université PARIS-SACLAY | GRADUATE SCHOOL Health and Drug Sciences

# Cardiovascular models :
## non-clinical, *in vivo* exploration

**Boris MANOURY**

**February 10, 2025**

Master 2 D2HP

Development of Drugs and Health Products

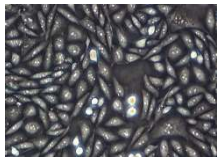
Compulsory Teaching Unit 11: Pharmacology/Toxicology

# I. Which experimental approaches?

## II. Methods for in vivo exploration of cardiovascular models

## III. Models of arterial hypertension

# Non clinical Cardiovascular Pharmacology



Pharmacokinetics

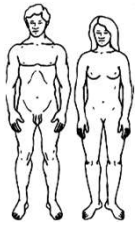
Chemistry

Toxicology

**Safety Pharmacology**

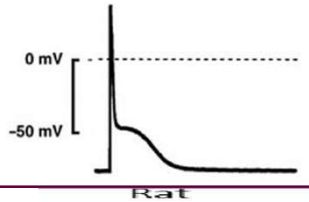
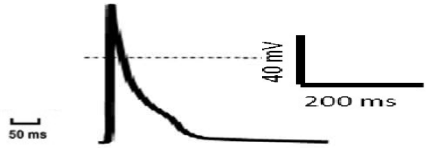
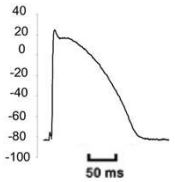
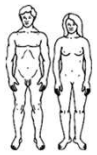
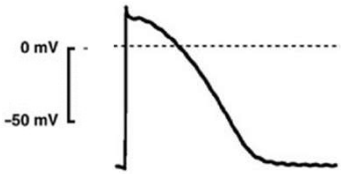
**Pharmacodynamics**

(***non clinical evaluation***)



1st trial in human (FIH ; Phase I)

# Which species do I need for a good model?

| species | Resting heart rate (bpm) | Ventricular action potential | Mean arterial pressure (mmHg) |
|---|---|---|---|
|  | 580 |  | 111 |
|  | 340 |  | 111 |
|  | 105 |  | 128 |
|  | 70 |  | 93 |

*Pulsen et al., 2017 Acta Physiol*

# Which model do I need to study CV function?

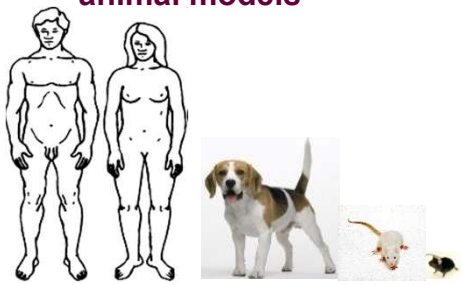
## non-clinical models

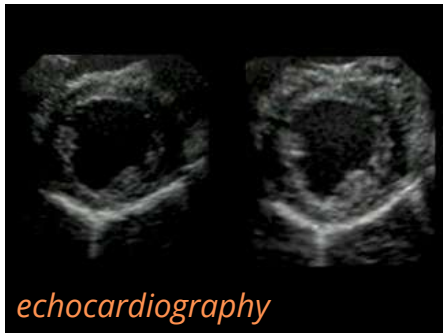
**Experimental model = a specific methodology applied on a biological system**

Example: *I'm interested in studying cardiac inotropism!*

### In vivo

- **clinical data**
- **animal models**



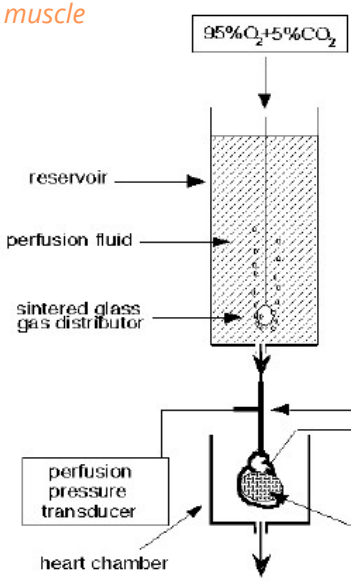


*echocardiography*

### Ex vivo

**isolated organ**

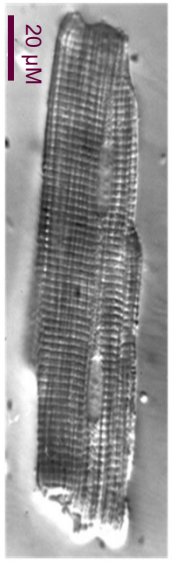
*contractility of an isolated muscle*




**de rodent**

### Ex vivo / In vitro

**Isolated cardiac cell**



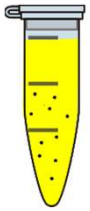

*Single cell contractility*

### In vitro

**biochemistry**

Ex : enzymatic ATPase assay

- (actin/ myosin/ATP)



# Which model do I need to study CV function?

## non-clinical models

## Pathological model

**Aim: To mimic the pathology in experimental models**

### *In vivo*

**Pathological animal models**

- Genetic selection: salt-sensitive Dahl rats, ob/ob mice…
- Genetic manipulation: knock out, overexpression of a transgene, mutation…
- Administration of a toxic or pharmacological compound (e.g. AngII)
- Surgery: ex. : myocardial infarction
- …

### *Ex vivo*

**isolated organ**

- Sampling an organ from patients or an animal model

### *Ex vivo / In vitro*

**Cellular models**

- Primary cell culture from patients or an animal model
- **Cell lines**
- **Re-programmed stem cells (iPS)**
- **Engineered Tissue**

### *In vitro*

**Molecular models**

Ex. :

- Modified Recombinant protein with mutation

# Etudes de pharmacodynamie

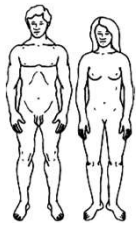
compound

Therapeutical target

**Pathological experimental model**

## Non-clinical « proof of concept »

- **Aim : to demonstrate <u>EFFICACY</u> of the therapeutic strategy in a <u>non-clinical model of the pathology</u>.**



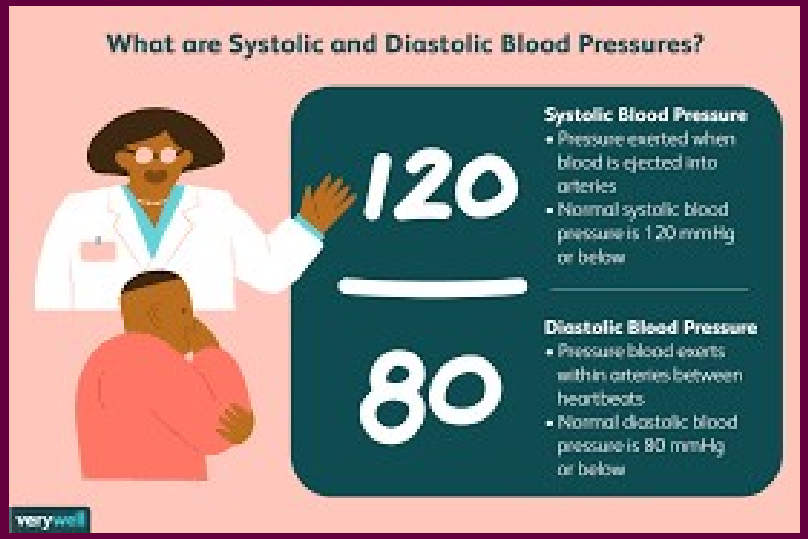
1st trial in human (FIH ; Phase I)

I. Which experimental approaches?

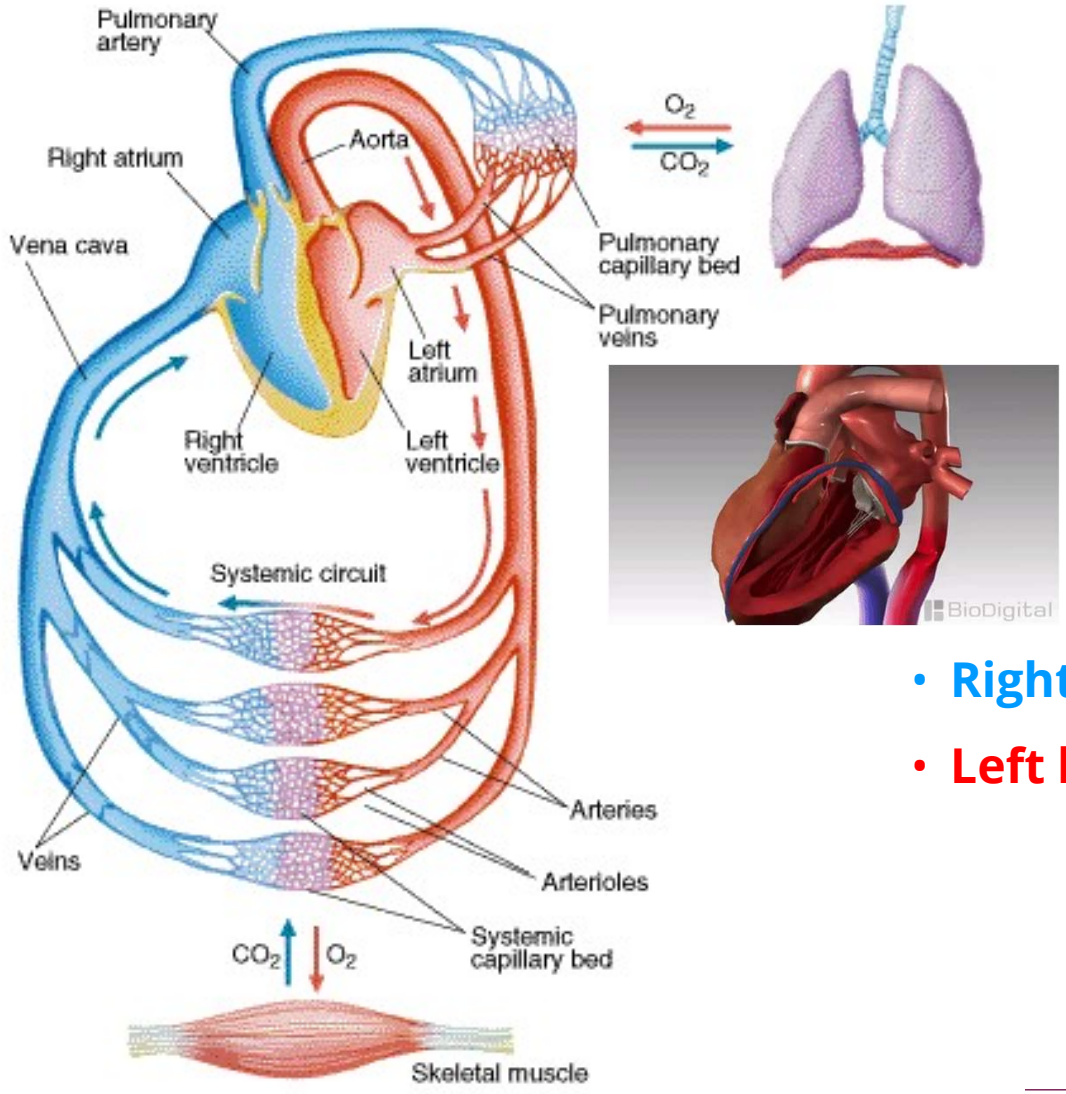
**II. Methods for in vivo exploration of cardiovascular models**

III. Models of arterial hypertension

# Blood pressure




# Organ perfusion is driven by ARTERIAL BLOOD PRESSURE ($\Delta P_A$)







$$\Delta P_A = Q_c \cdot PVR$$

$$Q_c = HR \times V_s$$

$Q_c$ : cardiac output (L/min)

