TU11 Pharmacology/Toxicology

GRADUATE SCHOOL
Health and
Drug Sciences

Animal models & pharmacology

Revision

Assistant Profs

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Why using an animal model?

To study :

- Basal biological / behavioral data
- A spontaneous or induced pathological process, with common features with a human pathology.
- Physiological mechanisms
- Physio-pathologic process
- To find new treatment diseases
- Treatments tolerance/efficiency

Experimental animals

Rodents	Non Rodents	Miscellaneous
Mouse/Rat Guinea Pig Gerbil Hamster	**Rabbit** Monkey Dog, Cat Pig	Amphibian Birds Zebra fish Reptile
hypertension, diabetes, cataracts, obesity, seizures, respiratory problems, deafness, Parkinson's disease, Alzheimer's disease, various cancers, cystic fibrosis, and acquired immunodeficiency syndrome (AIDS), heart disease, muscular dystrophy, and spinal cord injuries. behavioral, sensory, aging, nutrition, drug efficacy, and toxicity, teratogenicity and genetic studies …	human pregnancy, skin and eye irritation studies, toxicity and safety testing of substances. **production of polyclonal antibodies for use in immunology research** atherosclerosis, osteoporosis, ocular, cardiovascular study, development of vaccine, pharmacologic studies for teratogenicity testing of novel pharmaceutic compounds, Dental, and periodontal disease and surgery, orthopedic surgery and skeletal physiology, and radiation oncology…	Developmental biology biochemistry and molecular biology, cell biology, neurological sciences, and genetics embryonic development, metamorphosis, regeneration, physiology aging, memory, parasitology, atherosclerosis, reproduction, and infectious disease…

Animal model quality check

- The experiments have to demonstrate the model relevance which includes its analogy with the human pathology
- Necessity of validation according to well defined criteria
- The model has to be adapted across time

Method to study human diseases in animals

Which general methodology will you apply to study the therapeutic potential of the newly synthetized drug XXXTRA ?

Method of study of human diseases in animals

Which general methodology will you follow to study the therapeutic potential of the newly synthetized drug XXXTRA ?

- *Choose the species*
- *Model of the disease (TG, Pharmacological, Spontaneous)*
- *Validation of the model (symptoms, mechanism, …)*
- *Choose the test/parameter*
- *Choose the reference (positive/negative control)*
- *Route of administration*
  - *Doses*
  - *Acute vs. chronic*
- *Toxicology testing*

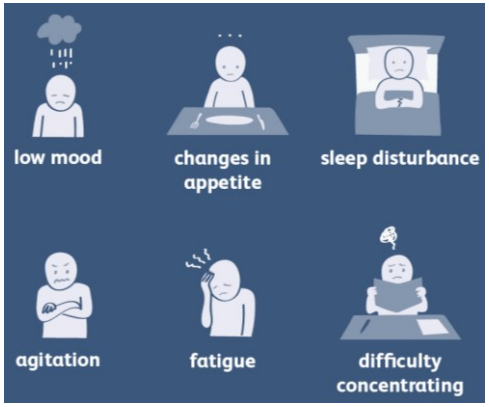
Models of anxiety & depression in animals

Depression

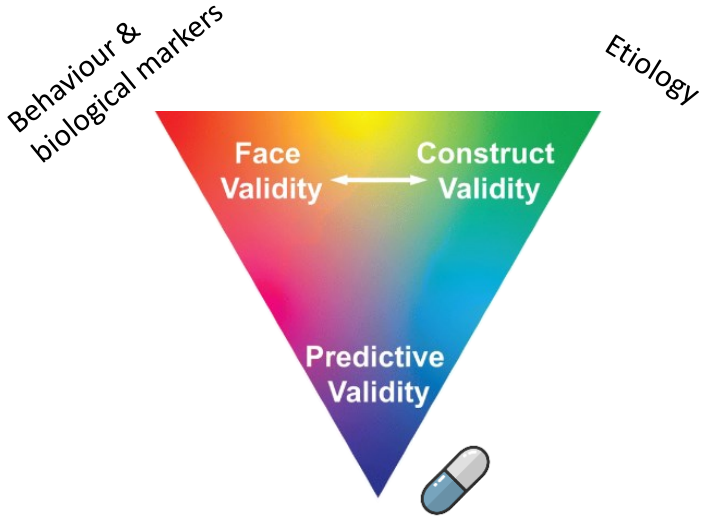
Global burden of disease

Treatment resistance

Core symptoms



- Candidate neural circuits
- Neurophysiological systems
- Molecular targets



Using DSM criteria to construct a mouse model of mental illness

"To illustrate the challenges involved in using DSM criteria to construct animal models, consider two individuals with the diagnosis of major depression. ***Patient one*** *might have depressed mood, weight loss, insomnia ,psychomotor agitation, and suicidal thoughts, while* ***patient two*** *might have markedly diminished pleasure, weight gain, hypersomnia, psychomotor retardation, and fatigue."* No symptoms in common!

