

Experiments on frog cardiovascular and respiratory system

Outline

- experiments on animals
 - rules and laws
 - types of experiments
 - species used
- basic frog functional anatomy
- exploring the cardiovascular and respiratory systems of the frogs

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)

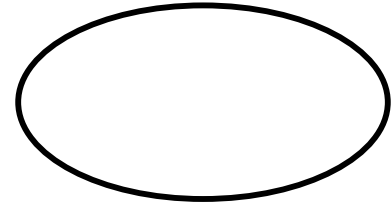
Animal experimentation - Laws

- 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



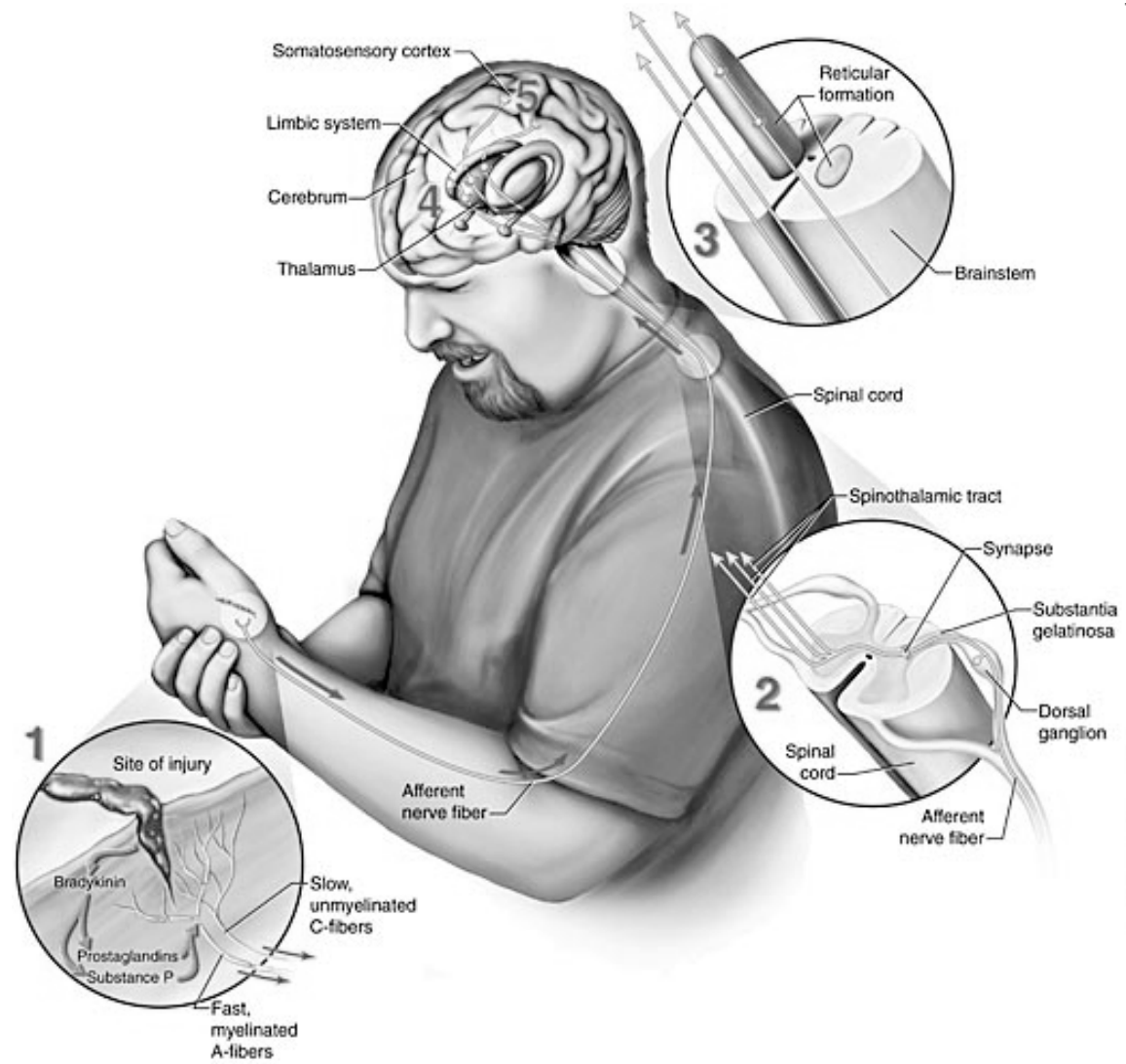
pain

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

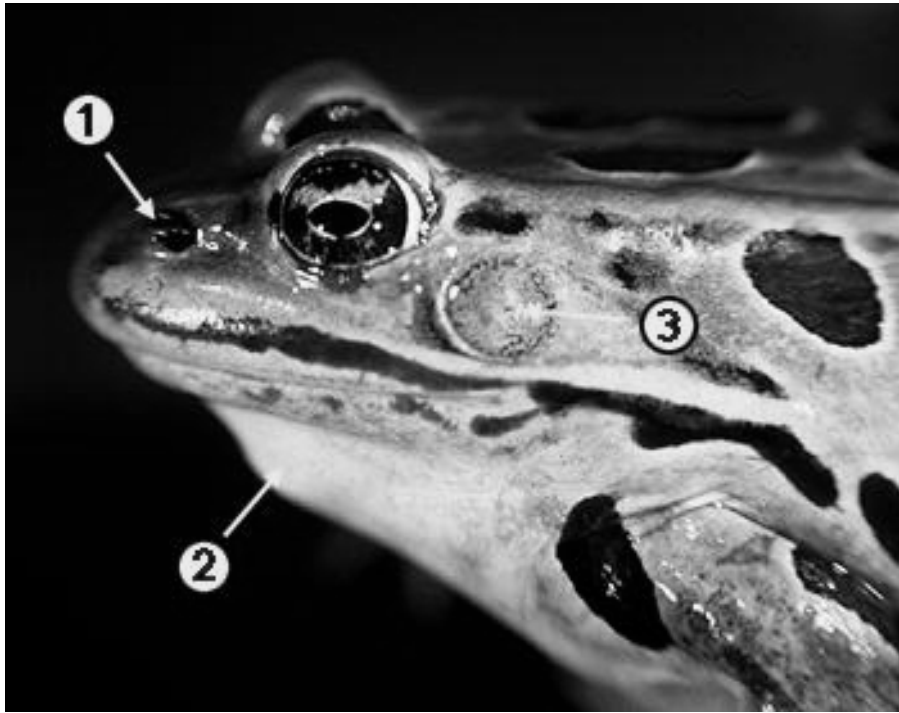
- pharmacological
 - local anesthetics: membrane stabilizing drugs (i.e. Na⁺ channel blockers - Lidocaine)
 - general anesthetics: reversible loss of consciousness
- other (only for animal research)
 - decapitation (guillotine, scissors)
 - when using frogs destroying spinal cord as well