PVR : peripheral vascular resistances

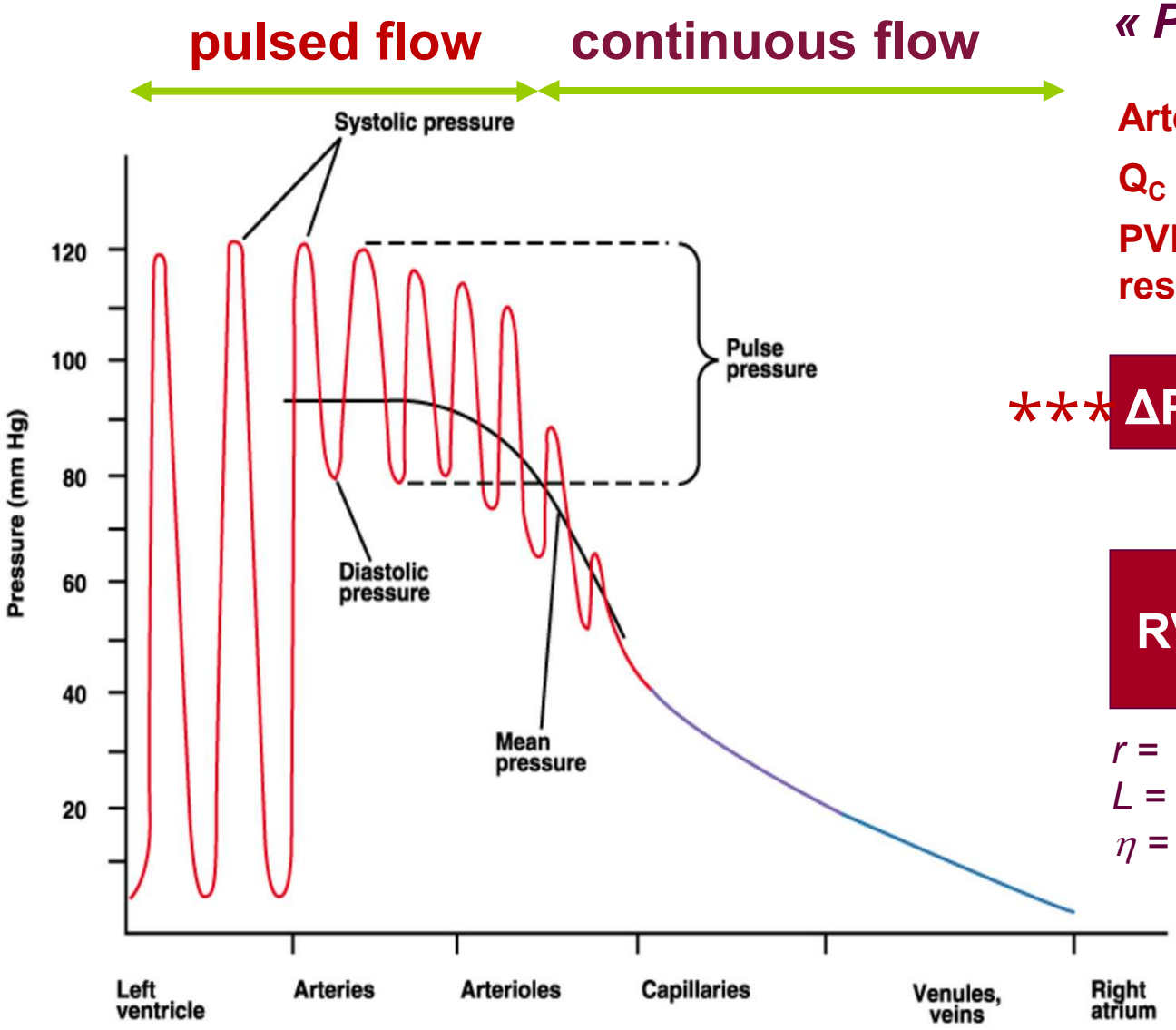
HR : heart rate

$V_s$: systole ejection volume

- **Right heart** : low pressure system
- **Left heart :** high pressure system

# Blood pressure (BP) throughout the cardiovascular system

*Blood pressure = force exerted on the vessel walls (Pa/mm$^2$ or mmHg)*




***« Poiseuille's law »***

**Arterial pressure (ΔP)**

**$Q_C$ = cardiac output**

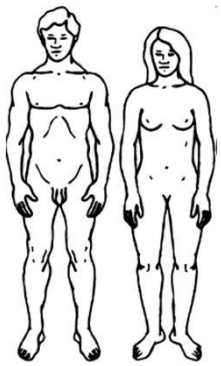
**PVR : peripheral vascular resistances**

*** **$\Delta P = Q_C \times RVP$**

$$RVP = \frac{8\eta L}{\pi r^4}$$

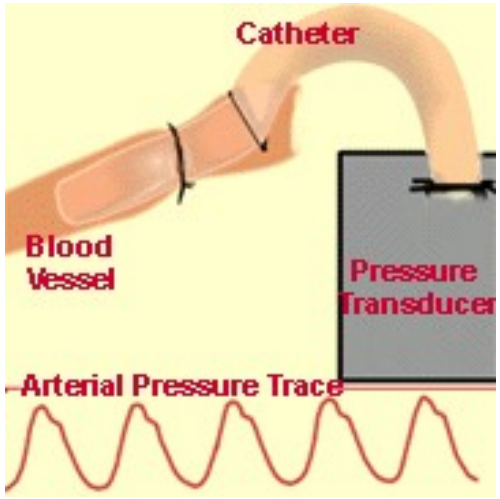
*r = radius of the vessel*
*L = length*
*$\eta$ = viscosity*

# Hemodynamics recording : ex : blood pressure



## Invasive

Catheterism



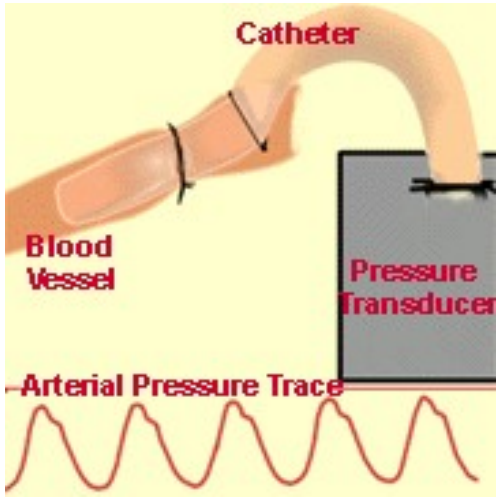

http://www.medicine.mcgill.ca/physio/vlab/cardio/back.htm

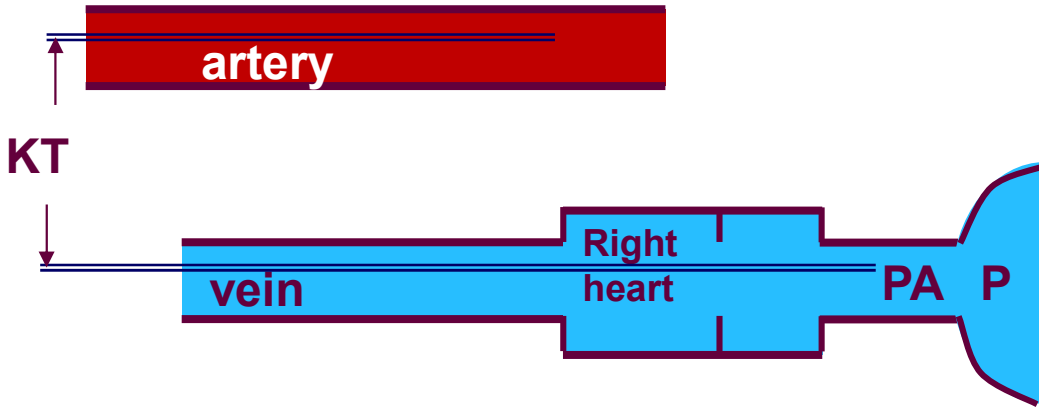
## Non-Invasive

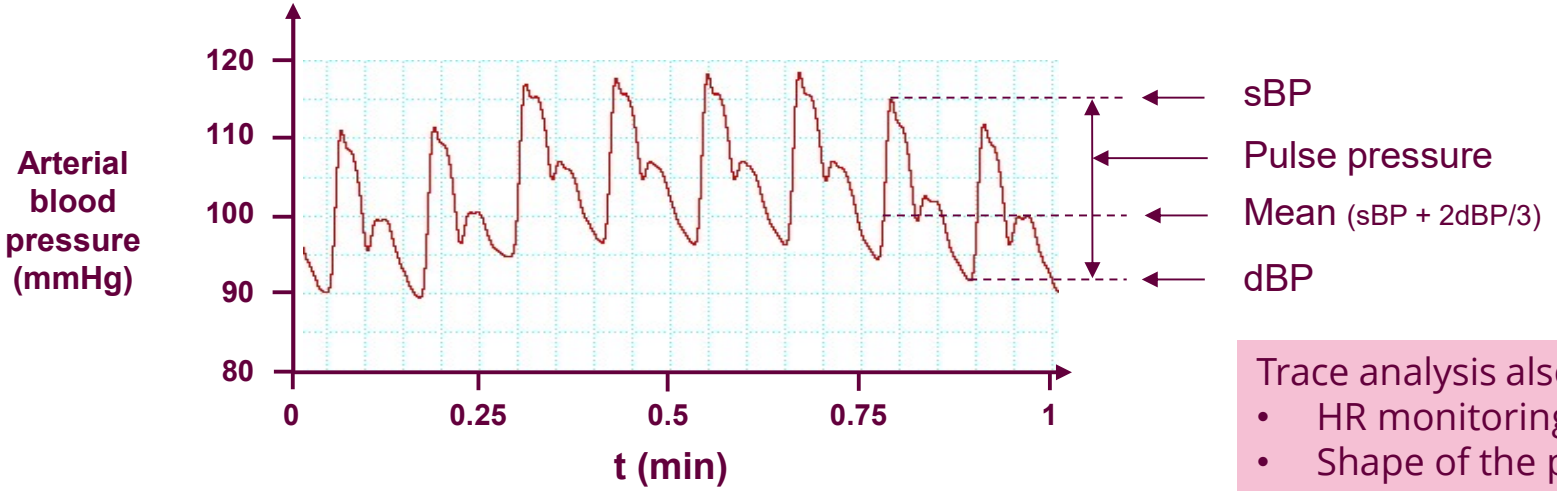
Sphygmomanometer

| | | |
|---|---|---|
| **Advantages** | • Direct mesurement of pressure<br>• Collection of blood samples | • Recording in vigil (non-anesthetized) « animals » |
| **Limits** | • Anesthesia may be required<br>⇒ influence heart rate, blood pressure…<br>• Risk of complications : thrombosis, infection | • Indirect measurement |

# Direct measurement of blood pressure : catheterism




Trace analysis also provide with :

