Recording of electrical and mechanical activity of rat papillary muscle
Outline

• experiments on animals
  – rules and laws
  – types of experiments
  – species used
• basic rat functional cardiovascular anatomy
• microelectrodes and methods
• recordings
Animal experimentation - purpose

- numbers: 50 to 100 million vertebrate animals worldwide
- species: zebrafish -> primates
- sources: purpose-bred, some caught in wild
- research conducted by: universities, medical schools, pharmaceutical companies, defense establishments
- why: education, research (genetics, developmental biology, behavioral studies, biomedical research, drug testing and toxicology tests, including cosmetics testing)
Animal experimentation - Laws

- EU Directive 86/609/EEC
- Romanian laws regarding animal welfare
- university's local policy
Animal experimentation - reasoning

• virtually every medical achievement in the 20th century relies on the use of animals in some way
• even sophisticated computers are unable to model interactions between molecules, cells, tissues, organs, organisms, and the environment, making animal research necessary in many areas
• medical students:
  – appraise the tremendous knowledgebase created by means of animal testing
  – honor the possibility to learn and experiment
  – use common sense and conscience, be responsible
Animal experimentation - principles

- Animal testing should cause as little suffering to animals as possible, and
- Animal tests should only be performed where necessary.

- Reduction: methods to obtain comparable levels of information from fewer animals, or to obtain more information from the same number of animals
- Replacement: preferred use of non-animal methods over animal methods whenever it is possible to achieve the same scientific aim
- Refinement: methods that alleviate or minimize potential pain, suffering or distress

- Pain
- Distress
- Immobilization
Animal experimentation - PAIN

- **Definition:** physical pain is an unpleasant feeling that typically consists of negative affect and aversion, and has location, duration, intensity and a distinctive quality (e.g., burning, stabbing)
Animal experimentation - ANESTHESIA

• Definitions:
  – condition of having sensation (including the feeling of pain) blocked or temporarily taken away
  – reversible lack of awareness
    • local (including spinal)
    • general
  – pharmacologically induced reversible state of amnesia, analgesia, loss of consciousness, loss of skeletal muscle reflexes and decreased stress response
Animal experimentation - ANESTHESIA METHODS

• pharmacological
  – local anesthetics: membrane stabilizing drugs (i.e. Na\(^+\) channel blockers - Lidocaine)
  – general anesthetics: reversible loss of consciousness

• other (only for animal research)
  – decapitation (guillotine, scissors)
    • when using frogs destroying spinal cord as well
Animal experimentation –
Types of experiments

- **in vivo**: “within the living” (experimentation using a whole, living organism)
- **in situ**: “in the place” (examine the phenomenon, organ exactly in place where it occurs, i.e. without moving it to some special medium)
- **in vitro**: “within the glass” (outside of the living organism, in a controlled environment, such as in a test tube or Petri dish)
Introduction

• in vitro experiment
• invasive method to investigate the cardiovascular system – recording simultaneously the electrical and mechanical activity
• determine membrane potential, excitability, refractory periods, etc
Method – choosing species

• when choosing an animal species to work with one has to consider:
  – original hypothesis to test
  – availability of literature data to compare with
  – availability of resources
  – economics

• usually small animals are preferred
  – regarding the cardiovascular system: mouse, rat, guinea pig and rabbit

• must consider inter-species differences
Method – choosing species
Method - dissection

• deep anesthesia: Ketamine/Xylazine
  – test effectiveness: reflexes, response to pain
• open chest and remove whole heart (after sectioning major arteries and veins)
  – must work quickly, survivability of tissue decreases with every second
  – place heart in Petri dish with oxygenated Tyrode solution (isotonic solution used in physiological experiments and tissue culture. It contains magnesium, a sugar (usually glucose) as an energy source and uses bicarbonate and/or HEPES as buffer; it is typically gassed with 95% Oxygen 5%)
• dissect until isolation of papillary muscle
Method - dissection

Rat Thoracic Arteries

- Trachea
- Esophagus
- R. Common Carotid A.
- L. Common Carotid A.
- R. Subclavian A.
- L. Subclavian A.
- Innominate A.
- Aorta
- L. Ventricle
- L. Atrium
- R. Atrium
- L. Lung
- R. Lung
- Diaphragm

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Method - dissection
Method - dissection
Method – Steiert bath
Method – Steiert bath

Continuous oxygenated (95% O₂, 5% CO₂) Tyrode solution flow: 10 ml/min
Method – preparation of muscle

- keep oxygenated
- keep at 36°C
- stretch gently to create preload (0.5 mN)
- test stimulation
- record electrical and mechanical activity
Method – microelectrodes

- A very small, fine electrode (tip under 1µ):
  - borosilicate glass capillaries
  - fix both ends, heat at middle
  - pull (horizontal or vertical) until breaks
  - polish tip if necessary
  - visual and electrical (measure impedance) check
Method – sharp electrode
Method – voltage clamp
Method – patch clamp
Electrophysiological recordings

- unipolar recordings (one active electrode – microelectrode and one passive - reference)
- lower electrode using micromanipulators
  - when electrode is only in Tyrode solution: no electrical potential is measured
  - when microelectrode penetrates the cell membrane: sudden drop in potential from around 0mV to approximately 90mV = resting membrane potential
  - if stimulated: action potential
Electrophysiological recordings
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Electrophysiological recordings - determine threshold voltage

- threshold: is the membrane potential to which a membrane must be depolarized to initiate an action potential
Electrophysiological recordings - determine threshold voltage

- all-or-nothing law: responding only to a stimulus above a specific threshold and then responding with the maximum discharge
Electrophysiological recordings - determine threshold voltage

- stimulate muscle with 0.5Hz
- start with low stimulus amplitude and increase gradually
- if stimulus intensity is under threshold: no response
- if stimulus intensity is above threshold: action potential followed by contraction
Electrophysiological recordings - determine refractory periods

- stimulate muscle with 2 consecutive stimuli
- start with small interstimulus interval and increase gradually
- if time between stimuli is small: only one AP (second stimulus arrives when tissue is in ARP)
- if time between stimuli is greater: both stimuli will create AP (initially different – relative refractory period)
Electrophysiological recordings - determine refractory periods

- refractory period: the amount of time it takes for an excitable membrane to be ready for a second stimulus once it returns to its resting state following excitation
Frank-Starling mechanism

• if the heart fills with more blood than usual, the force of the muscular contractions will increase: an increase in the load experienced by each muscle fiber due to the extraneous blood entering the heart

• stretching of the muscle fibers increases the affinity of troponin C for calcium -> a greater number of cross-bridges form within the muscle fibers -> increases the contractile force of the cardiac muscle

• the force that any single muscle fiber generates is proportional to the initial sarcomere length (known as preload), and the stretch on the individual fibers is related to the end-diastolic volume of the ventricle
Frank-Starling mechanism - measurement

- stretch gradually papillary muscle
- watch and measure contraction amplitude