

# Experimental Methods



Experimental Methods

Bioengineering 6000 CV Physiology

## Literature of Physiology

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

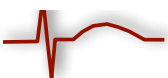


### **A Systematic Review of Randomized Trials Comparing Radiofrequency Ablation with Antiarrhythmic Medications in Patients with Atrial Fibrillation**

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. WITKAMPF, 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.\*

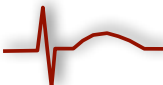
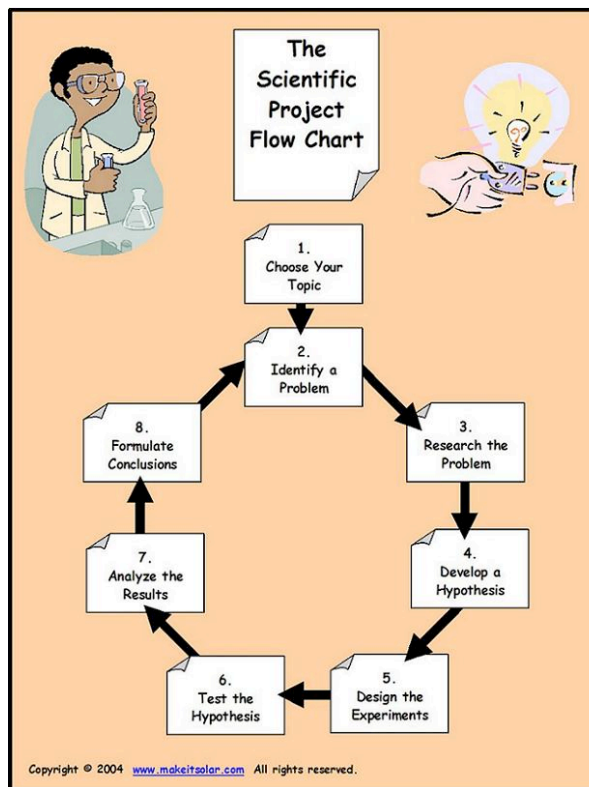


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# Literature of Physiology

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



# Review Process



WWW.PHDCOMICS.COM

## ADDRESSING REVIEWER COMMENTS

BAD REVIEWS ON YOUR PAPER? FOLLOW THESE GUIDELINES AND YOU MAY YET GET IT PAST THE EDITOR:

### Reviewer comment:

"The method/device/paradigm the authors propose is clearly wrong."

### How NOT to respond:

✗ "Yes, we know. We thought we could still get a paper out of it. Sorry."

### Correct response:

✓ "The reviewer raises an interesting concern. However, as the focus of this work is exploratory and not performance-based, validation was not found to be of critical importance to the contribution of the paper."

### Reviewer comment:

"The authors fail to reference the work of Smith et al., who solved the same problem 20 years ago."

### How NOT to respond:

✗ "Huh. We didn't think anybody had read that. Actually, their solution is better than ours."

### Correct response:

✓ "The reviewer raises an interesting concern. However, our work is based on completely different first principles (we use different variable names), and has a much more attractive graphical user interface."

### Reviewer comment:

"This paper is poorly written and scientifically unsound. I do not recommend it for publication."

### How NOT to respond:

✗ "You #@% reviewer! I know who you are! I'm gonna get you when it's my turn to review!"

### Correct response:

✓ "The reviewer raises an interesting concern. However, we feel the reviewer did not fully comprehend the scope of the work, and misjudged the results based on incorrect assumptions."

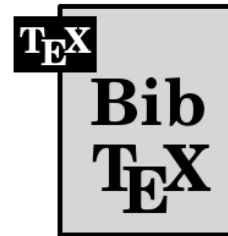
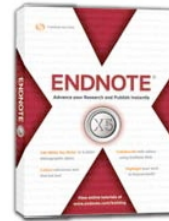
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# Literature of Physiology

- 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](http://www.sci.utah.edu/~macleod/litbase)



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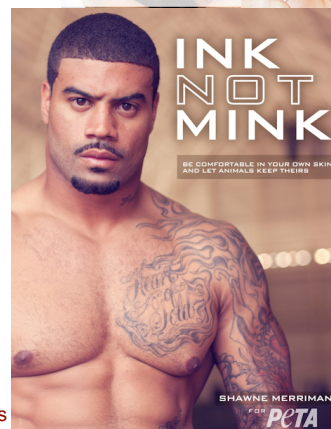
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# Animals in Experiments

- 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



The only difference between our "best friends" animals killed for their fur is how we treat them.