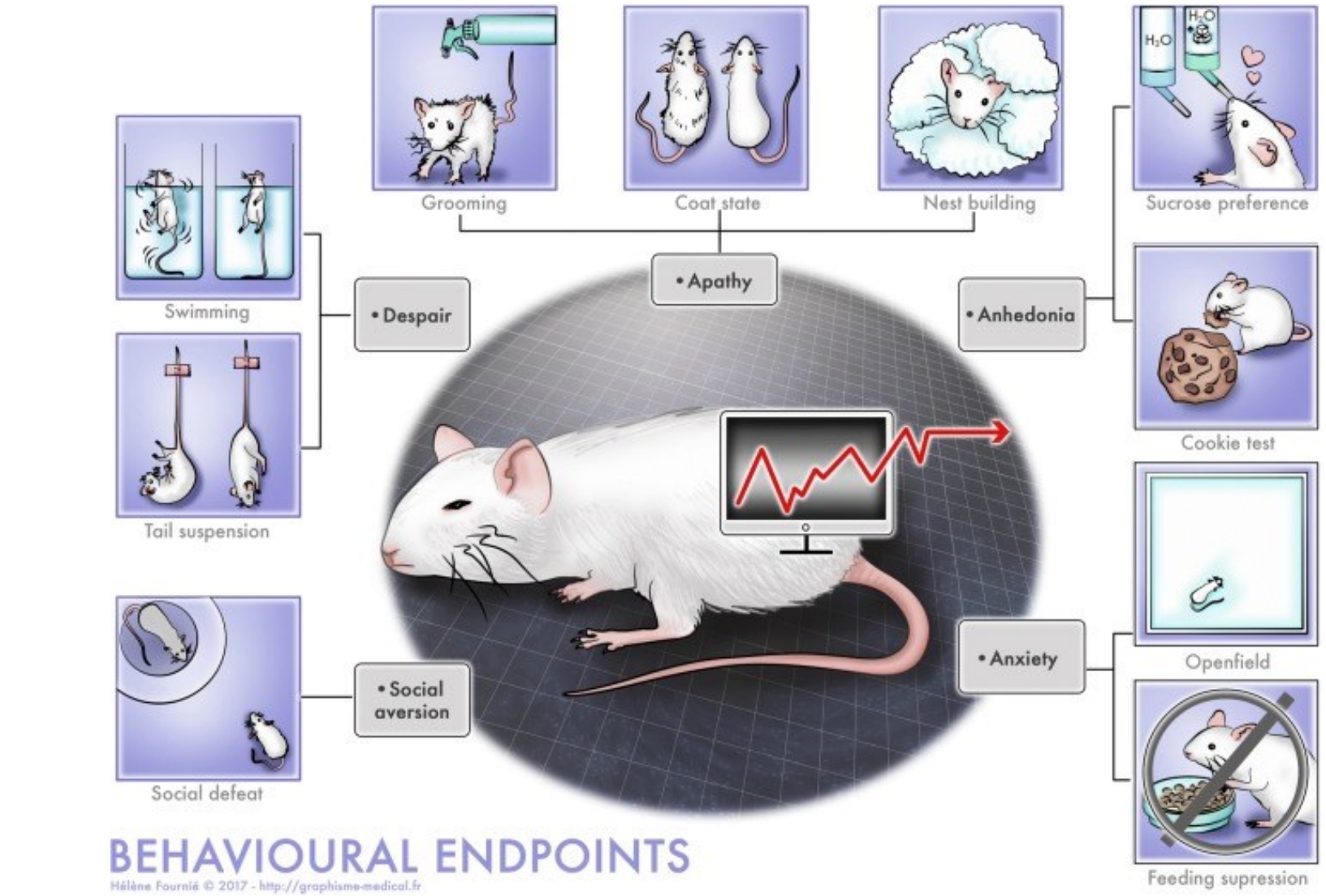
DSM V Criteria for Major Depressive Episode

