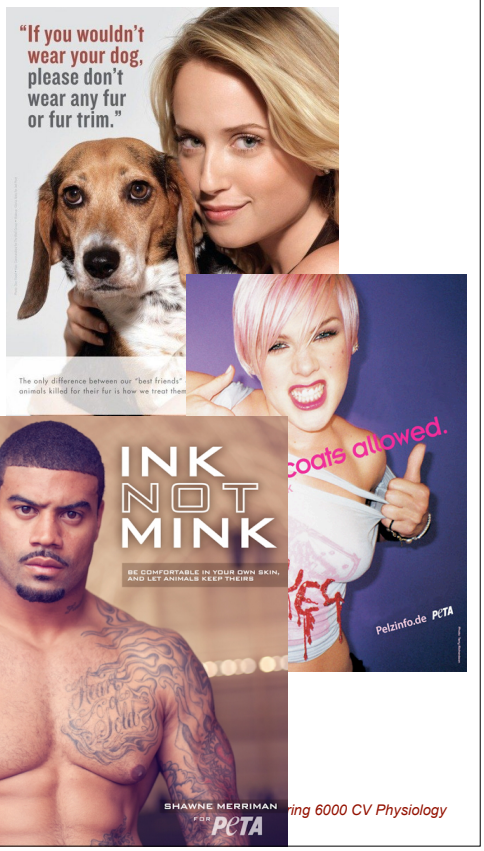
- Journals
  - General purpose versus narrow field
  - Original articles versus reviews
- Process
  - Perform experiments
  - Write and submit paper
  - Peer review
  - Iterate ad nauseum
- Purpose
  - Report results and ideas
  - Confirm or refute findings
  - Survive in science
- Read critically
- More info: www.sci.utah.edu/~macleod/litbase





Animals in Experiments

- Animal welfare versus Animal rights
- Benefits to science and medicine
  - Testing of physiological theories
  - Development of techniques
  - Evaluation of treatments
- Requirements
  - Protocols, animal review boards
  - National and international standards



Animals in Experiments

Nov 11

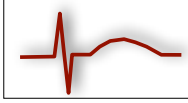
All in a Day's Work: Confinement, Torment, Killing in University's Labs

Posted at 04:46 PM | Permalink | Comments (129)

For more than eight months this year, a PETA investigator worked undercover inside **University of Utah** animal labs, where she documented the miserable conditions and daily suffering of dogs, cats, monkeys, rats, mice, rabbits, frogs, cows, pigs, and sheep. Today, ***The Salt Lake Tribune*** ran a story about the investigation, including the response from Tom Parks, the university's vice president for research. The response is (not so) stunningly callous: "None of the things she alleges are substantive. It's a remarkably banal list of ordinary events in an animal-care facility."

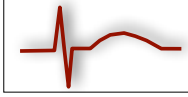
Here's a list of the things the university considers "banal"—part of an "ordinary" day in the "animal-care facility":

- Cutting the spinal cords and tender eyes of rabbits and tying off the nerves in the paws of rats to study pain
- Buying homeless cats from animal shelters, drilling holes into their heads, and injecting their kittens' brains with harmful chemicals
- Cutting into the chests of dogs from animal shelters and implanting medical devices for deadly heart experiments
- Drilling holes into monkeys' skulls, confining them in tiny cages, and keeping them constantly thirsty so that they will "cooperate" in experiments in exchange for a few drops of water
- Inflicting mice with tumors the size of golf balls that covered the animals' bodies



Experimental Design

- Hypothesis
  - Formulation
  - Testing and testability
- Methods
  - Choice of animal model and level of organization
  - Reductionism versus integration
  - Techniques and instrumentation
  - Signal processing
  - Statistics
  - Controls
- Analysis and Discussion

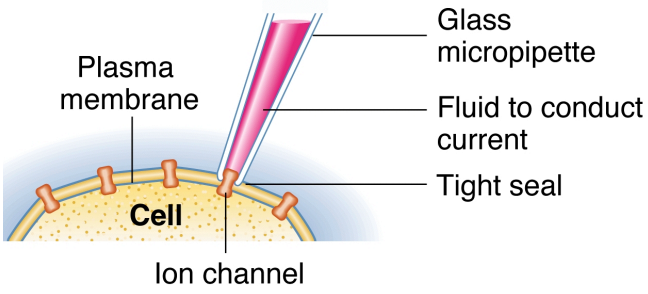


Molecular Techniques

- Radioactive tracers
  - Measure flow, distributions
  - Mimic naturally occurring molecules
  - Scintillation counters versus autoradiography
- Antibody markers
  - Link fluorescent molecule to an antibody for a specific antigen
  - Radioimmunoassay
- Genetic engineering
  - Recombinant DNA
  - Transgenic animals