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# 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



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# 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



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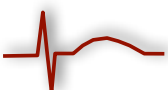
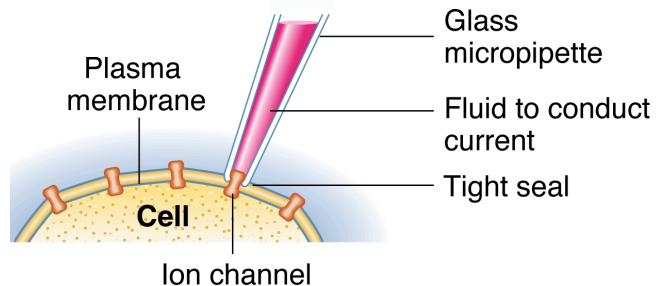
# Molecular Techniques

- 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



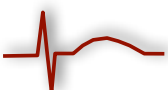
# Cellular Techniques

- 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



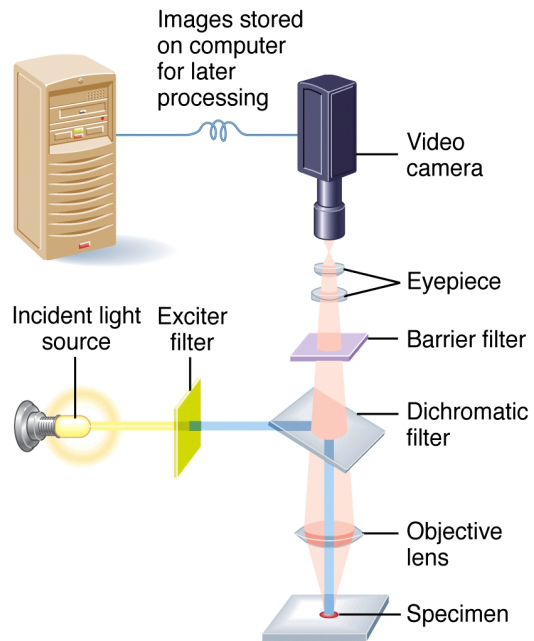
# Microscopy

- 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  $\mu\text{m}$ ) preps
    - embedding/freezing before sectioning

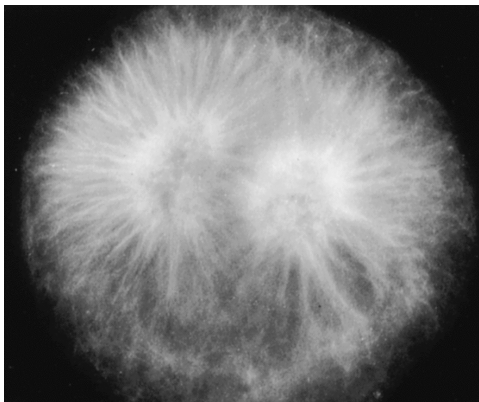


# Fluorescence and Confocal

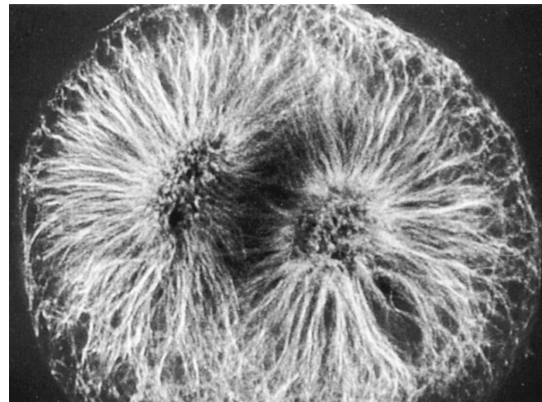
- Dyes
  - emit fluorescence when excited
  - linked to membrane, antibodies, or cell constituents
- Confocal
  - Focused excitation beam
  - Incident and reflected light follow same path
  - Two- and three-dimensional scanning
- Work on living preparations



## 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