A. At least 5 of the following are present simultaneously for at least 2 weeks (symptom 1 or 2 is necessary):

1. Depressed or irritable mood
2. Markedly diminished interest or pleasure in all, or almost all, daily activities
3. Significant weight loss or weight gain
4. Insomnia or hypersomnia nearly every day
5. Psychomotor agitation or retardation nearly every day
6. Fatigue or loss of energy nearly every day
7. Feelings of worthlessness or inappropriate guilt nearly every day
8. Diminished ability to think or concentrate nearly every day

Animal models of neuropsychiatric disorders. Nestler EJ1, Hyman SE. Nat Neurosci. 2010 Oct;13(10):1161-9.

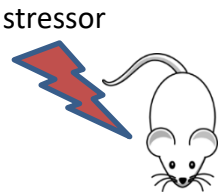
Behavioral endpoints



Planchez et al., 2019

Animal models of depression

1) Models based on application of stressors

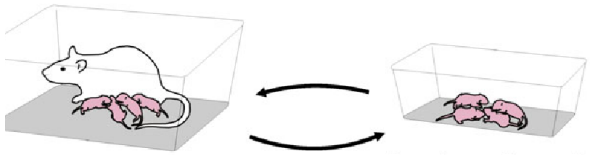


- In early life
- In adulthood

2) Biological causation models

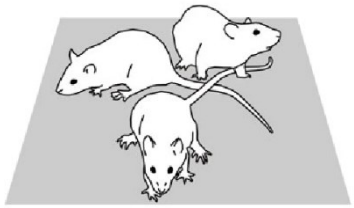
Planchez et al., 2019

Early life adversity – maternal separation

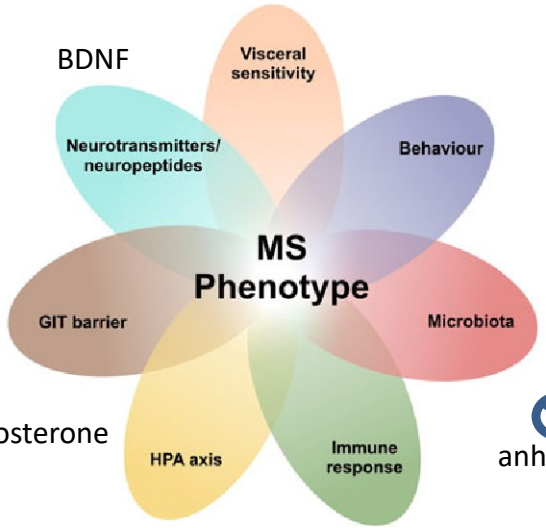


home cage

Separate pups
3h/day PND 2-12



Adult maternally separated rodents



corticosterone

anhedonia

Similar findings in rodents that undergo spontaneous deficits in maternal care

Support the findings from human research **<u>maternal neglect</u>** or a **<u>history of childhood abuse increases MDD</u>**

Use of newborn pups whose development stage corresponds more to a pre-natal human stage

(O'Mahony et al., 2010)

Stress applied during adulthood

- **Social defeat**

10 min/day
10 days

→ Social withdrawal & anhedonia

- Dysregulation of the prefrontal cortex (PFC)
- ↑ amygdala activity
- ↑ pro-inflammatory cytokines
- ↑ corticosterone
- Changes in neurotrophins