Animal experimentation – Types of experiments

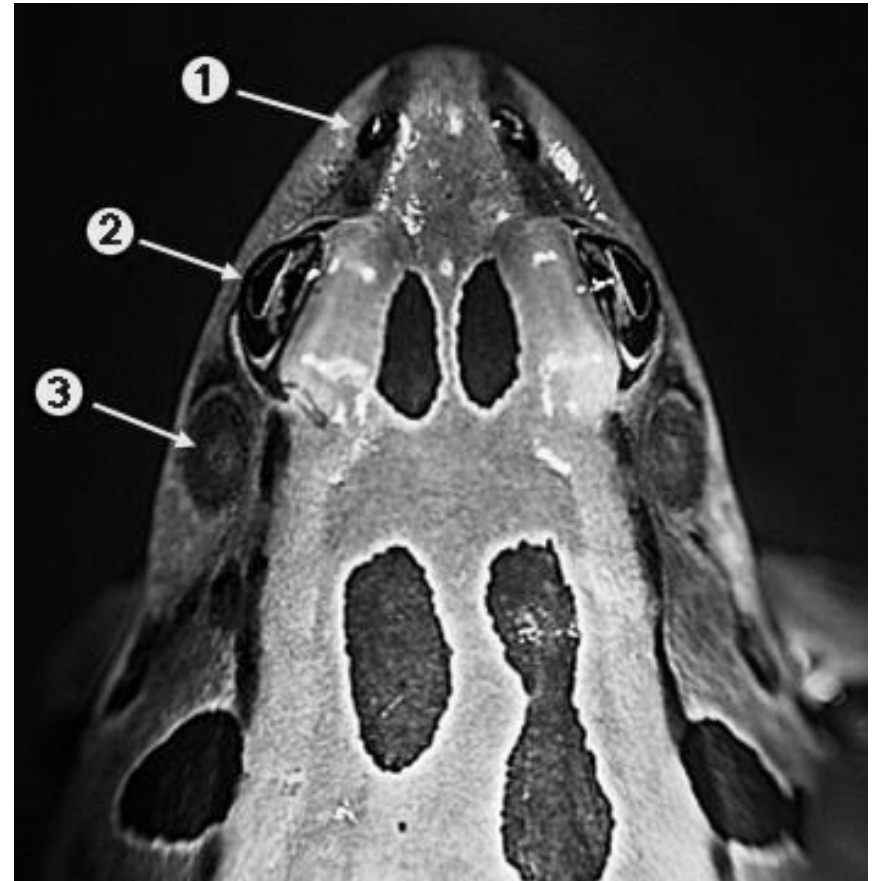
- in vivo: “within the living” (experimentation using a whole, living organism)
- in situ: “in the place” (examine the phenomenon, organ exactly in place where it occurs, i.e. without moving it to some special medium)
- in vitro: “within the glass” (outside of the living organism, in a controlled environment,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

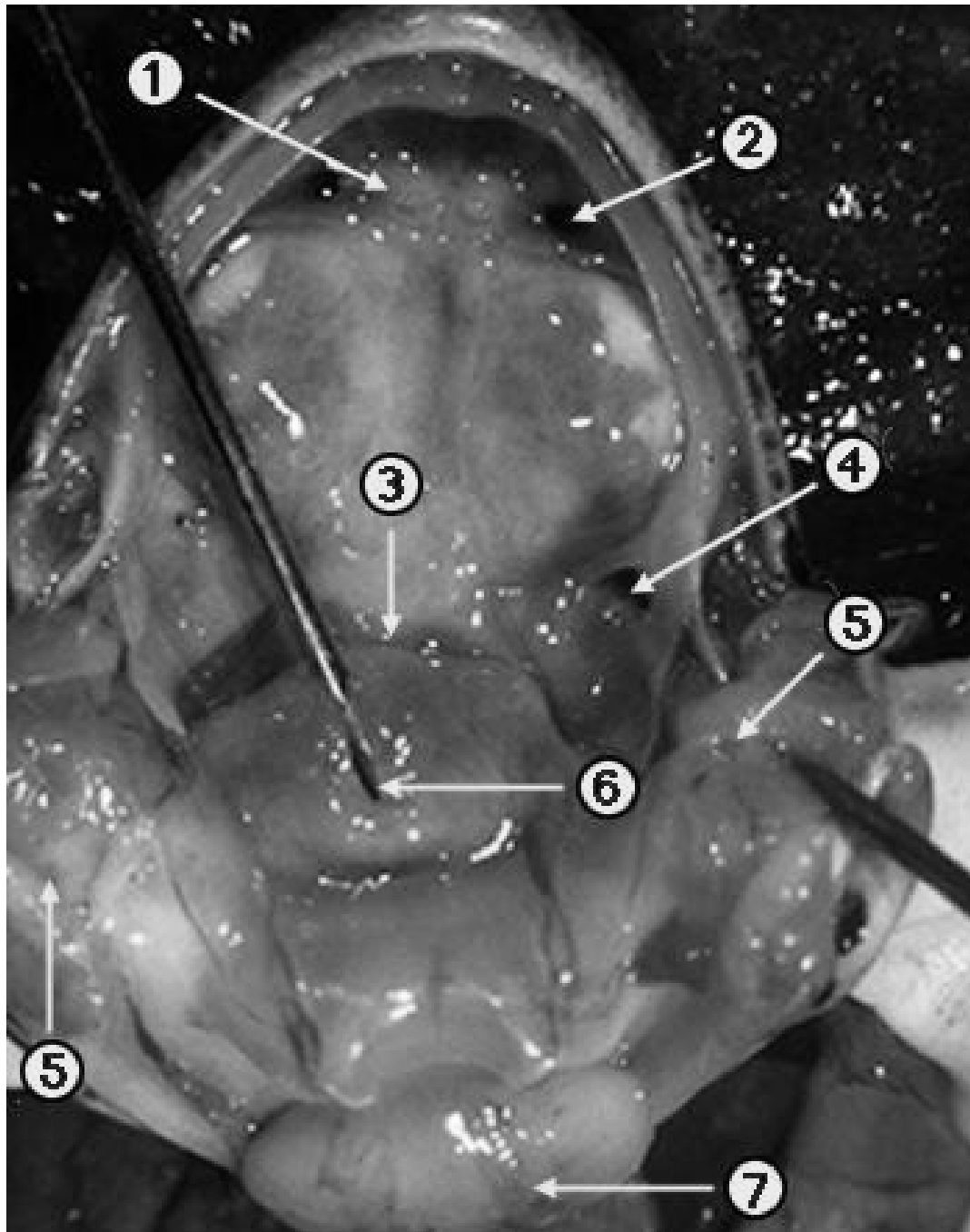
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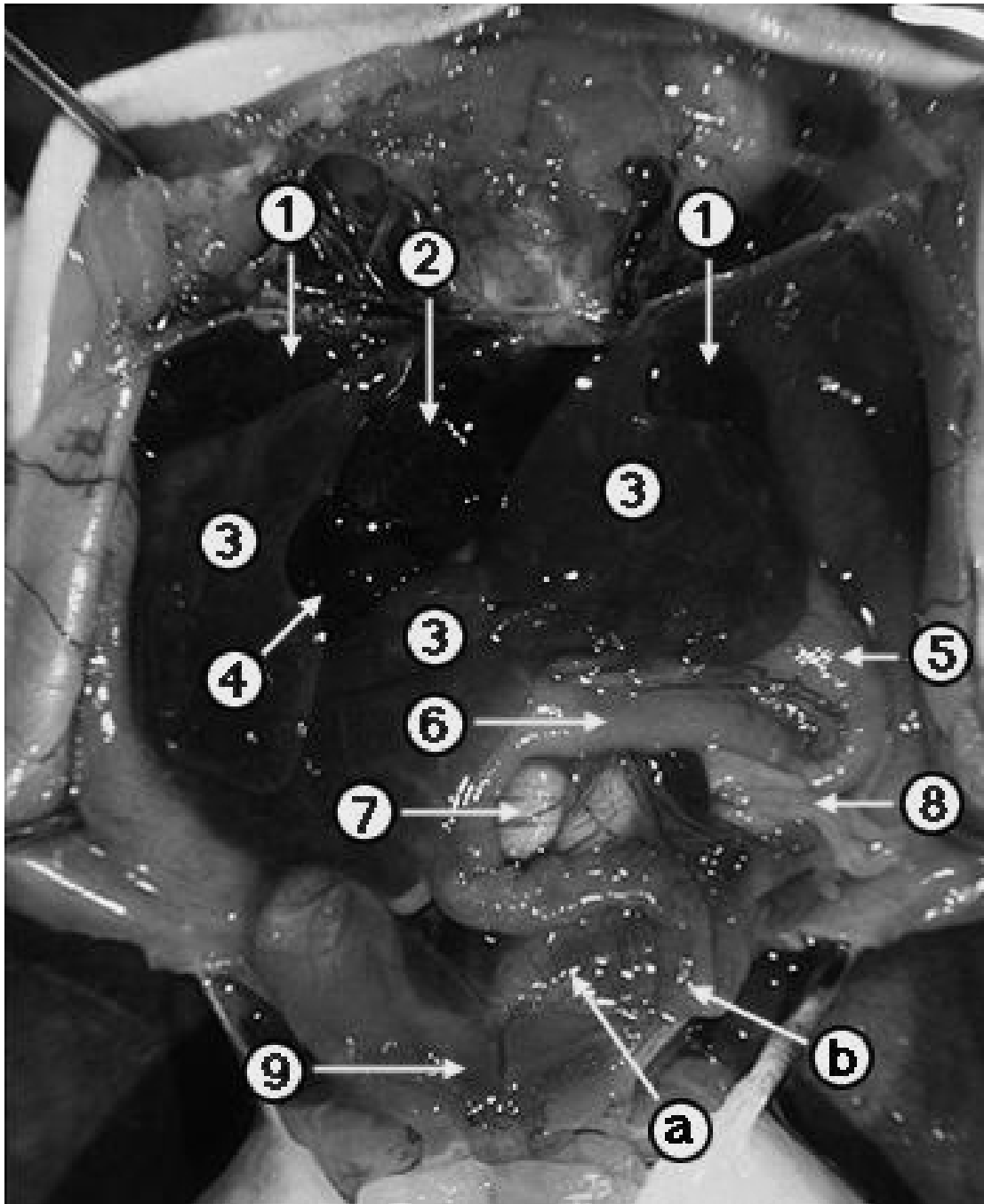