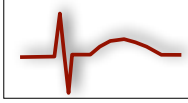
Cellular Techniques

- Glass Micropipette techniques
  - "Sharp" glass microelectrodes
    - impale the cells
  - Patch electrodes
    - fire polished and cleaned
    - Gigaseal
  - Measure voltages
    - high impedance



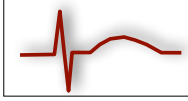
Cellular Techniques

- Ion sensitive electrodes
  - Semipermeable membranes
  - Ion ratios reflected in voltage (Nernst potential)
  - Used for ions, pH, gases
- Pressure
  - Microelectrodes filled with known solution
  - Resistance changes with content of electrode
  - Servo pressure system can balance (and thus record) solutions according to resistance
  - Used for microcirculation and kidney



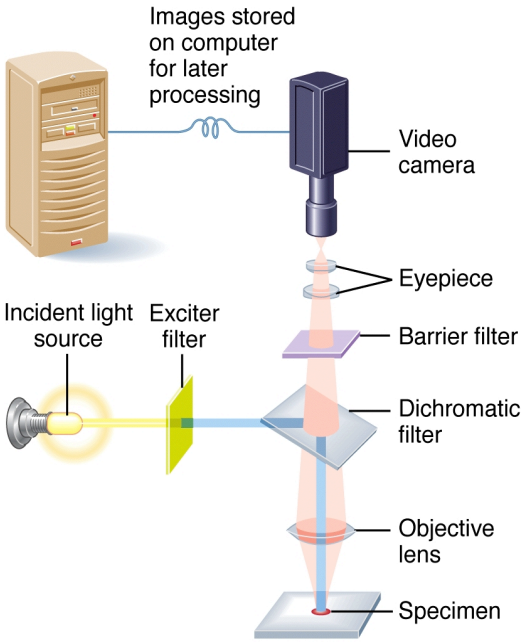
Microscopy

- Light microscopy
  - Resolution limited to a few microns
  - Fixation/staining of specimens often required
    - kills cells, stabilizes and reveals their structure
    - works only for small (1-10 μm) preps
    - embedding/freezing before sectioning

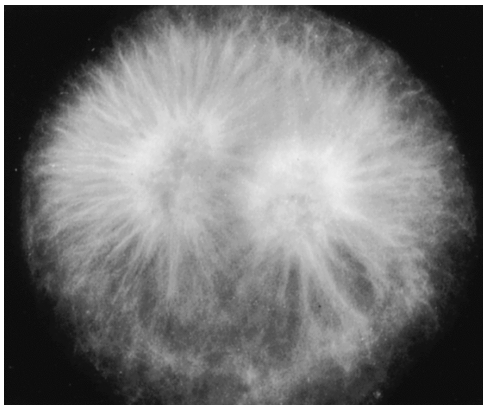


Fluorescence and Confocal

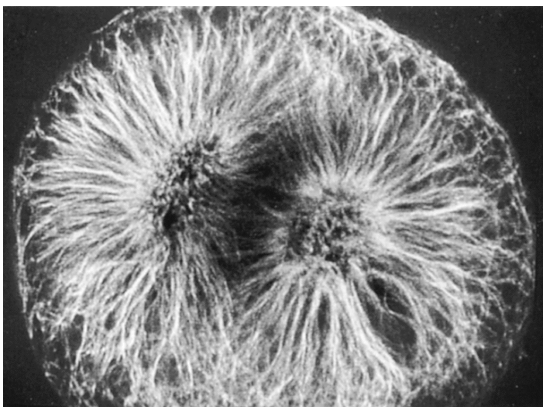
- Dyes
  - emit fluorescence when excited
  - linked to membrane, antibodies, or cell constituents
- Confocal
  - Focused excitation beam
  - Incident and reflected light follow same path
  - Two- and three-dimensional scanning
- Work on living preparations



Confocal Example



Standard fluorescence microscopy



Confocal fluorescence microscopy

Preparations

- Cell Culture
  - Can be challenging to maintain alive for more than few days (depends on species, cell type, age)
  - E.g., adult heart cells vs. embryonic
  - Stem cells
- In Situ organs
  - Physiologically intact
  - Naturally supported
- Isolated Organs
  - Heart beats on its own for hours-days
  - Research implications
  - Treatment implications