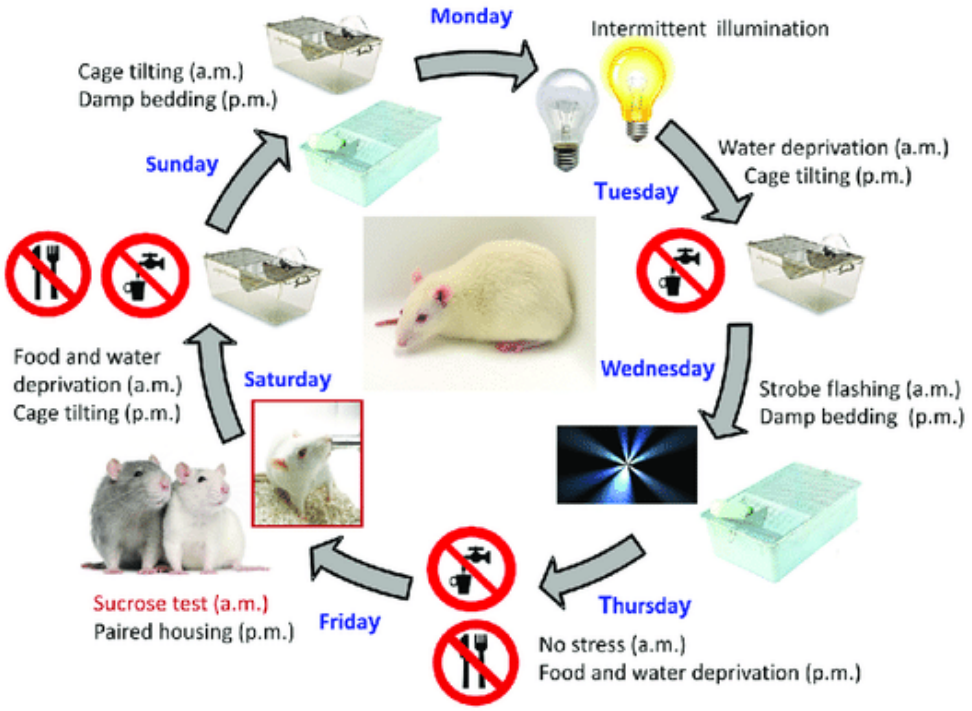
Resilient *vs* susceptible rodents

This model is sensitive to chronic SSRIs

Difficult to carry out in ♀ due to lower aggressiveness

Stress applied during adulthood

- **Unpredictable chronic mild stress (UCMS)**



- Deterioration of coat state
- ↓ grooming
- Anhedonia
- ↓ hippocampal neurogenesis
- ↓ 5-HT neurotransmission
- ↓ neurotrophins

Responsive to chronic antidepressants

Strain differences in susceptibility

Biological causation models

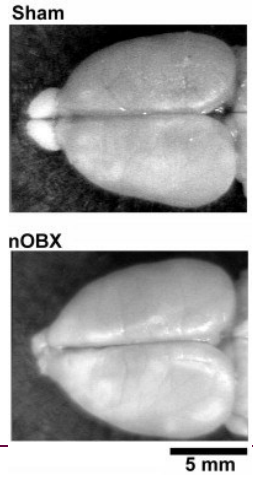
- **Lipopolysaccharide injection**

Bacterial endotoxin 0.5-0.83 mg/kg ➡ inflammation-related model of MDD

- ↑ brain cytokines (IL-1β, TNF-α)
- ↑ anhedonia & despair behaviour
- Reversed by antidepressants
- ↑ corticosterone
- ↓ neurotrophins