1. nostrils
2. floor of the oral cavity
3. ear drum

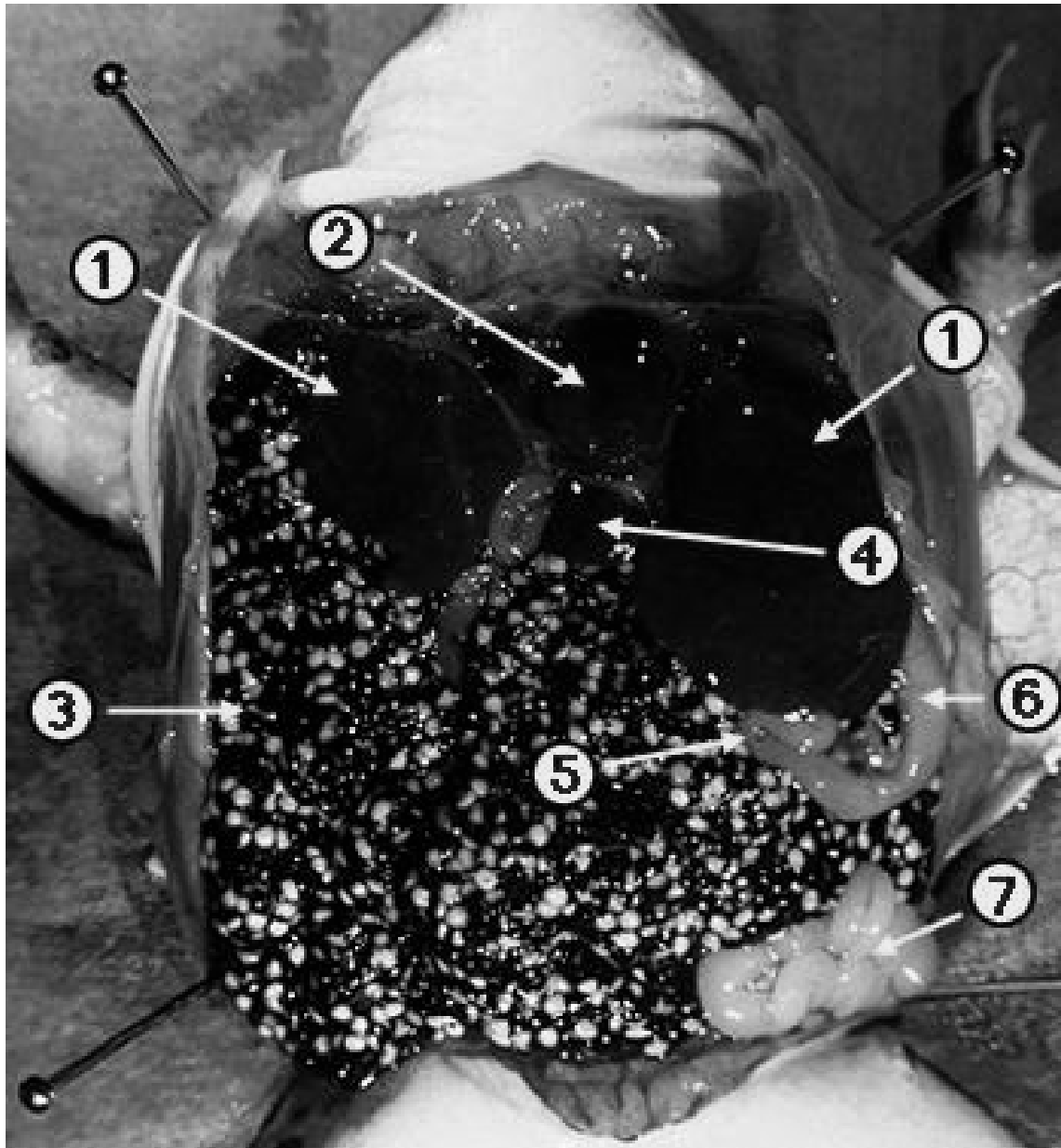




1. vomerine teeth
2. internal nare
3. opening to the esophagus
4. Eustachian tube opening
5. vocal sac opening
6. glottis
7. underside of the tongue



1. lungs
2. heart
3. liver
4. gall bladder
5. pyloric portion of stomach
6. duodenum of small intestine
7. testis
8. fat body
9. urinary bladder
- a. large intestine
- b. ileum



1. liver
2. heart
3. ovary with eggs
4. gall bladder
5. duodenum of small intestine
6. pyloric portion of stomach
7. oviduct

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Experiment

- type: in vivo, in situ
- species used: frog
- anesthesia: urethane, followed by decapitation and destruction of spinal cord