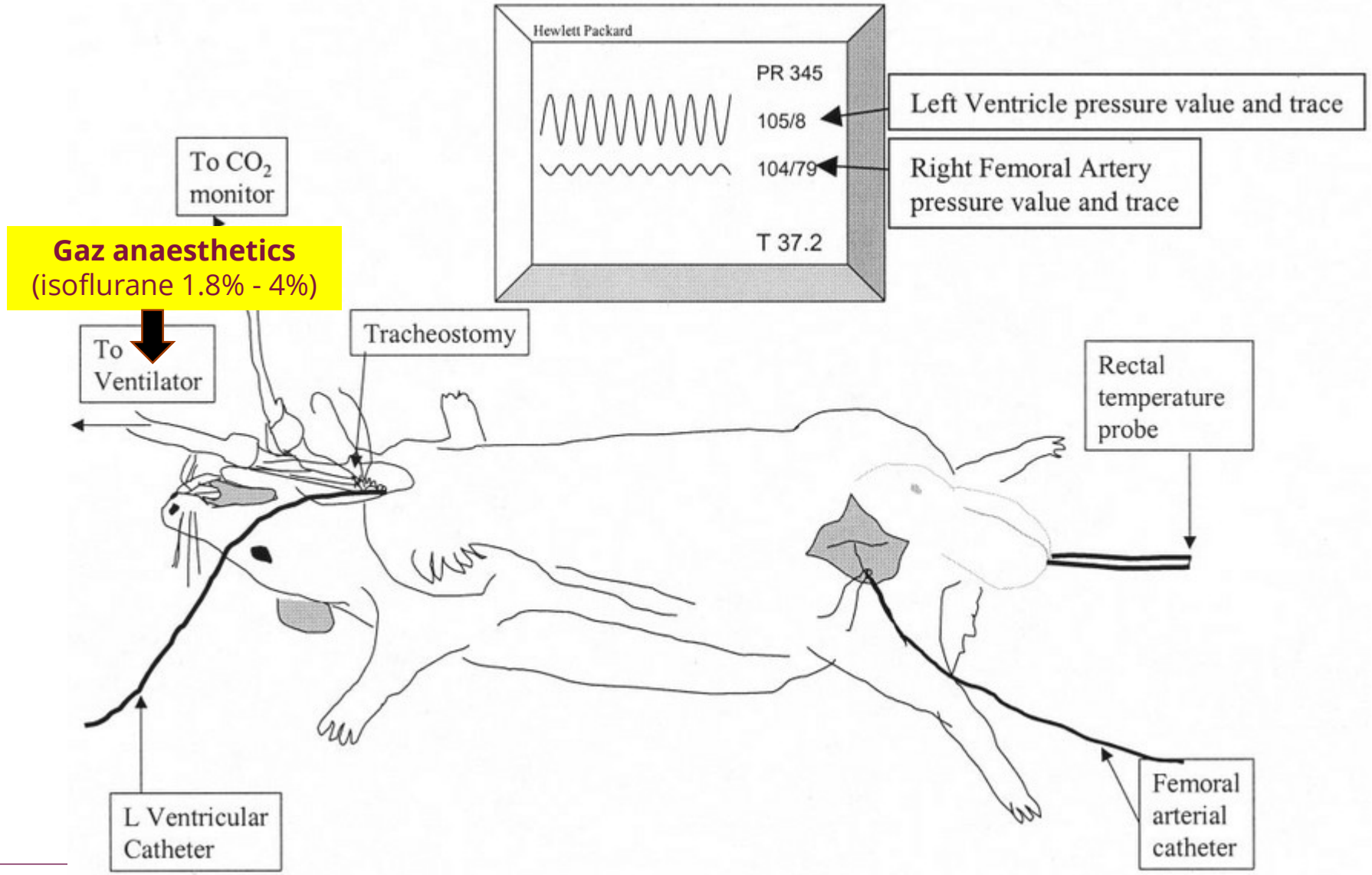
- HR monitoring
- Shape of the pulsewave form : depends on arterial stiffness

PA : pulmonary artery ; sBP : systolic blood pressure ; dBP : diastolic blood pressure; HR : heart rate

*http://www.medicine.mcgill.ca/physio/vlab/cardio/back.htm*

# Measure of blood pressure by catheterism

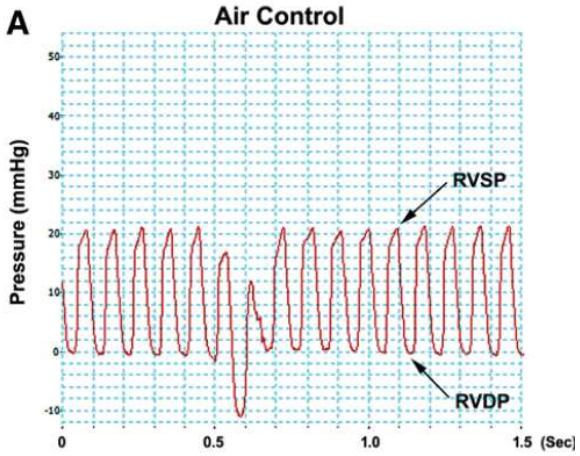
https://www.jove.com/v/3496/femoral-arterial-venous-catheterization-for-blood-sampling-drug

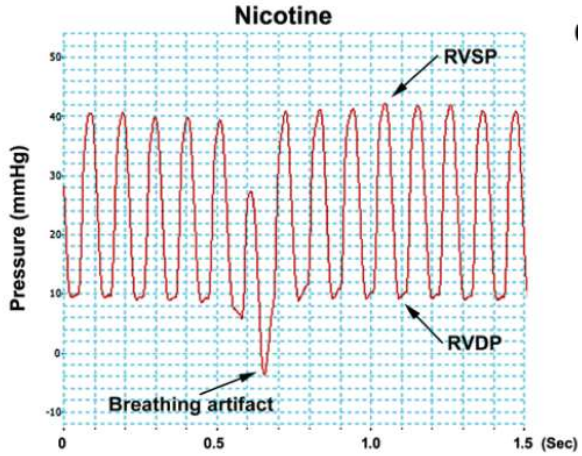


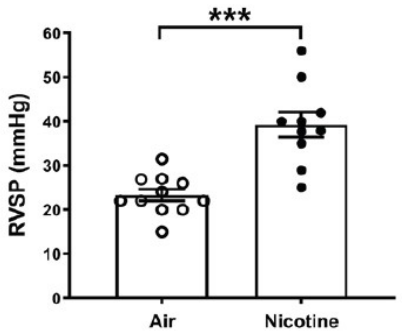

*Palmer et al., 2002 Anesth Analg 2002;95:1080 –6)*

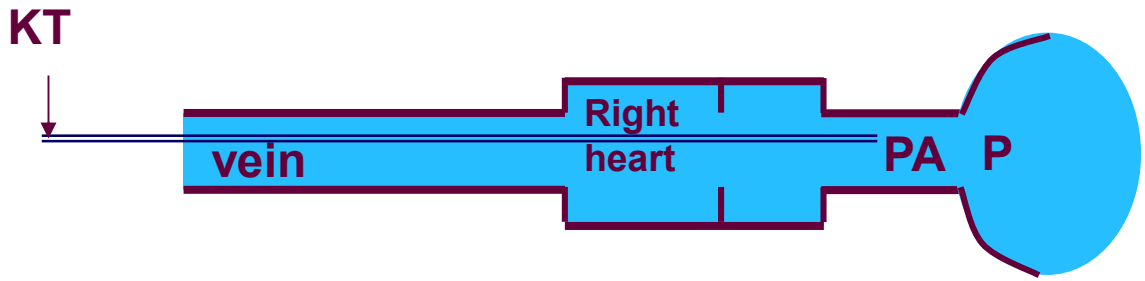
# Measure of blood pressure by catheterism

- Mouse model of 8-week exposure to inhalated nicotine or air
- Right ventricle catheter to measure RV blood pressure, as a surrogate of pulmonary artery pressure



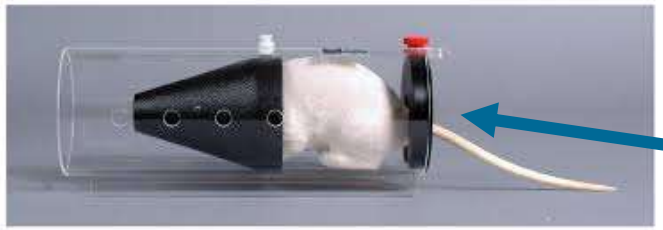




RVSP: right ventricle systolic pressure
RVDP: right diastolic systolic pressure

*Oakes et al., 2020 Hypertension* 75(5):1305-1314

# Non- invasive blood pressure measurement : tail cuff method

- **In rodent (rat, mouse) : tail cuff « CODA system »**
- This method consists of utilizing a tail-cuff placed on the tail to occlude the blood flow.
- Upon deflation, one of several types of noninvasive blood pressure sensors, placed distal to the occlusion cuff, can be used to monitor the blood pressure.
- Animals are vigil ;
- Necessitates an habituation period (generally 5 consecutive days, with “real” measurement on day 6-7)
- Limit : provides with a snapshot of BP value; no long term monitoring



https://www.jove.com/v/1291/measuring-blood-pressure-mice-using-volume-pressure-recording-tail

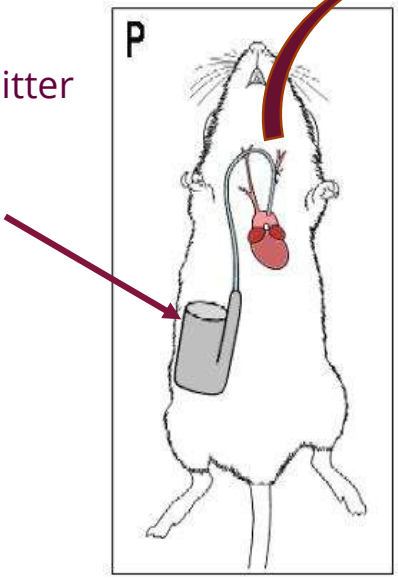
*Kent Scientific Corporation : https://www.kentscientific.com*

*Daugherty et al., 2009 J Vis Exp*

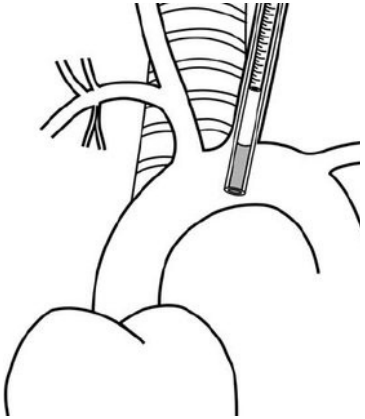
# The new gold standard : radiotelemetry (I)

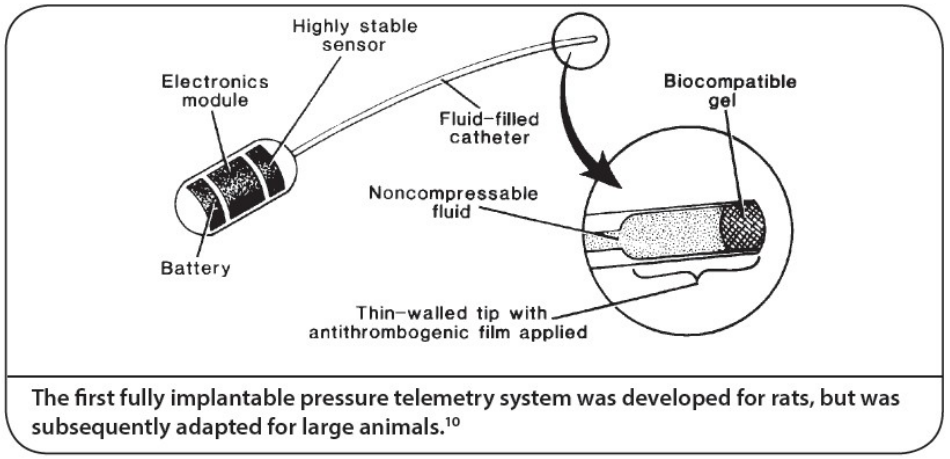
- Implant of a pressure probe in the animal during a surgery.
- Direct method : BP could be continuously monitored, resulting in a nuanced picture of BP changes over an extended period of time
- Radio transmitter sends the BP data while the animal is conscious and free
- Adapted to rodents and large animals (dog, pig, monkey…)
- limit : battery life

Radio transmitter body



Catheter tip advanced to the aortic arch

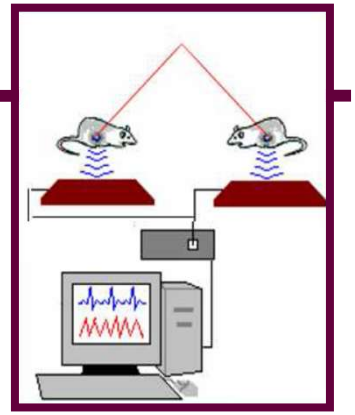





The first fully implantable pressure telemetry system was developed for rats, but was subsequently adapted for large animals.[10]