Other models

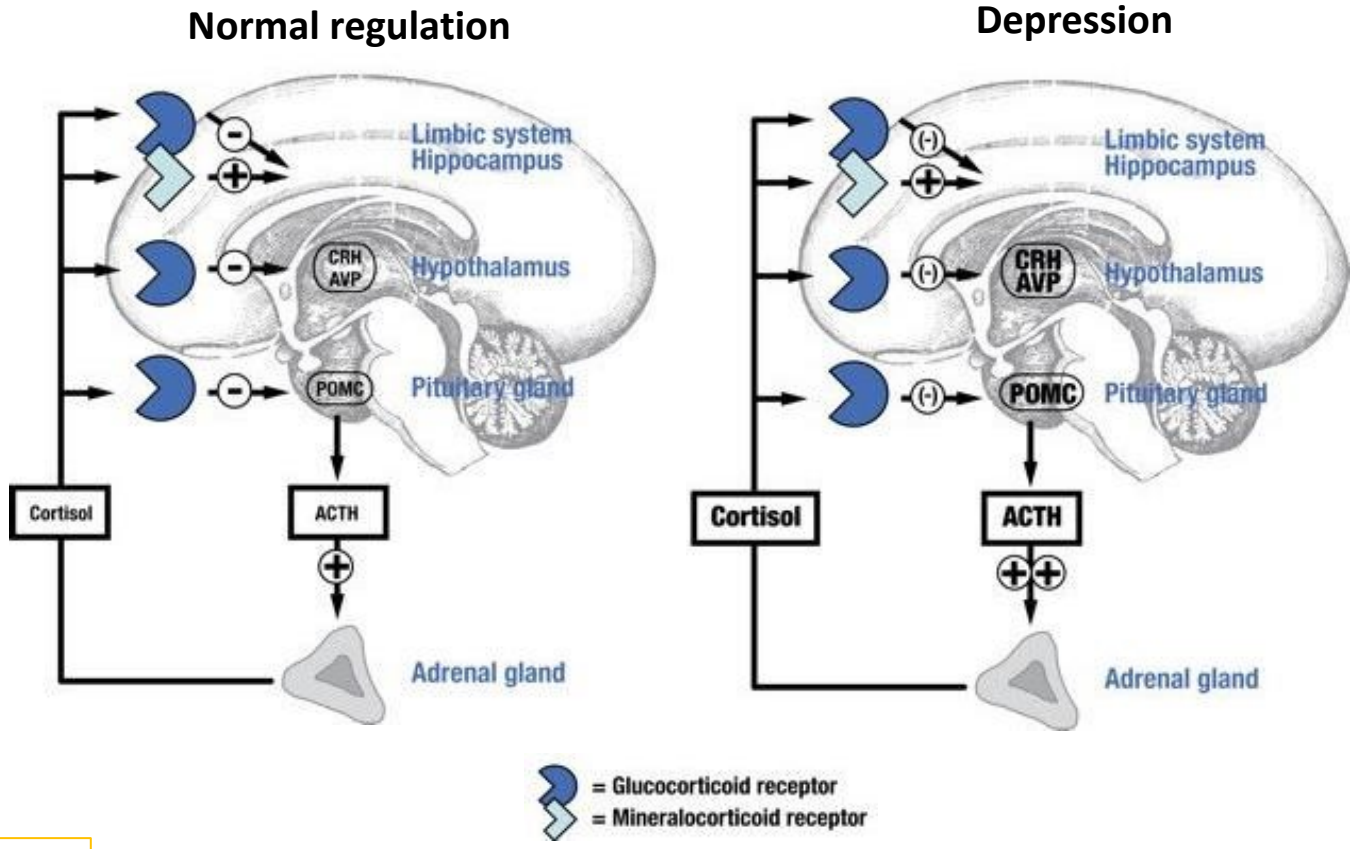
Olfactory bulbectomy



Poor face validity but strong predictive validity

GR (Glucocorticoid Receptor) knock-out mice: Role of HPA axis

‘cortisol hypothesis’
↑ CRH
↓ negative feedback



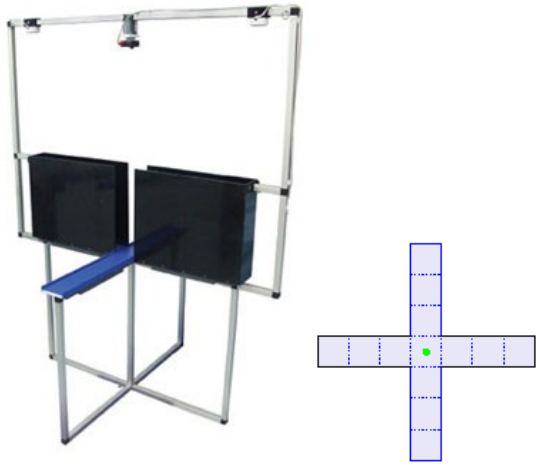
Forebrain-specific
GR knockout mice

Depressive-like behaviour

CRH = corticotropin releasing hormone
AVP = arginine vasopressin
POMC = proopiomelanocortin
ACTH = adrenocorticotrophic hormone

EXERCISE 1 : Test of GABA-R subunits function

A



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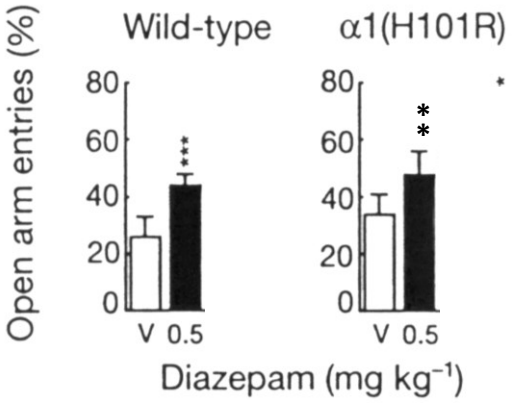