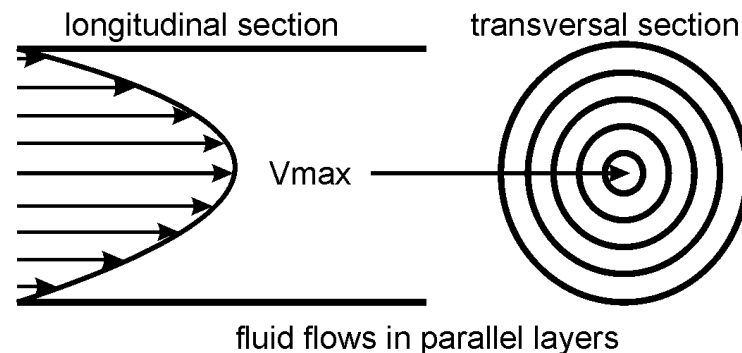
Background and purpose

- frog cardiovascular and respiratory systems present several functional similarities
- purpose:
 - mucociliary clearance
 - in vivo circulation on frog tongue
 - recording the electrical and mechanical activity of the heart simultaneously
 - influences on cardiac output (thermal, chemical)
 - conduction, AV block, overdrive suppression
 - refractory period

In vivo circulation on frog tongue

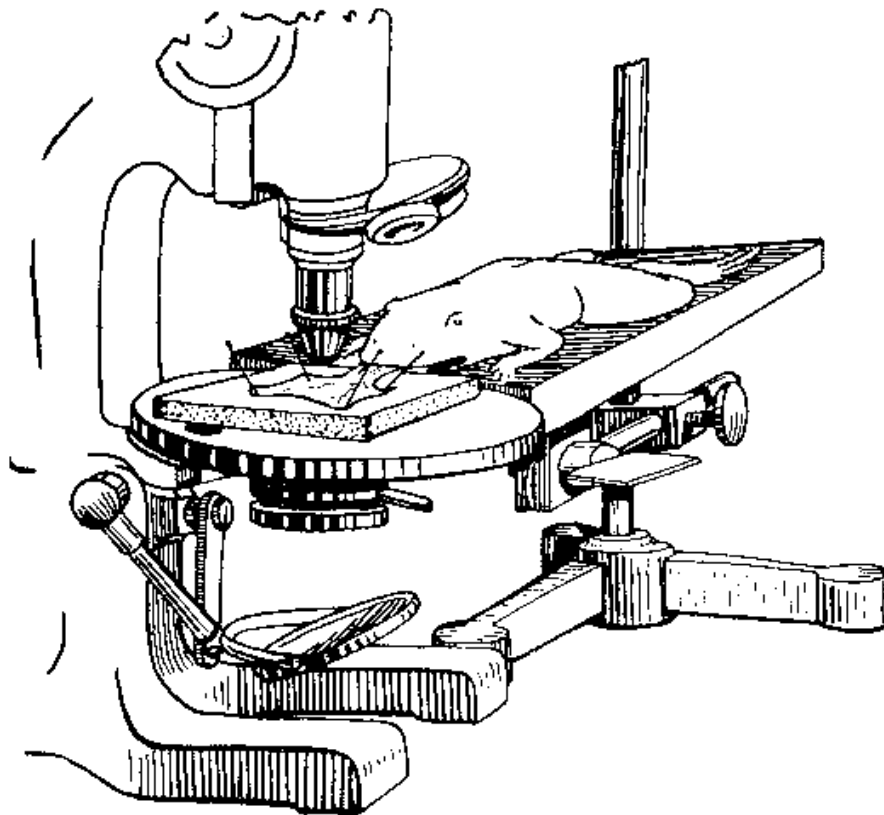
- background -

- explore and identify:
 - microcirculation: arterioles, capillaries, venules
 - main vessels: arteries, veins
 - laminar flow
- artery: velocity \uparrow , pulsate, flow to ramifications
- vein: velocity \downarrow , no pulsations, flow from ramifications
- laminar flow: velocity of core $>$ parietal velocity



In vivo circulation on frog tongue - experimental protocol -

- i.p. anesthesia: urethane 10% 1ml/50g
- check reflexes: flip reflex, cornea reflex
- fixate frog and examine with 10x objective:



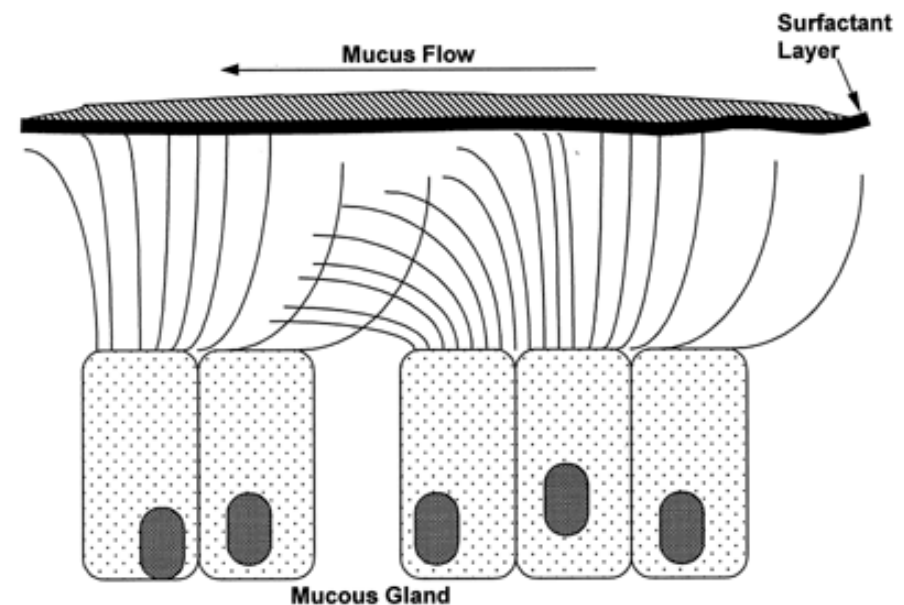
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Mucociliary clearance

- background -

- respiratory tract is lined with ciliated epithelium on top of which is a viscous film of mucus: foreign particles and microorganisms get stuck-> transported to pharynx -> swallowed
- approximately 200 cilia/cell, 10-20 beats/sec, speed: approximately 1cm/min