*Kurtz et al., 2005 Hypertension ; Aubdool et al., 2017 Circulation*

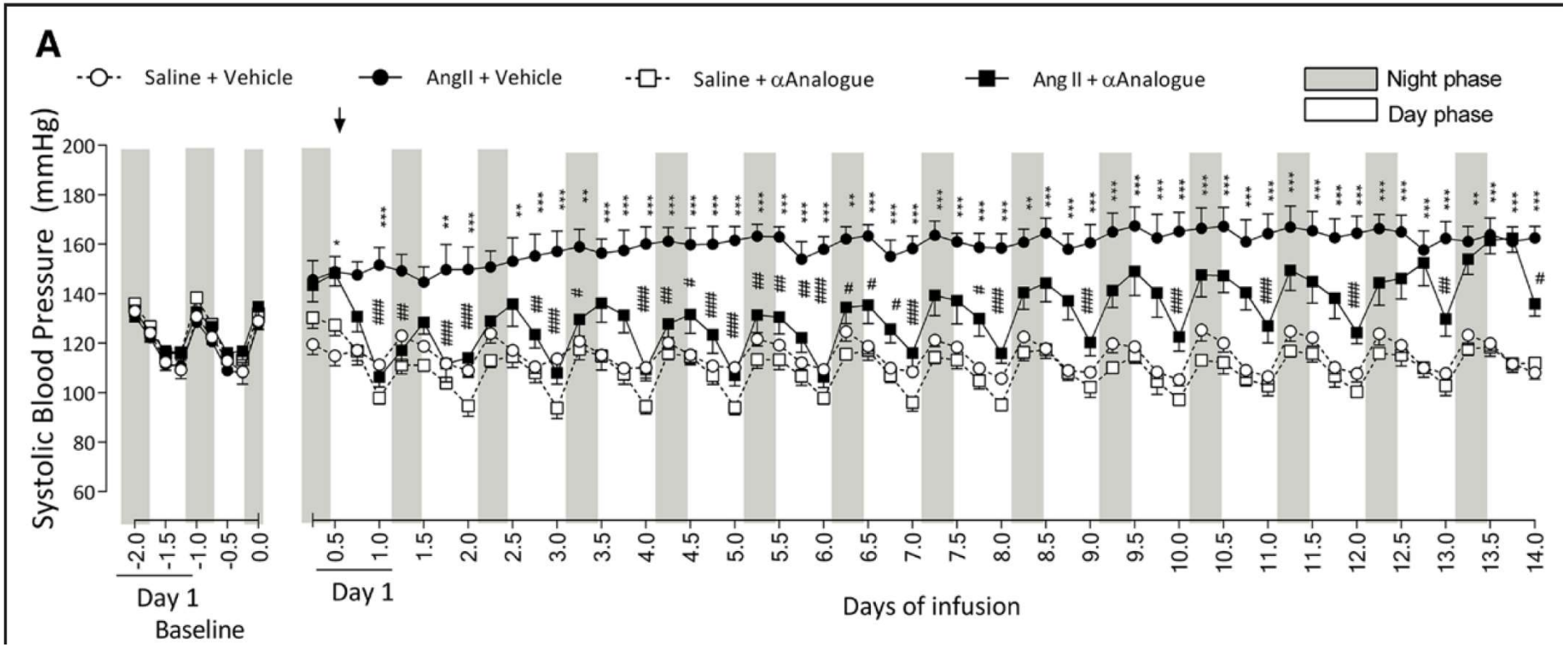
# The gold standard : radiotelemetry (II)



***Example:***

Monitoring of BP in mice receiving :

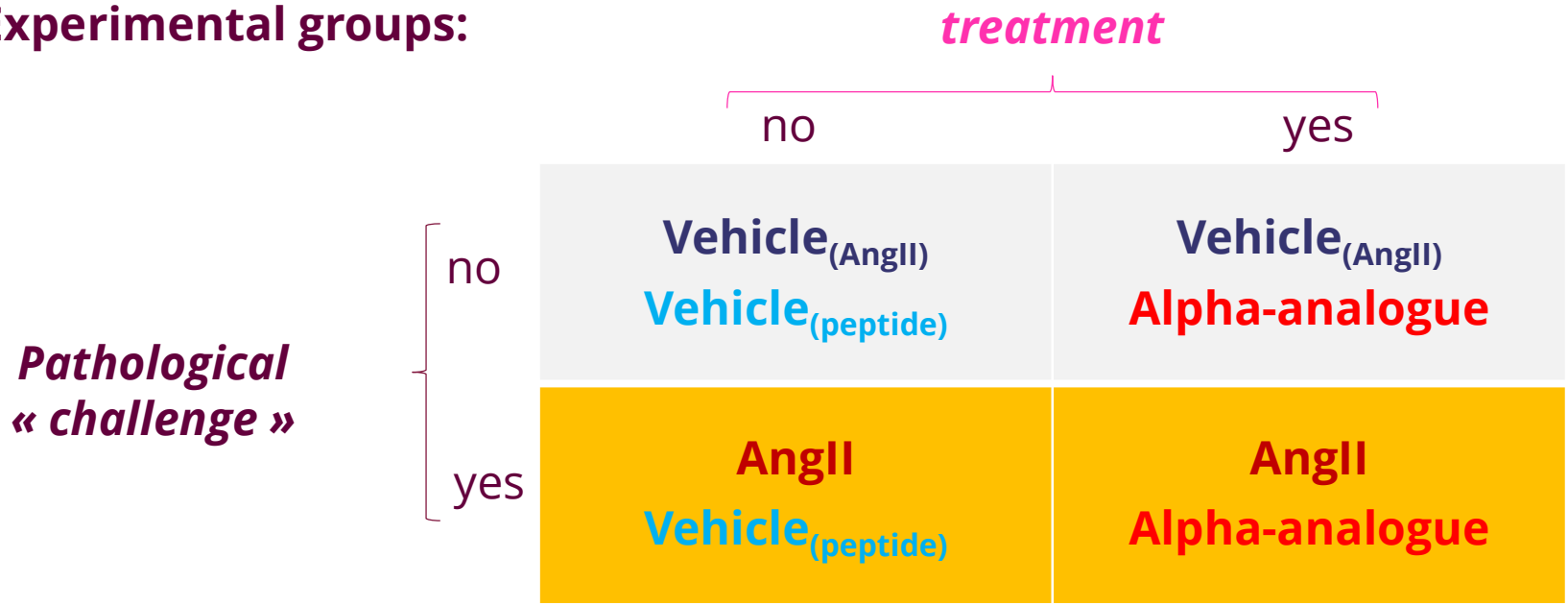
- AngII OR vehicle
- a vasodilatory peptide (alpha analogue) OR vehicle




*Aubdool et al., 2017 Circulation*

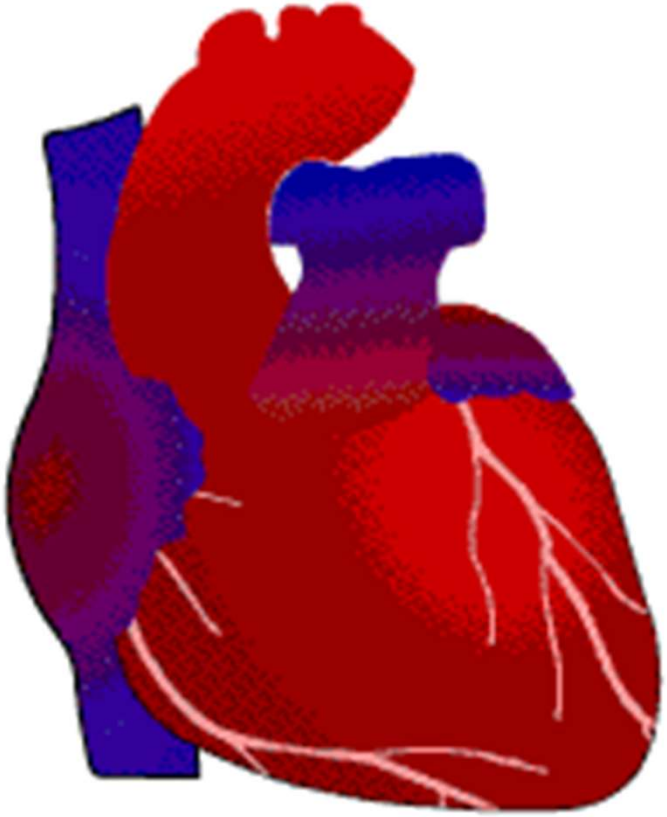
# NB! Experimental design

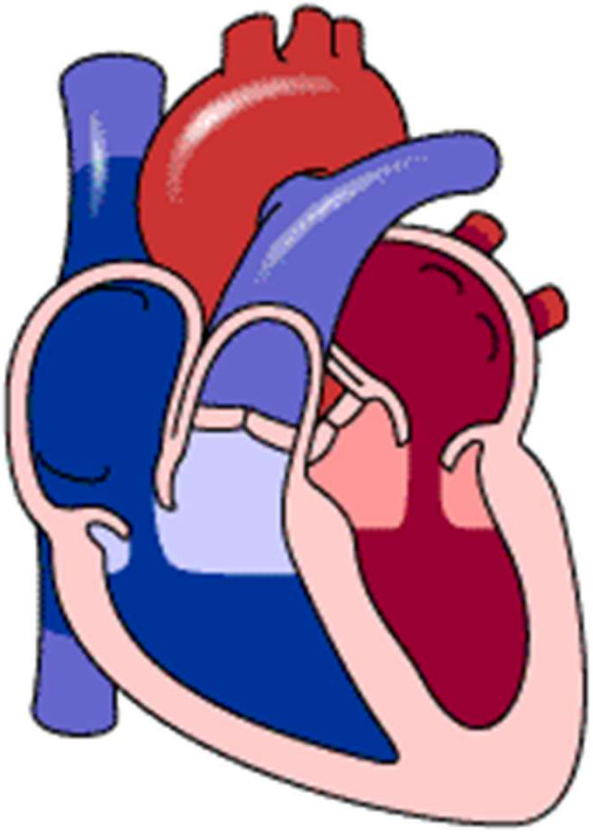
- **Experimental groups:**




- **Treatment : carefully chose:**
  - **dose (or concentration of compound)**
  - **administration route, frequency (pharmacokinetics)**
  - **vehicle**
  - **timing of treatment (when should it start/end)**
- **Plan to use a POSITIVE CONTROL with a <u>reference compound</u>**

# The heart

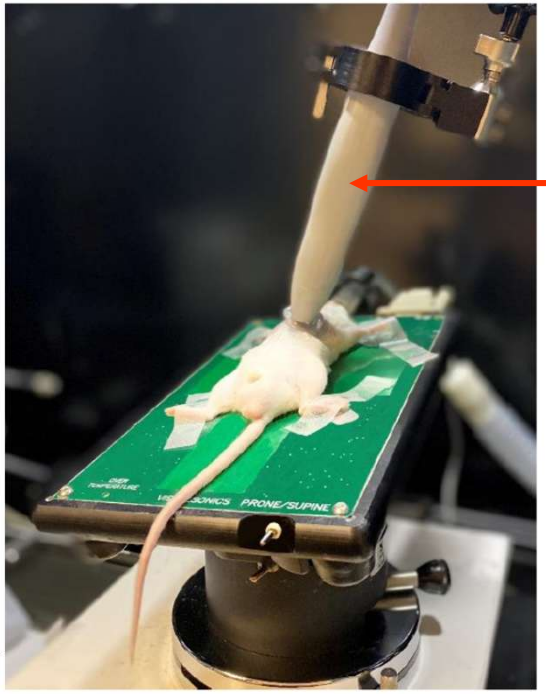




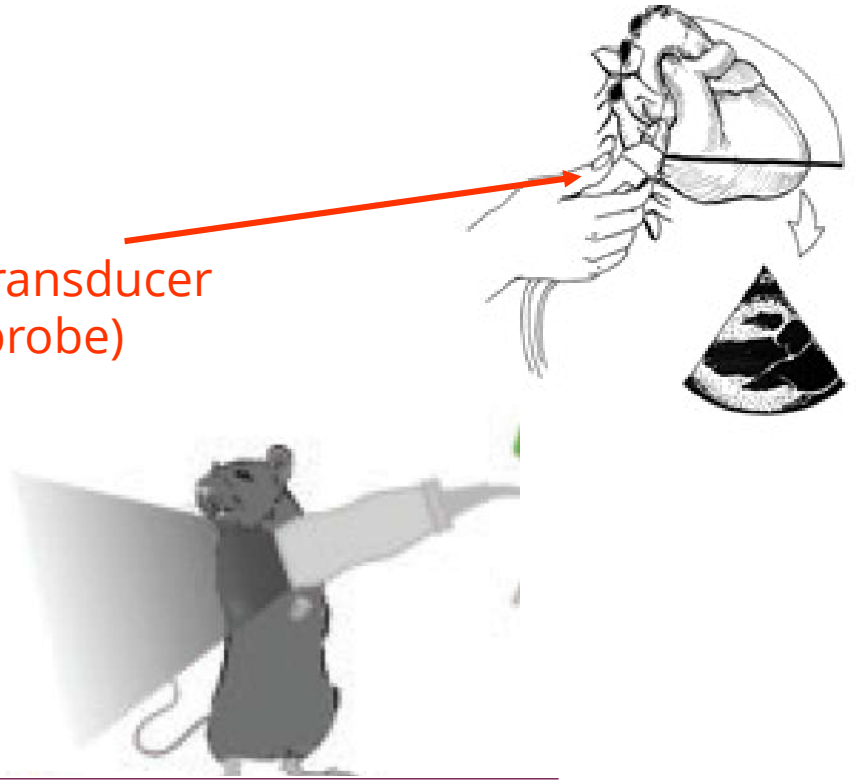
# Monitoring heart function and morphology with echocardiography (I)