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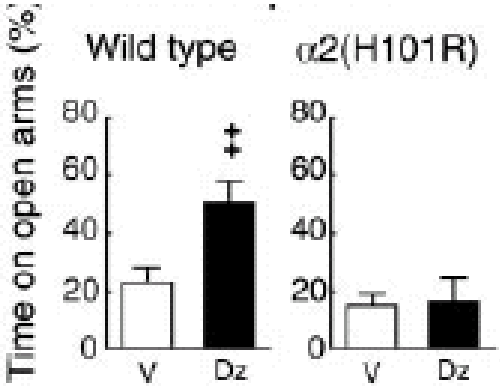
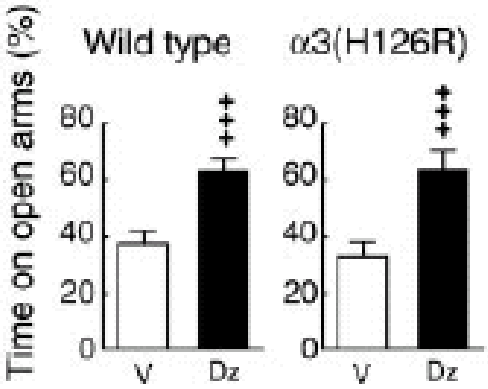


D



1- What type of behavioral test was used in this experiment?

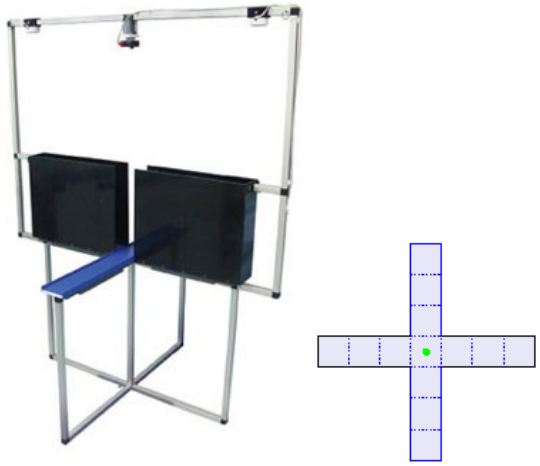
2- What does it allow to quantify? What can you conclude from the results?

3- Propose another test that could be used to measure the same parameter.

From Rudolph et al., 1999

EXERCISE 1

A



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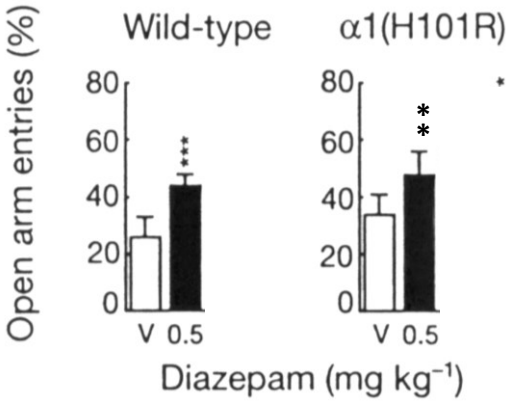

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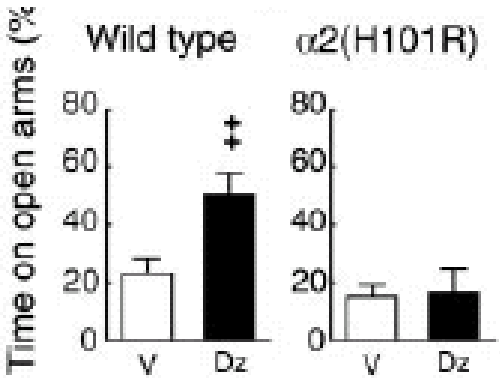
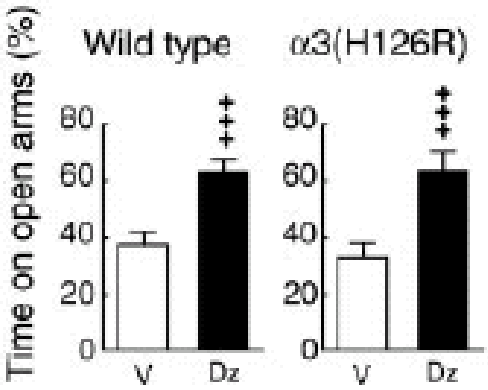