Mucociliary clearance

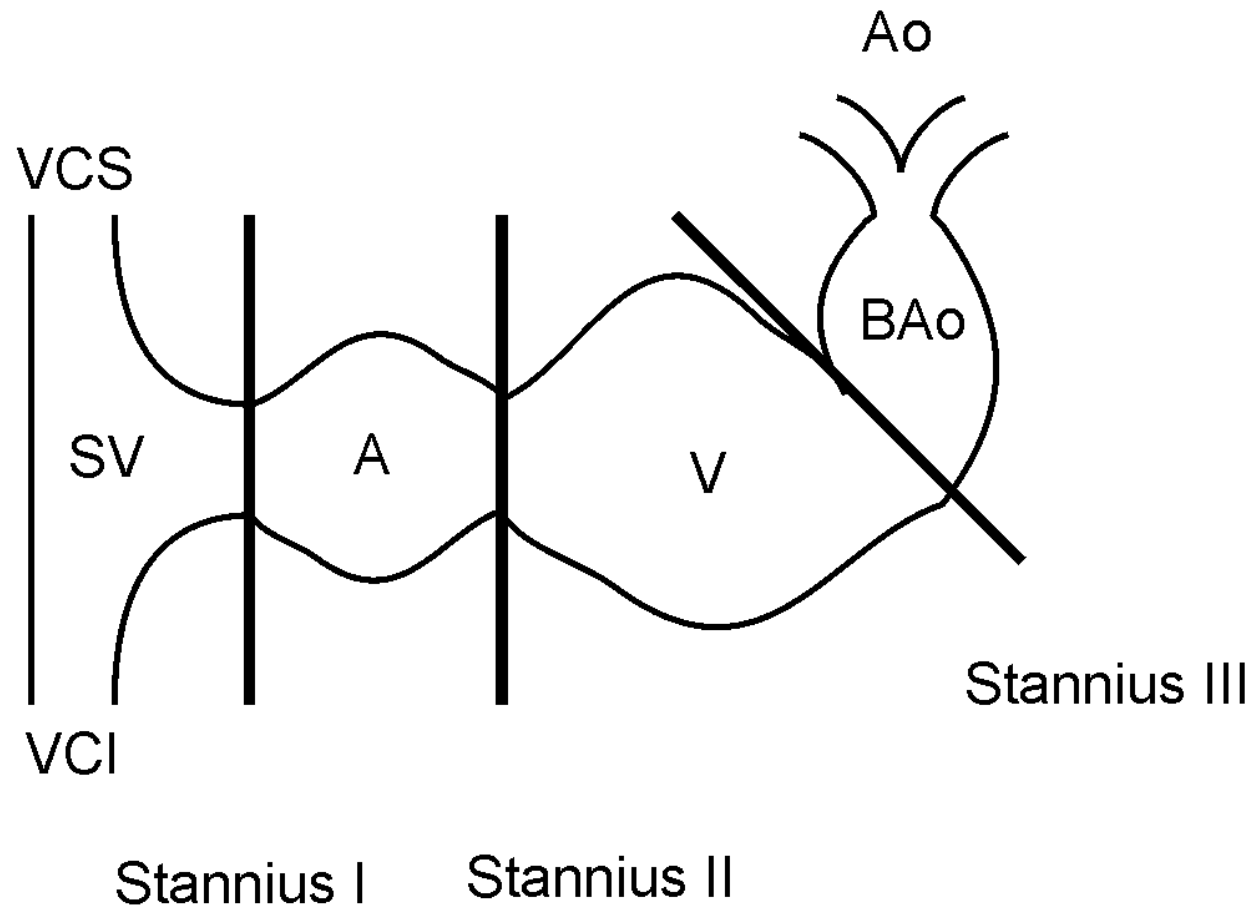
- experimental protocol -

- decapitation (followed by destruction of spinal cord)
- pipet Ringer's solution with suspended charcoal particles on frog palatum durum
- observe

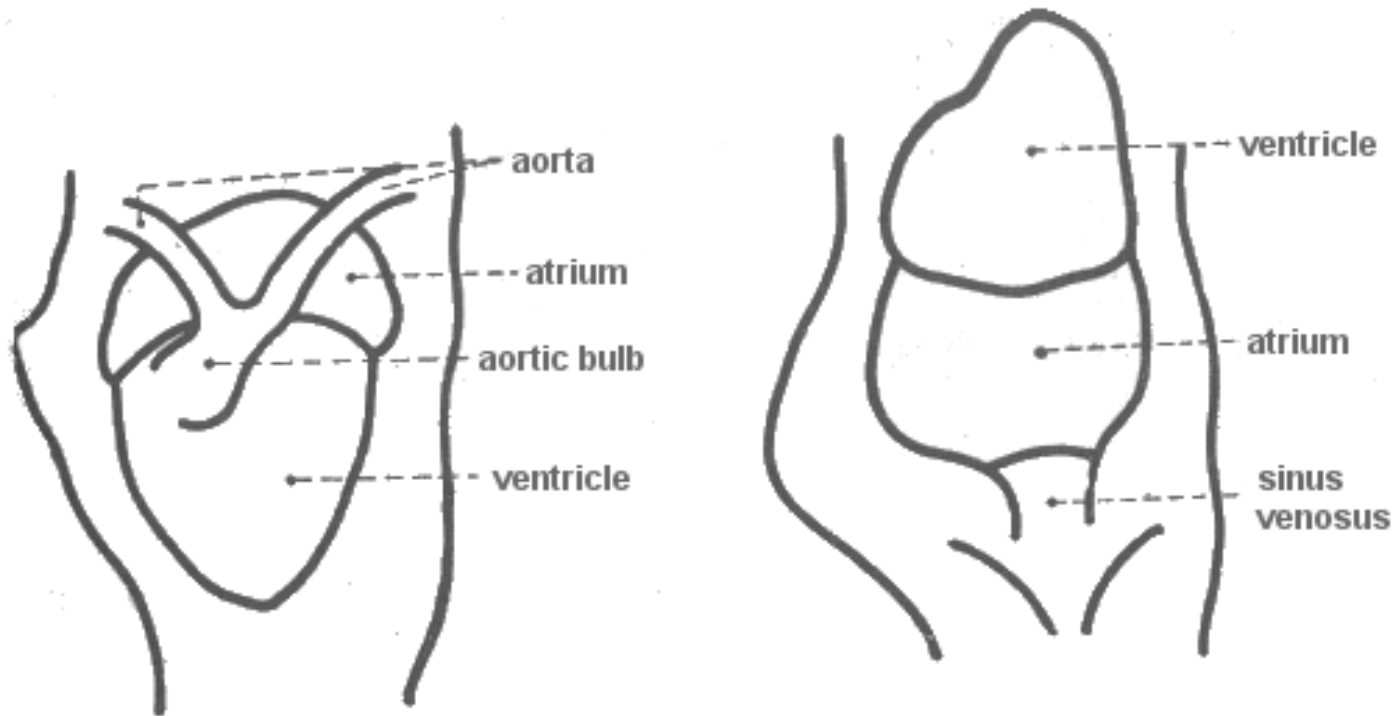
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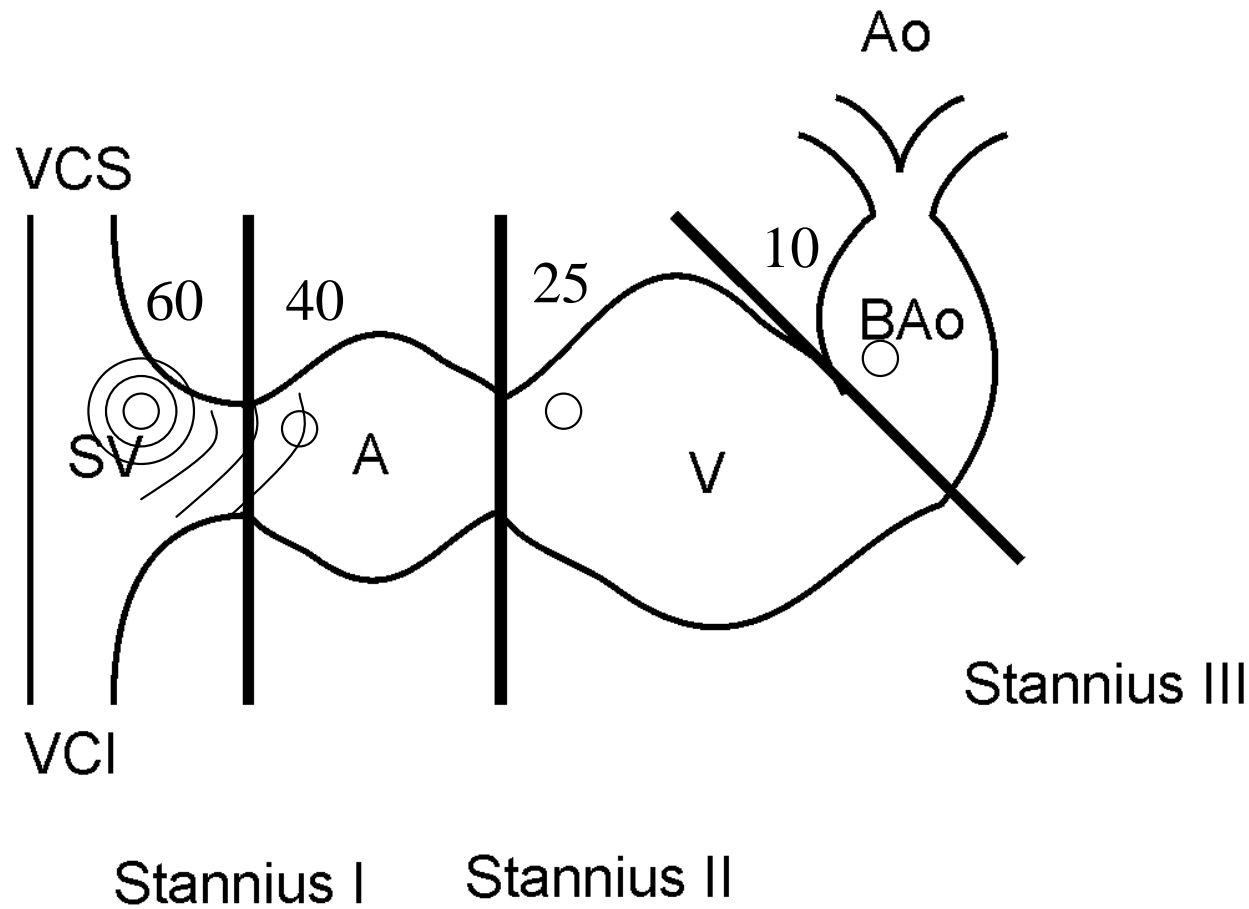
Recording the electrical and mechanical activity of the heart