- **Non invasive technique to explore cardiac morphology and function using ultra sounds.**
- A probe emits ultrasounds at high frequency (15-40 MHz) toward the organs
- The probe receives back the echoes, which are translated in electrical signal and amplified.
- Used for morphological exploration of the **heart chambers and large vessels**
- Conducted on **anaesthetized, unconscious animals** (typically using isoflurane, a **gaseous anaesthetics**)

- ***EXAMPLE : "parasternal, long axis, view"***




Transducer (probe)



*Fujufilm Visualsonics ; Zacchigna et al., 2020 Cardiovasc Res*

# Monitoring heart function and morphology with echocardiography (II

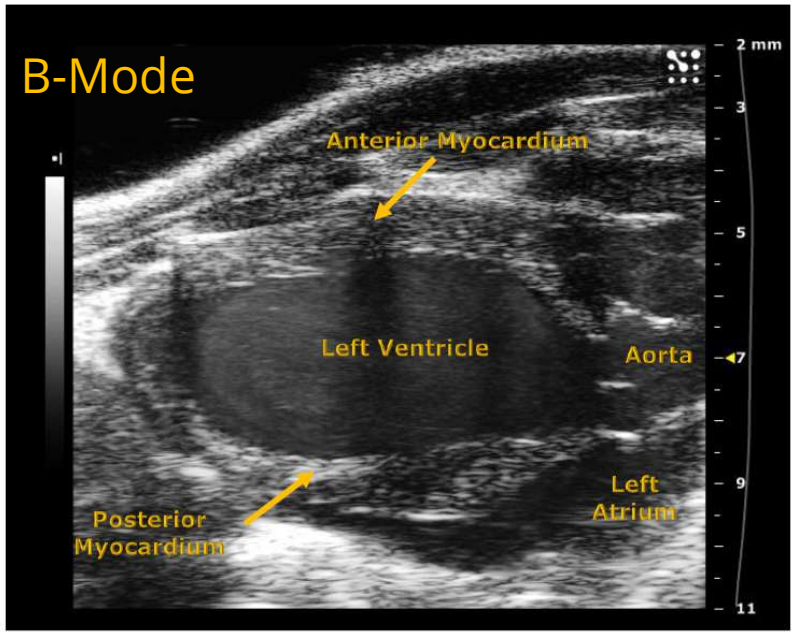



**Figure 3** - B-Mode image of the left ventricle in the parasternal long axis view.

B-Mode image of the left ventricle in the parasternal long axis view.

- ***EXAMPLE : parasternal, long axis, view***

LV: left ventricle

- **Morphology :**

Images => dimension measurements

=> calculation of:

  - LV end systolic volume (LVESV)
  - LV end diastolic volume (LVEDV)
  - LV mass

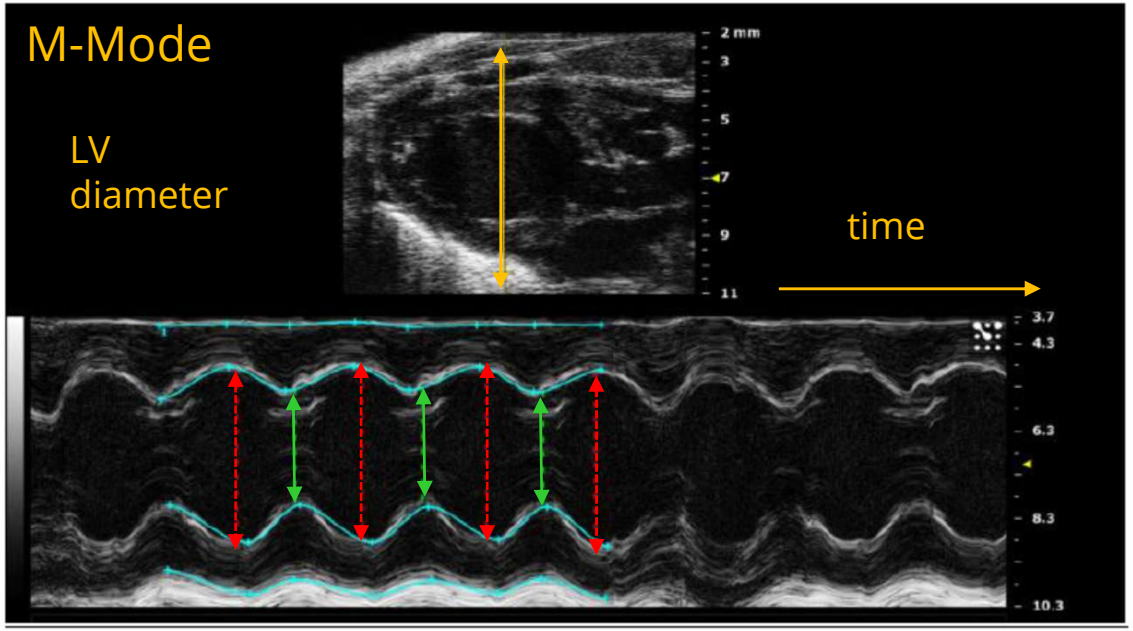
- **Function :**
  - **Stroke volume (SV) :** LVEDV - LVESV
  - **ejection fraction (EF)** :

    EF (%) = SV / LVEDV x100
  - **Cardiac output :** SV x HR

*Fujufilm Visualsonics ; Zacchigna et al., 2020 Cardiovasc Res*

# Monitoring heart function and morphology with echocardiography (

- ***EXAMPLE : parasternal, long axis, view***




M-mode image of parasternal long axis view displaying motion of the anterior and posterior walls.

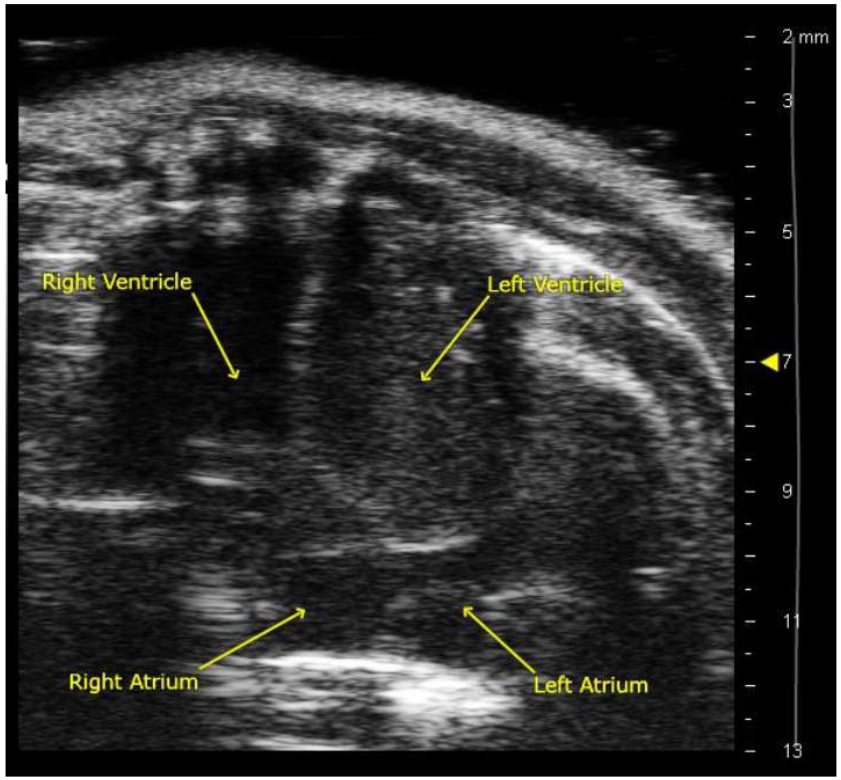
LV internal diameter in systole (LVIDd)

LV internal diameter in diastole (LVIDs)

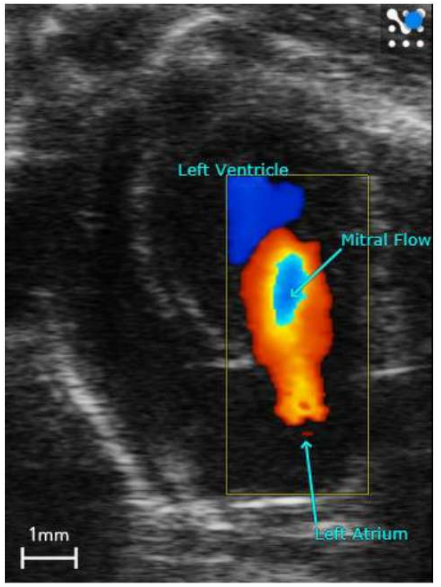
*Fujufilm Visualsonics ; Zacchigna et al., 2020 Cardiovasc Res*

# Monitoring heart function and morphology with echocardiography (IV)

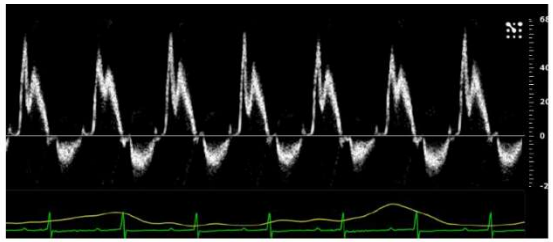
- ***EXAMPLE : apical, four chambers, view***




B-mode apical 4 chamber image displaying the left and partial right ventricles, left and right atrium.




Color **Doppler** mode image of mitral flow from the LA to LV through the mitral valve.



Pulse wave (PW) Doppler Mode waveform of mitral valve flow in the apical four chamber view. **=> indicator of the diastolic function**

Echo + Doppler mode : visualisation of **flow velocity** (mm/s)

https://www.jove.com/v/60404/echocardiographic-assessment-of-cardiac-anatomy-and-function-in-adult-rats

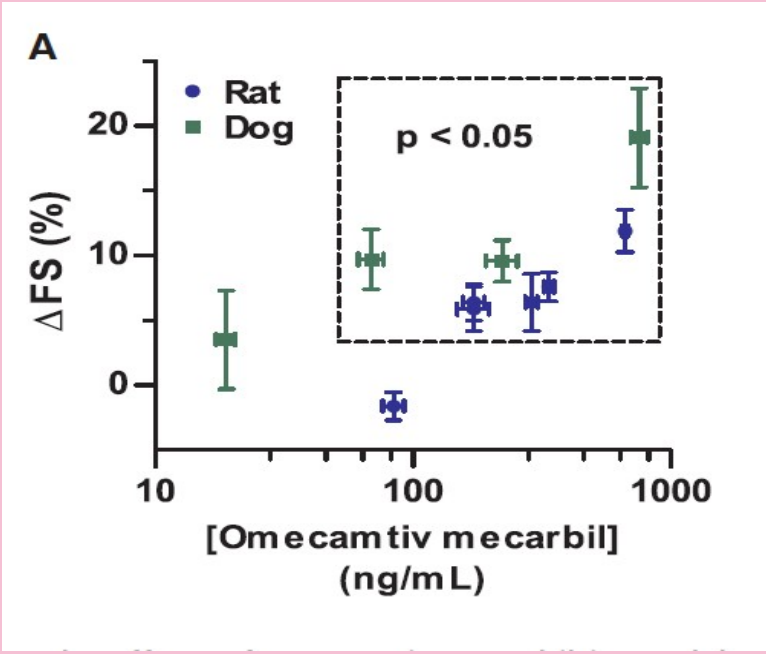
*Fujufilm Visualsonics ;Zacchigna et al., 2020 Cardiovasc Res*