D



1- What type of behavioral test was used in this experiment? Elevated Plus Maze

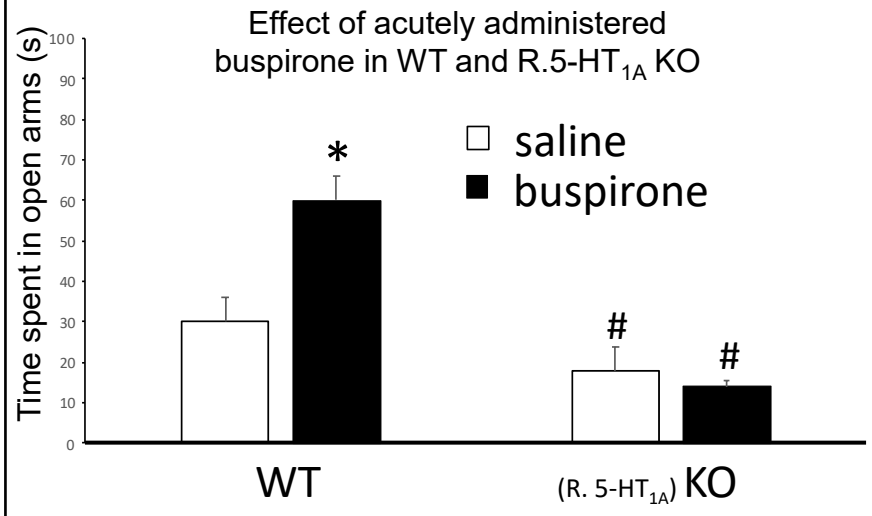
2- What does it allow to quantify? What can you conclude from the results? Behavioural assessment of anxiolytic-like actions of diazepam regulated by alpha 2 subunit.

3- Propose another test that could be used to measure the same parameter. Open field test.

From Rudolph et al., 1999

EXERCISE 2

The effects of buspirone were tested in an elevated cross maze model after acute administration (5 mg/kg), in control mice (WT) and knockout mice (KO) for the gene encoding receptor 5 -HT1A (R. 5-HT1A) KO. The results are shown in the figure below.



***** : statistically significant difference between the group treated with buspirone and the "saline" group.

: statistically significant difference between the KO group and the WT group within the same treatment.

1) Explain the principle of this behavioral test.

2) Describe the results obtained, the effects observed and propose a conclusion.

3) What additional parameter is necessary to validate this conclusion?

EXERCISE 2

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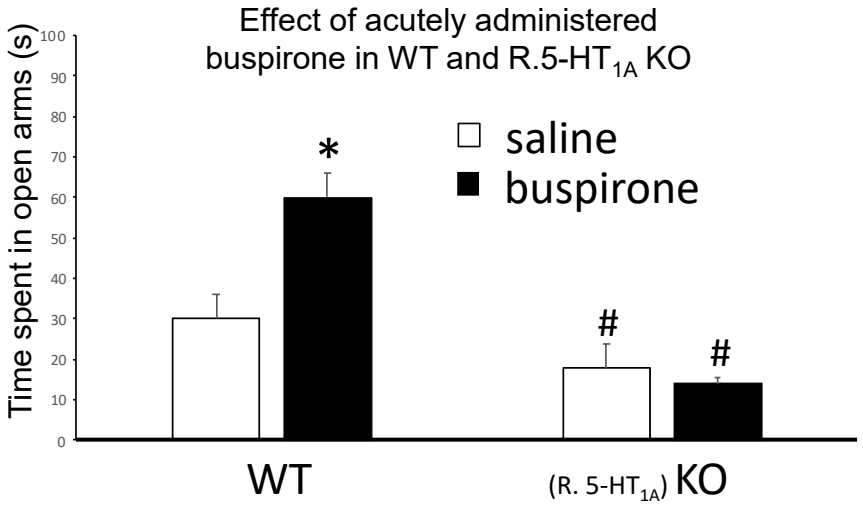


***** : statistically significant difference between the group treated with buspirone and the "saline" group.

: statistically significant difference between the KO group and the WT group within the same treatment.