Recording the electrical and mechanical activity of the heart

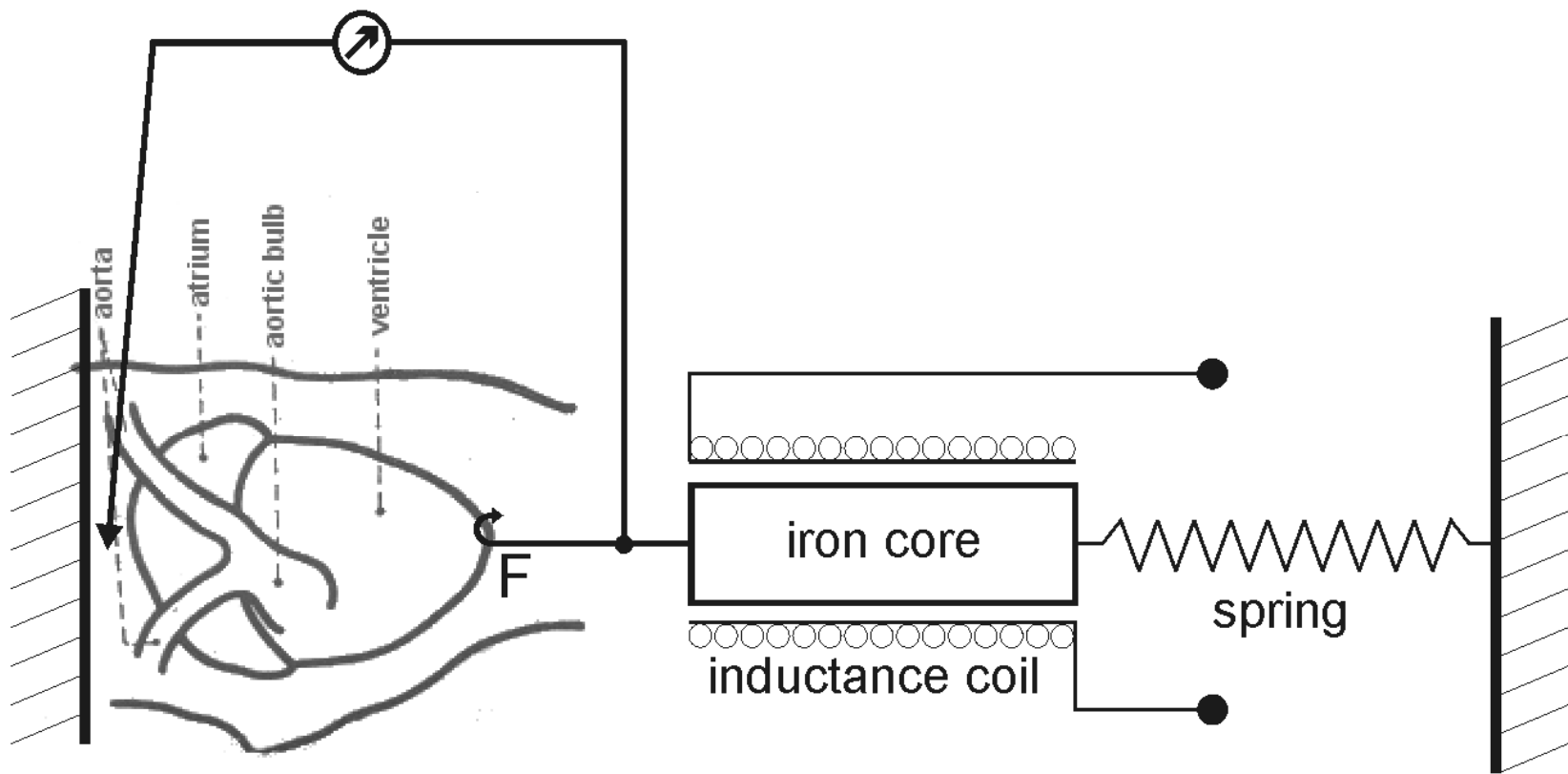


Recording the electrical and mechanical activity of the heart

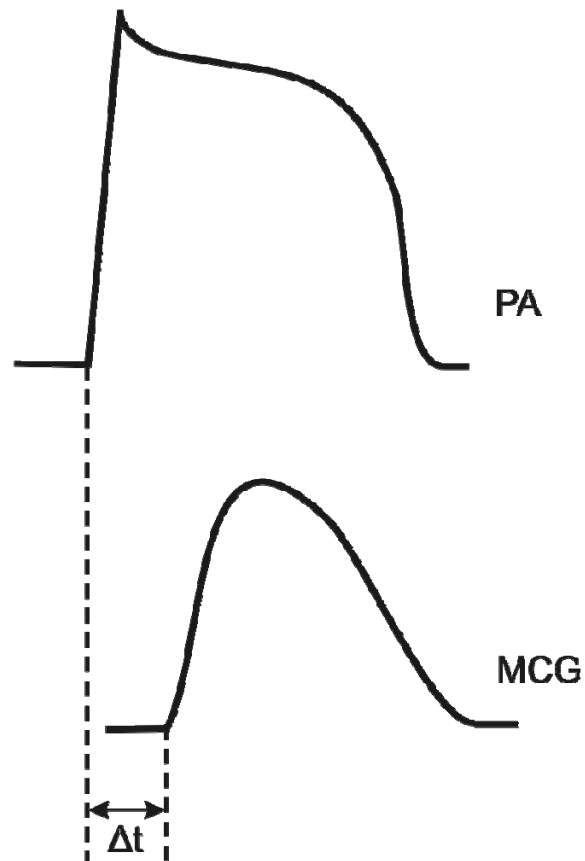


- pacemaker
- overdrive suppression

Recording the electrical and mechanical activity of the heart

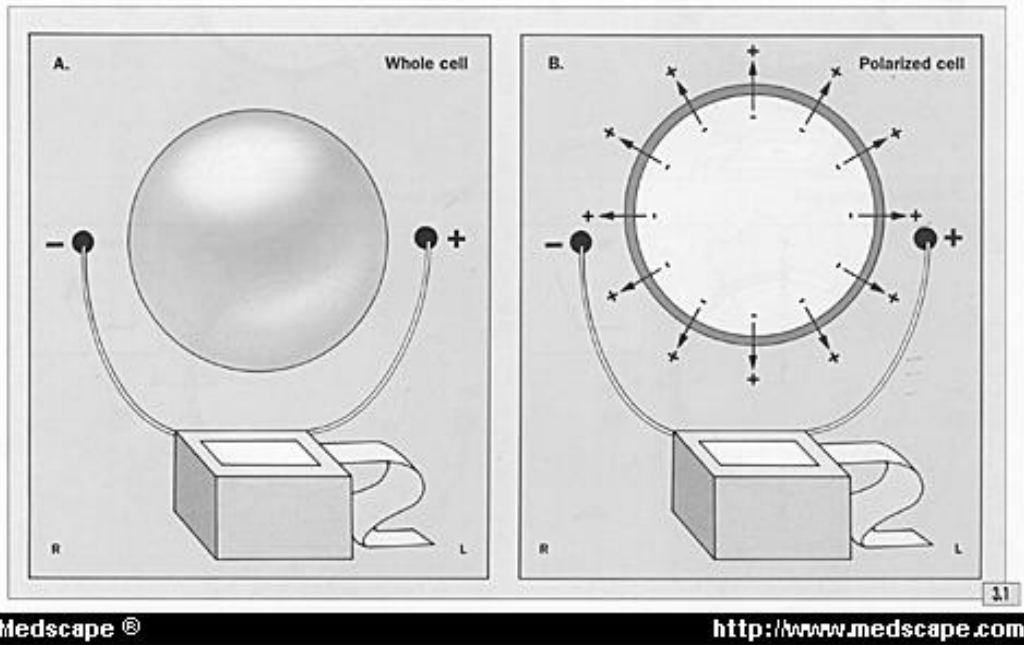


Recording the electrical and mechanical activity of the heart



AP – action potential: transient alteration of the membrane potential across an excitable membrane in an excitable cell generated by the activity of voltage-gated ion channels embedded in the membrane
 Δt - electromechanical coupling