# Example : characterization of inotropic drugs in vivo :

control **omecamtiv mecarbil (OM)**

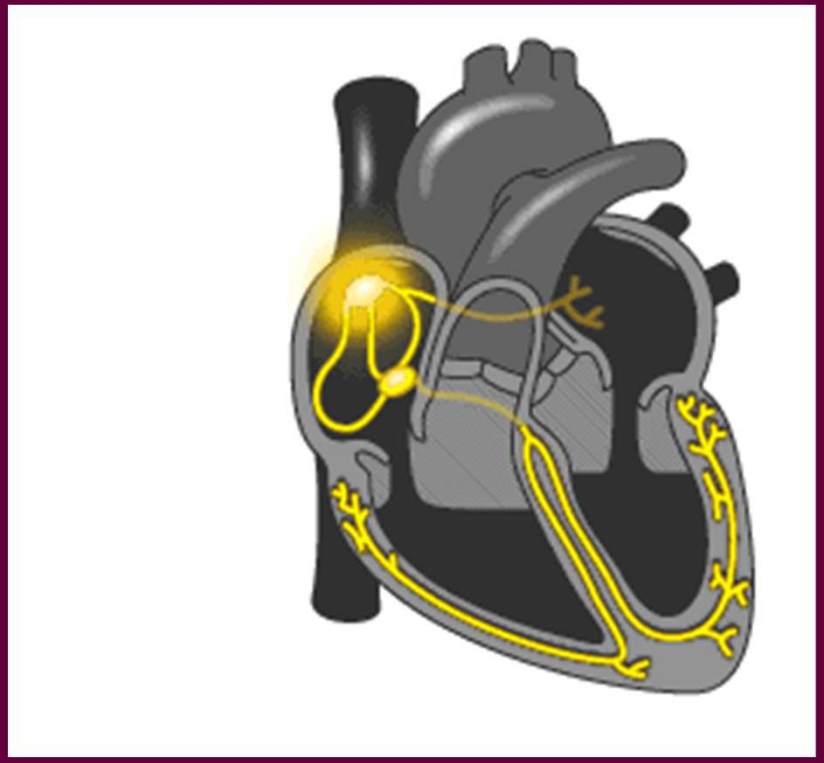
- Echocardiograms in parasternal, short axis mode
- Recording before or after admnistration i.v. of OM during 1 h in anaesthetized dog.

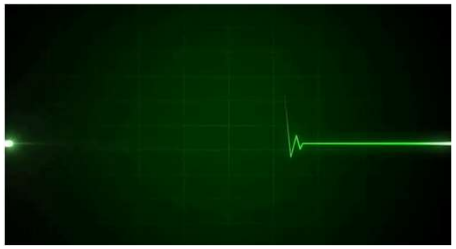
Increase in fractionnal shortening (FS) In function of OM plasmatic concentration in rat (blue) or dog (green).




*Malik et al., 2011 Science*

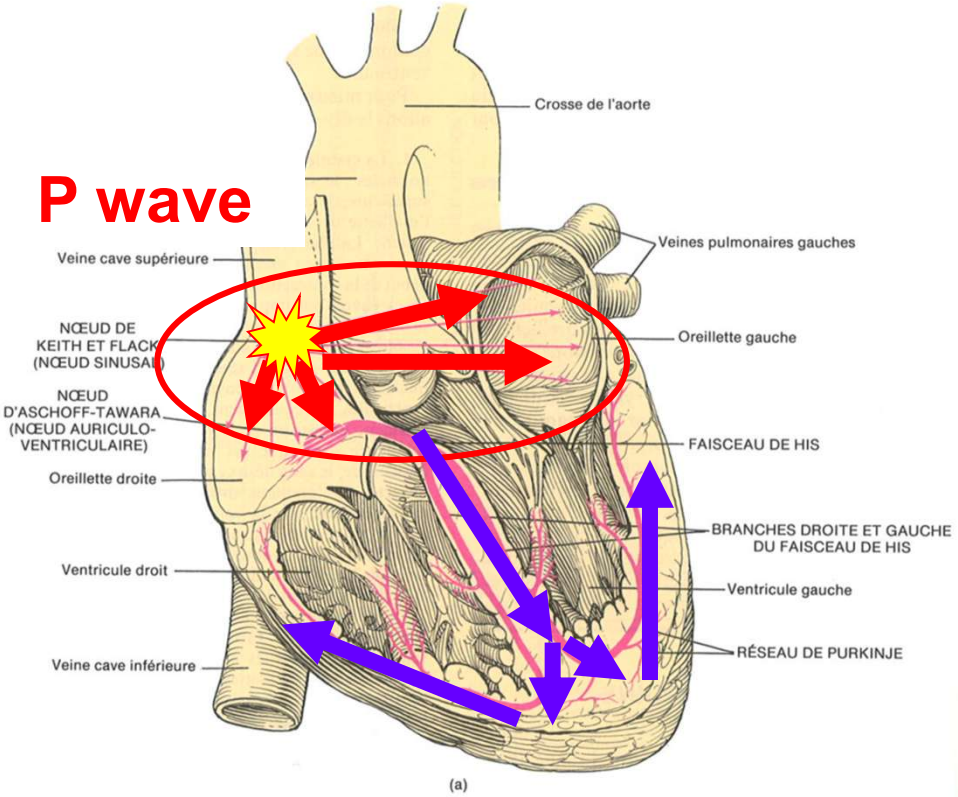
# Electrical activity



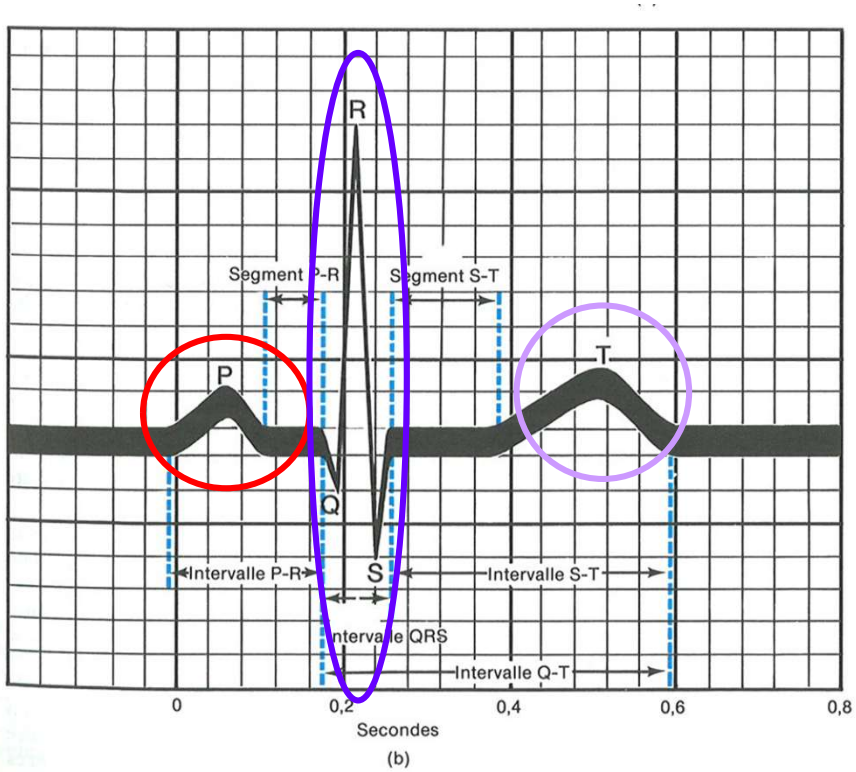


# Electrical activity in the heart : the ECG

Adapted from: Tortora & Anagnostakos, Principes d'Anatomie et de Physiologie




**P wave**




**QRS (ventricular depolarisation)**

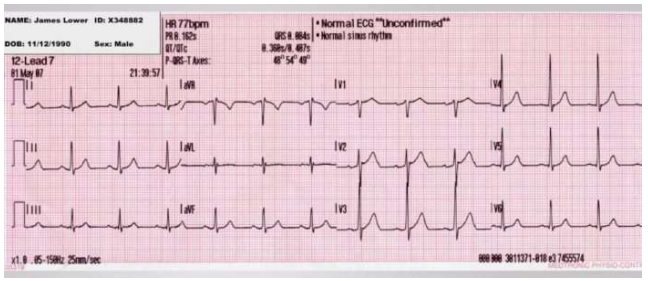
**Onde T (ventricular repolarisation)**

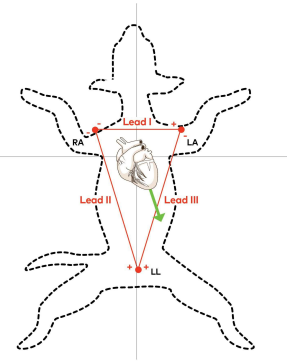
QT interval

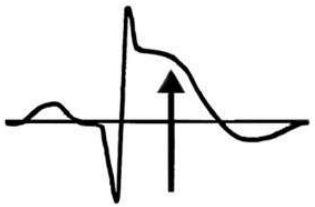
# ECG recording

**Measurement of electrical activity : yields data on**

- **Heart rate**
- **ECG anomalies, arrythmia (long QT)**
- **Ischemia (alterations in the ST segment )**





**Modalities :**

- **external electrodes (A)**
- **Implanted radiotelemetry transmitter (B)**

**(A)**

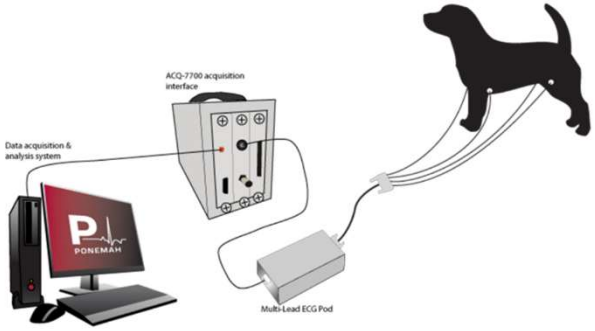



Figure 5: Hardwired ECG System Setup for Large Animals

**(B)**

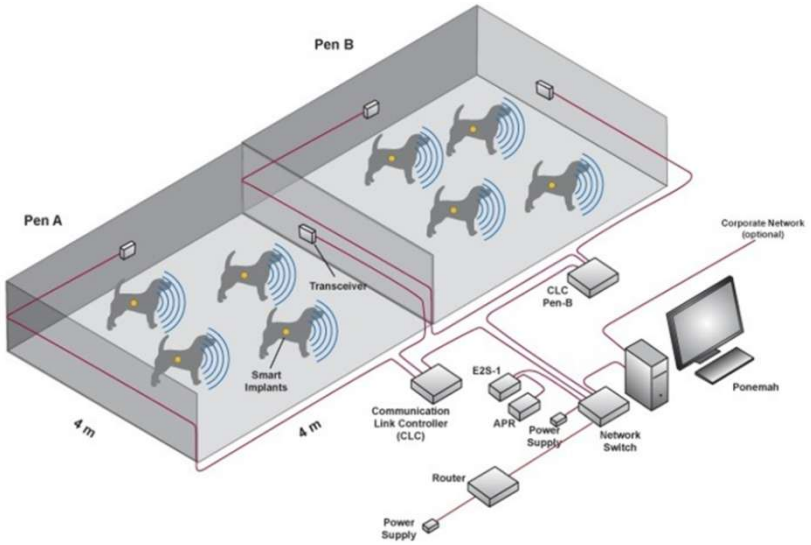


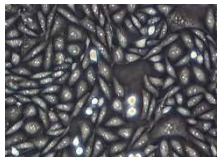

Figure 2: PhysioTel Digital Implantable Telemetry System Setup for Large Animals

*https://www.datasci.com/solutions/cardiovascular/*

*Cesarovic et al., 2011 JOVE*

https://www.jove.com/v/3260/implantation-radiotelemetry-transmitters-yielding-data-on-ecg-heart