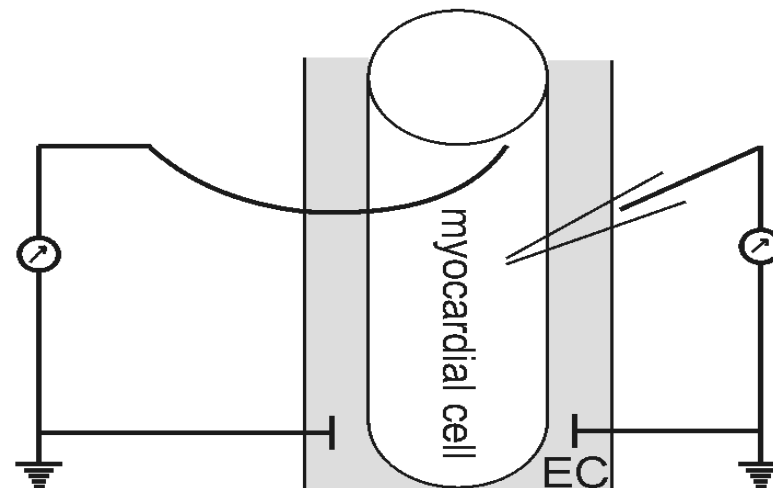
Recording the electrical and mechanical activity of the heart



$$I = \frac{V}{R}$$

$$R = \frac{L}{S} \times \rho$$

recording
frog heart



recording
of action
potentials

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Thermal effect

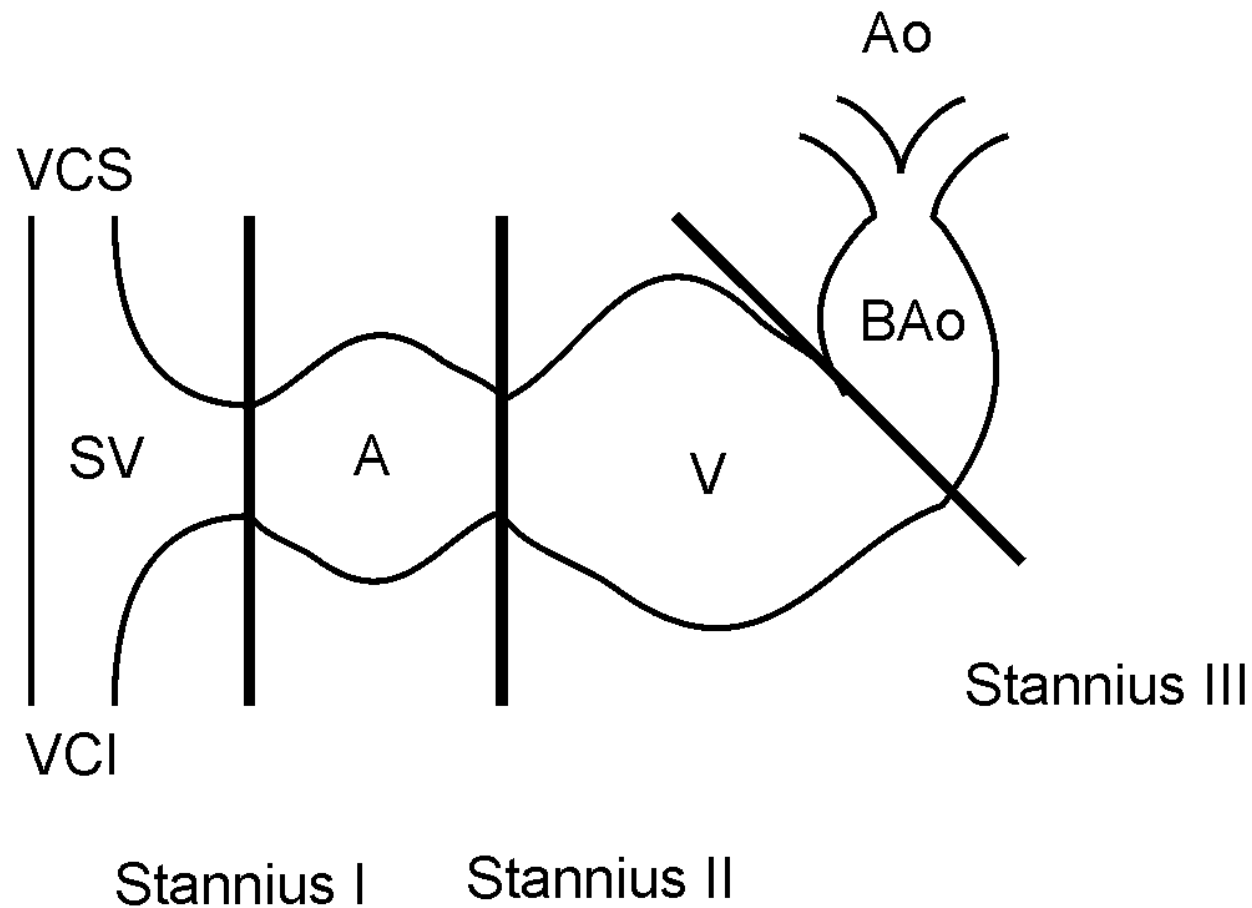
- increase in temperature: increase of frequency and contractility -> increase of cardiac output
- decrease in temperature: decrease of frequency and contractility -> decrease of cardiac output

Contractility and excitability are both dependent on enzymatic systems and such are influenced by temperature variations.