# Non clinical Cardiovascular Pharmacology



Pharmacokinetics

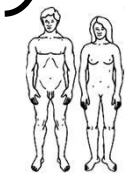
Chemistry

Toxicology

**Safety Pharmacology**

**Pharmacodynamics**

***non clinical evaluation***

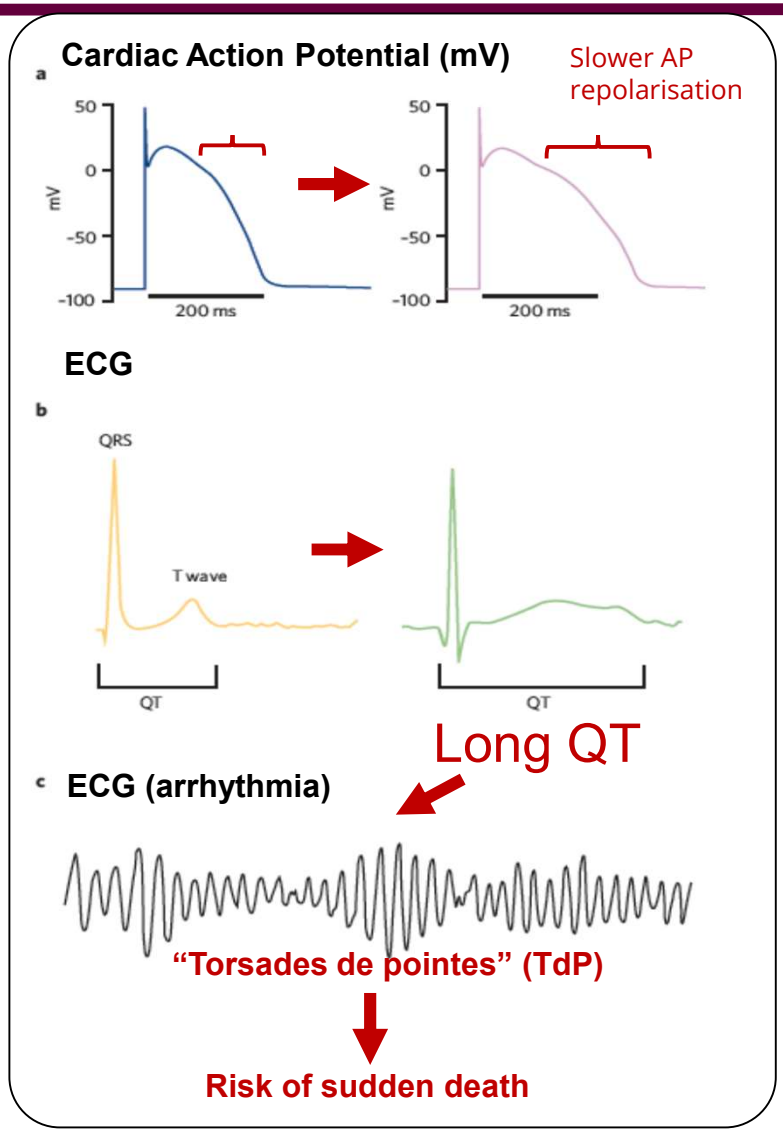


**1er trial in human (FIH ; Phase I)**

**•Safety Pharmacology :**

- Investigating possible unwanted pharmaco-dynamical effects due to acute exposition at therapeutic and supra-therapeutic doses of the compound.
- **before first studies in man : non-clinical assessment**
- **"core battery" include studies on CNS, respiratory function, cardiovascular function**
- **emphasis on arrhytmogenic potential of the compound**

# Long QT syndrome : cardiac repolarisation defect



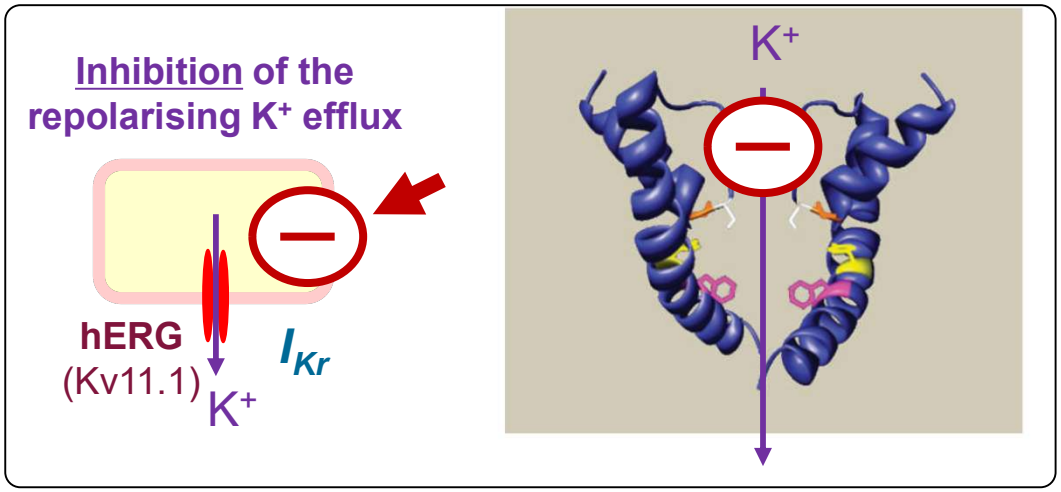

**Cause of LQTS?**

**Inherited ion channel dysfunction**

**or**

**Pharmacological inhibition of $K^+$ channels : drug-induced long QT syndrome** (iatrogenic) :

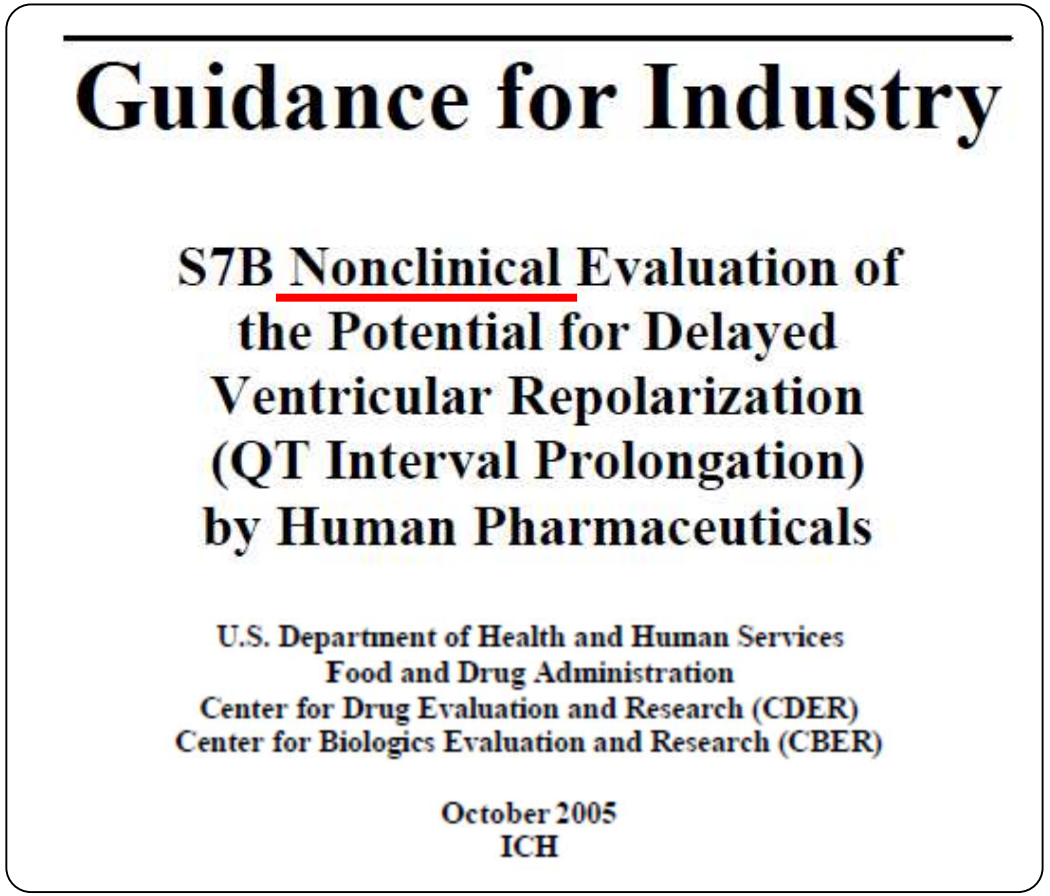
- anti-arythmic drugs (classe III)
- myriad of small molecules from various chemical families : histamine R.antagonists, neuroleptics, antibiotics…
- One of the culprit :
- the **hERG $K^+$ channel**
- **Inhibition** of this channel slows cardiac action potential repolarisation