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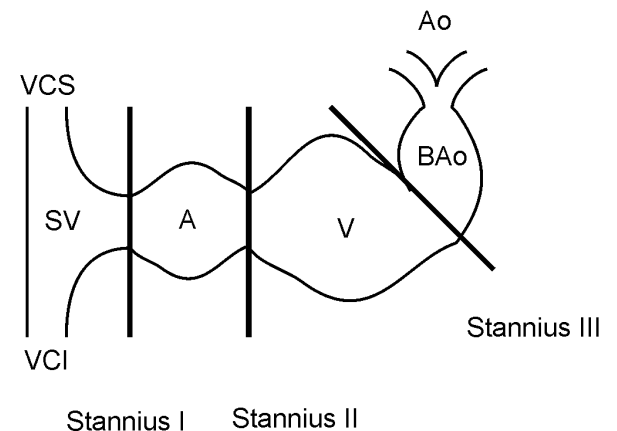
Stannius ligatures



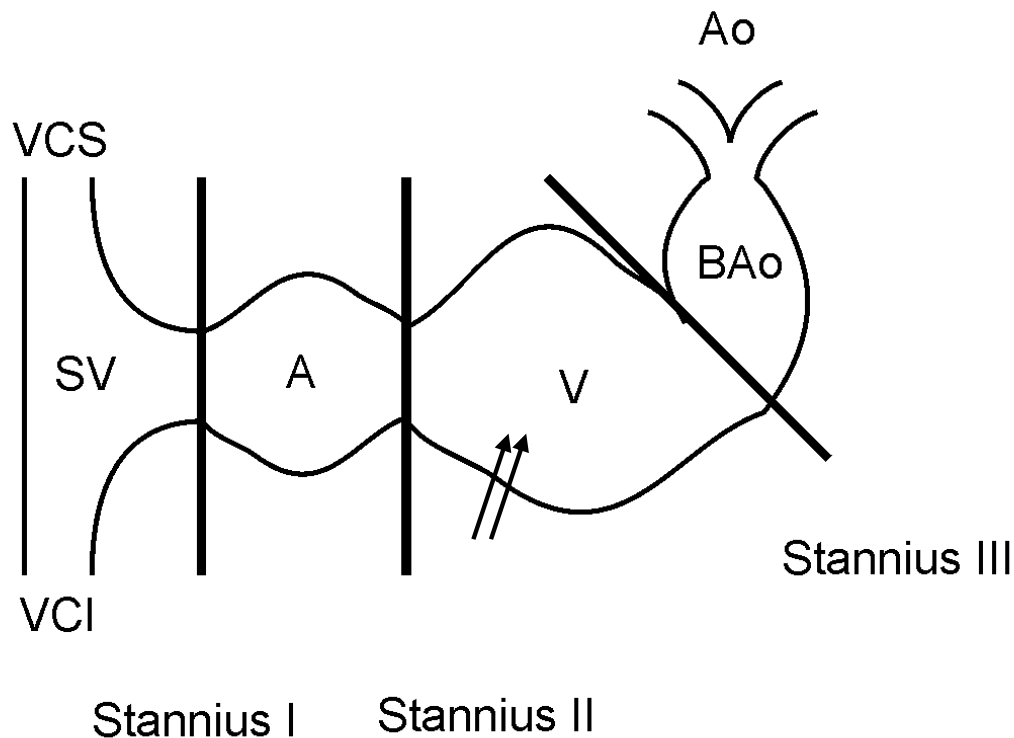
ligature
(constriction)
placed at the
junction of
adjacent
compartments

Stannius ligatures

- demonstrates:
 - possibility to block electrical conduction from one compartment to other (complete, i.e. third degree AV block)
 - successive chambers possess automaticity since each may continue to beat (but in own rhythm!)
 - interruption of overdrive suppression



Stannius ligatures – possibility to use stimulator for excitation

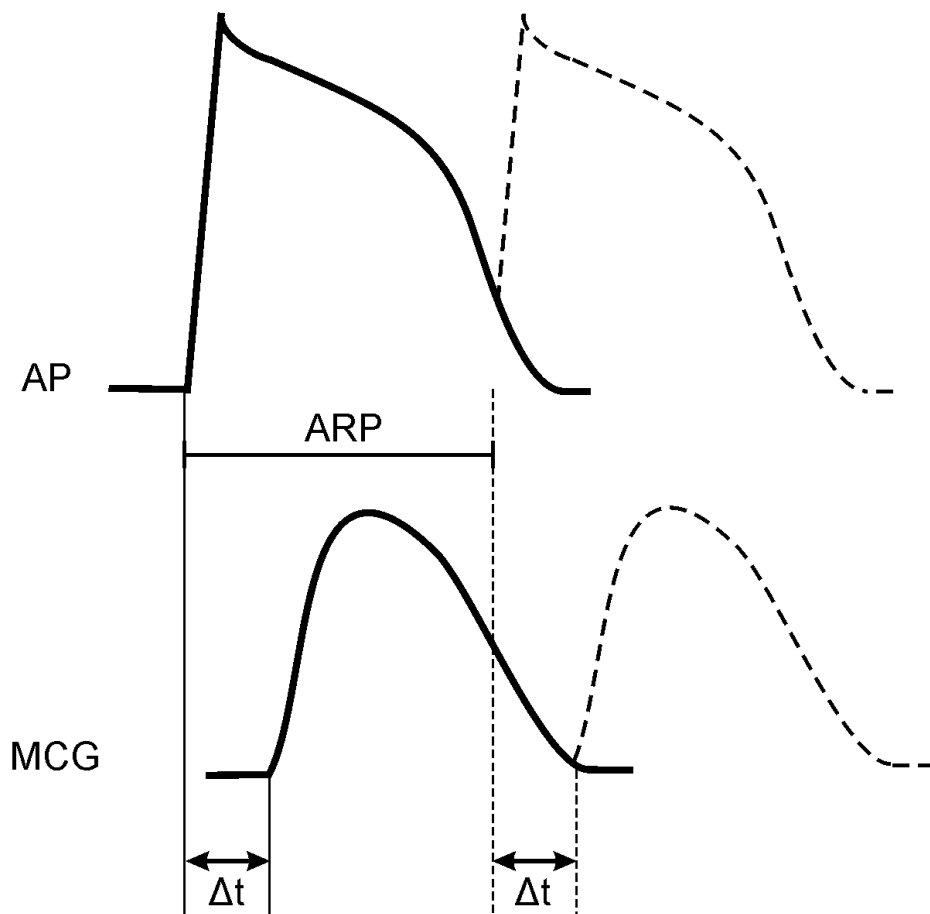


- demonstrates:
 - external pacing

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Recording the electrical and mechanical activity of the heart



ARP – absolute 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 an excitation