*Sanguinetti et Tristani-Firouzi 2006 Nature*

# Cardiac repolarization studies in safety pharmacology

## S7B guideline from ICH (International Conference for harmonization)



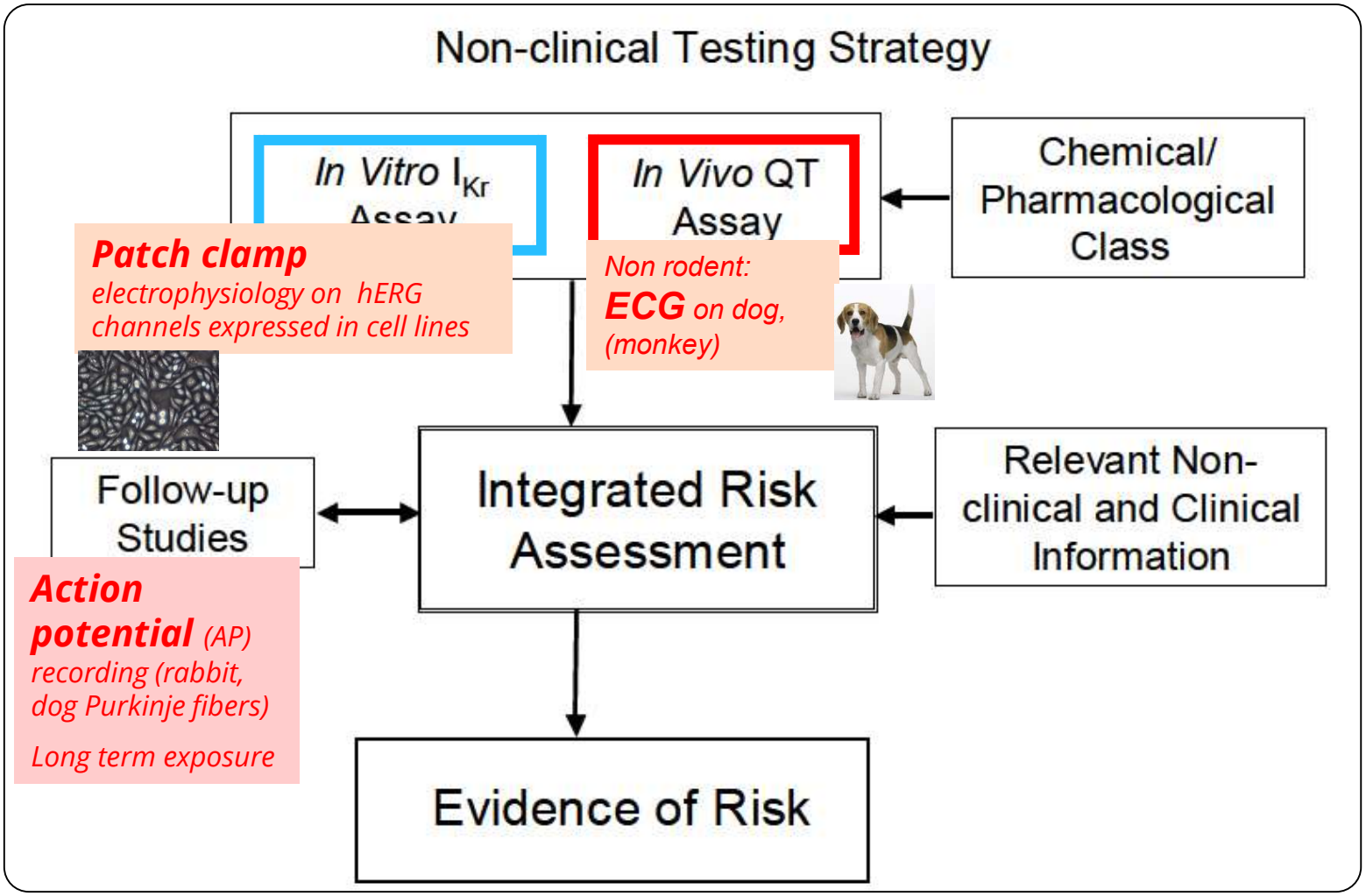
Guidance for Industry

S7B Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

October 2005
ICH

# S7B guideline from ICH (extracts)



017Circulation

I. Which experimental approaches?

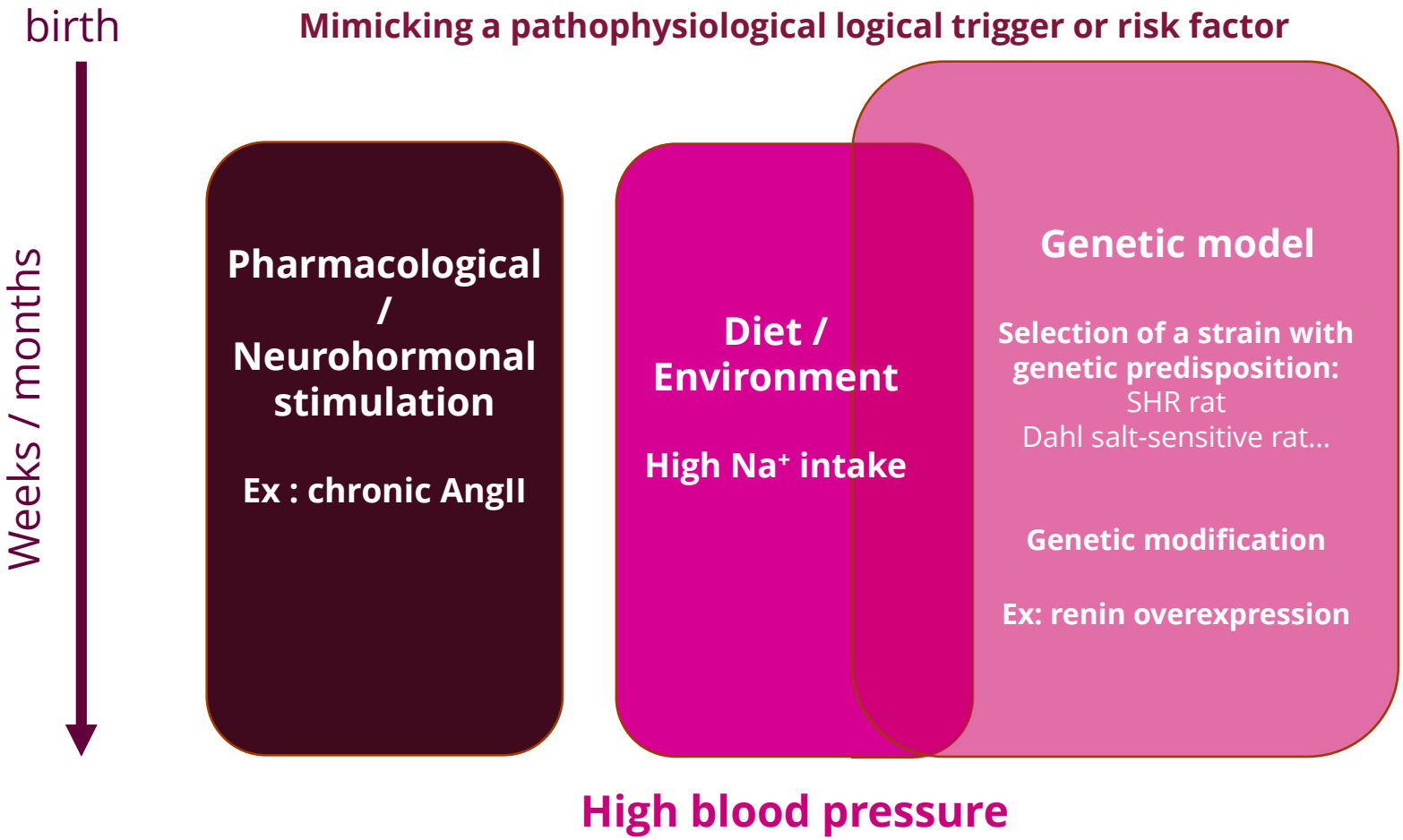
II. Methods for in vivo exploration of cardiovascular models

**III. Models of arterial hypertension**

# Arterial Hypertension

- Definition :
  - Systolic BP > 140mmHg
  - And/or diastolic BP > 90 mmHg.
- Prevalence :
  - 30-45% in adults, >60% if > 60 year-old
  - Male : 24% ; Female : 20% (age-standardized)
  - Prevalence in high around the world
- Aetiology :
  - Often « essential », or « primary » (no identified cause)
  - Risk factor : sedentarity, body weight
- Major risk for cardiovascular and renal events
  - Responsible for $10^7$ deaths in 2015
  - Risk factor for ischemic heart disease, haemorrhagic stroke, ischaemic stroke
  - Continuous relationship between BP and CV risk

# models of arterial hypertension




*Aubdool et al., 2017 Circulation*
*Dornas and Silva 2011 J. Biosci.,*
*Cowley et al., 2017 Hypertension*

# Genetic hypertension models

## Spontaneous hypertensive rat (SHR)

- **Origin:** Okamoto, at the Kyoto School of Medicine in 1963, started with an outbred Wistar Kyoto male with marked elevation of blood pressure and mated him to a female with slightly elevated blood pressure.
- **Characteristics of the strain:**
  - **Hypertension developing spontaneously at 4-6 weeks of age**
  - **SBP > 165 mmHg at 10 week-old**
  - Early stage : high CO, normal PVR
  - Progression : normal CO, high PVR (arterial wall thickening), cardiac hypertrophy
  - Metabolic disturbance : Insulin Resistance, dyslipidaemia…
- **Control strain : Wistar Kyoto (WKY)**
- **Most commonly used genetically hypertensive rat model.**

## Stroke prone SHR (SHRSP)

- **Origin:** bred from SHR, with even higher BP
- **Characteristics of the strain:**
  - **strong occurrence of stroke (80% in males, 60% in females)**
  - **extensive arteriosclerosis**
- **Control strain : Wistar Kyoto (WKY)**

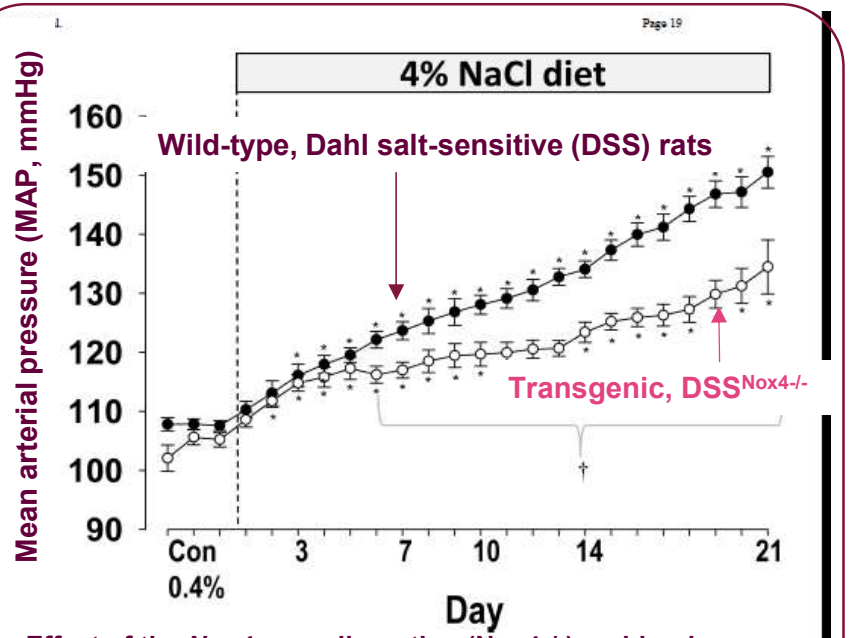
**CO**: cardiac output ; **PVR**: peripheral vascular resistance

*Dornas and Silva 2011 J. Biosci., Cowley et al., 2017 Hypertension*

# Genetic + environment (diet)

## Dahl salt-sensitive rat model

- **Origin:** derived from Sprague-Dawley stock (Dahl et al., 1962)
- **Characteristics of the strain:**
  - **A genetic model of hypertension with the feature of salt sensitivity.**
  - **Dahl Salt-sensitive (DSS) : develop rapidly high BP under a high-salt diet (e.g. switch from 0,4% to 4%)**
  - suppressed plasma renin activity (due to high Na reabsorption), low aldosterone
  - **Diastolic heart failure, nephropathy**
- **Control strain : normal rat, with high salt diet**
- **Mimics salt-sensitive HT in patients**




**Effect of the *Nox4* gene disruption (Nox4-/-) on blood pressure under a high-salt diet in DSS rats.**
Mean arterial pressure (MAP) measured on 0.4% NaCl diet for 3 days and for 21 days after the diet was switched to **4.0% NaCl diet** in SS (closed circles; n=8) and $DSS^{Nox4-/-}$ (open circles; n=9).

† : P<0.05, SS vs $SS^{Nox4-/-}$
* : P<0,05 vs 0,4% NaCl

CO : cardiac output ; PVR : peripheral vascular resistance
*Dornas and Silva 2011 J. Biosci., Cowley et al., 2017 Hypertension*

# Transgenic hypertension model

## Transgenic hypertension model

- **Transgenic rats : overexpression of a gene involved in BP regulation**

- **Ex: TGR(mREN2)27 transgenic rat**

*(Millins et al., 1990 ; Ganten et al., 1992 PNAS) :*

  - Overexpression of the mouse Ren-2 (**renin gene**)
  - Heterozygotes : $^{TGR/+}$ : SBP up to 240 mmHg at 10 weeks of age
  - Homozygotes : $^{TGR/TGR}$ : SBP up to 300 mmHg, high mortality rate

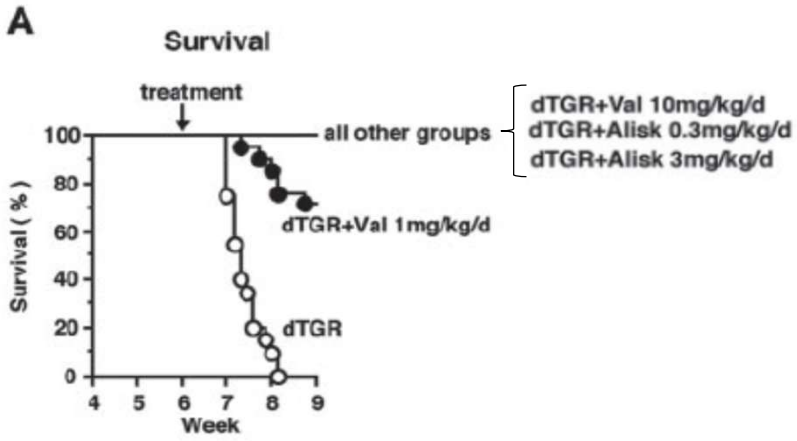
- **Double transgenic mice or rat**

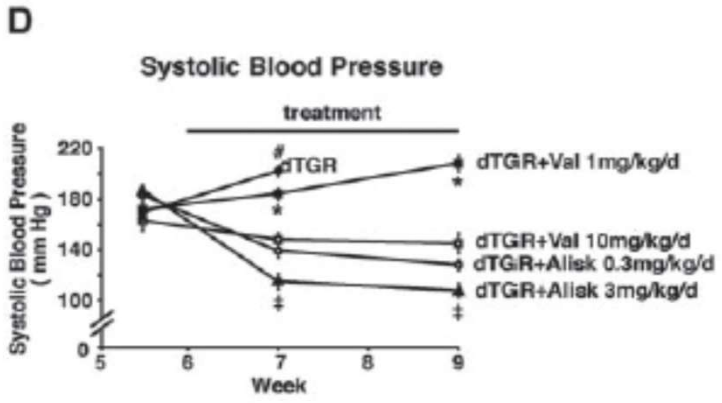
(*Fukamizu et al., 1993 J Biol Chem ; Pilz et al., 2005*)

  - Double transgene :
    - human renin
    - human angiotensinogen
  - used to test antihypertensive effect of the human renin inhibitor, aliskiren

**Val** : valsartan, AT1 R. antagonist 1 or 10 mg/kg/d

**Alisk** : aliskiren, 0.3 or 3 mg/kg/d



# Conclusion : what do I need to know?

- How can arterial BP be studied in vivo (2 methods)?
- How can an effect on cardiac repolarisation be studied?
- What is the advantage of studying conscious animals?
- Describe 2 models used to mimic hypertension in rats?
- List and explain 3-4 important parameters that could be examined to explore the effect of a drug on the cardiovascular system.