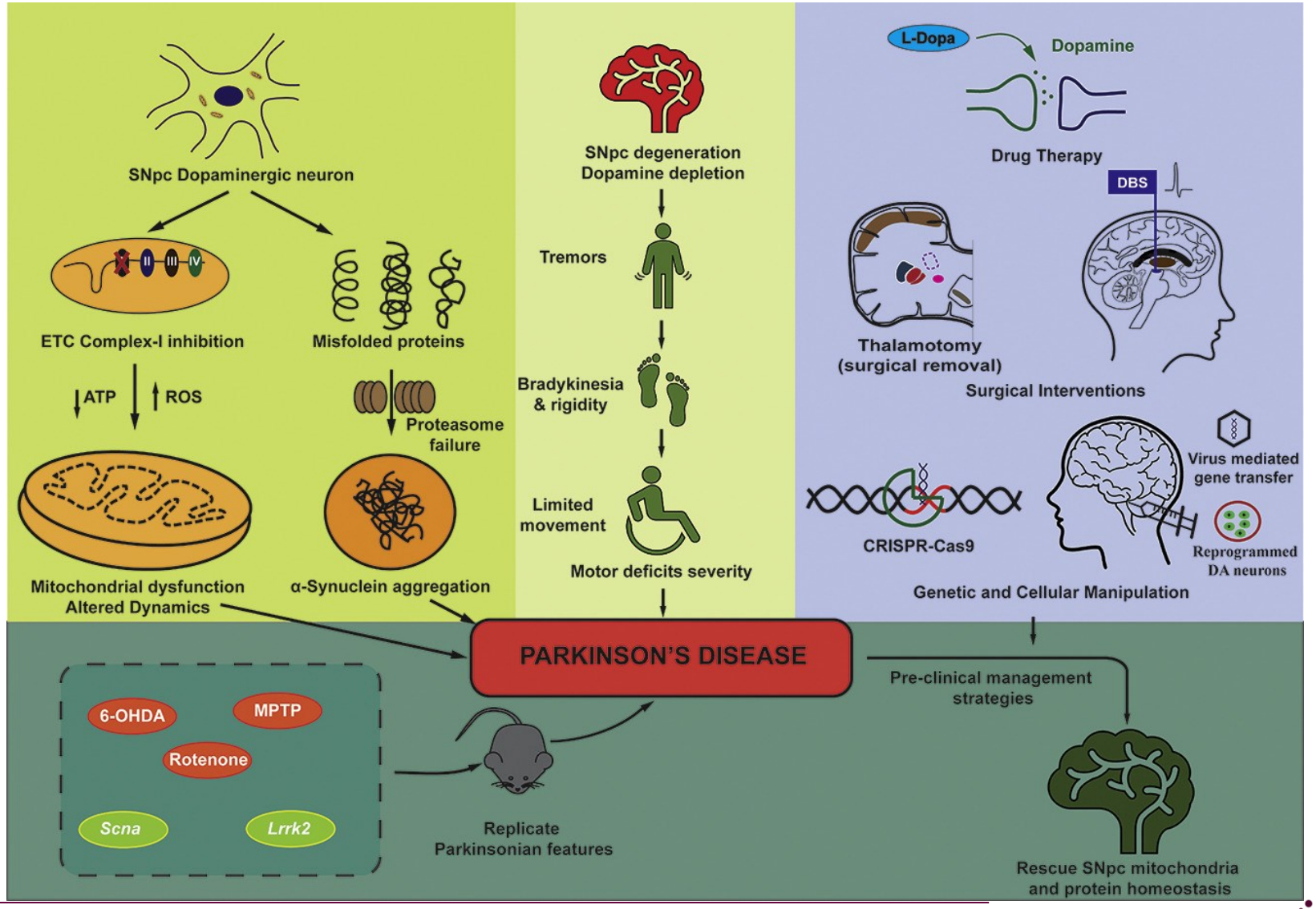
1) Explain the principle of this behavioral test. This is the raised cross labyrinth. It has two open arms and two closed arms in which the mouse can move freely. We measure the time spent in each type of arm. An increase in the time spent in open arms reflects an anxiolytic activity of a molecule.

2) Describe the results obtained, the effects observed and propose a conclusion. Buspirone therefore has an anxiolytic effect in WT; this effect is lost in KO mice for 5-HT1A. R. 5-HT1A is therefore involved in the mechanism of the anxiolytic effects of buspirone in this model. We can then hypothesize that buspirone is a ligand of this R. The absence of R. 5HT1A is anxiogenic: therefore the activity of R. is anxiolytic; buspirone is believed to be an R. 5HT1 agonist.

3) What additional parameter is necessary to validate this conclusion? Measure the number of entries that assess the locomotor activity of animals; variations in this parameter could also influence the time spent in open arms.

Models of Parkinson disease in animals

Overview of Parkinson Disease



Animal models in Parkinson disease

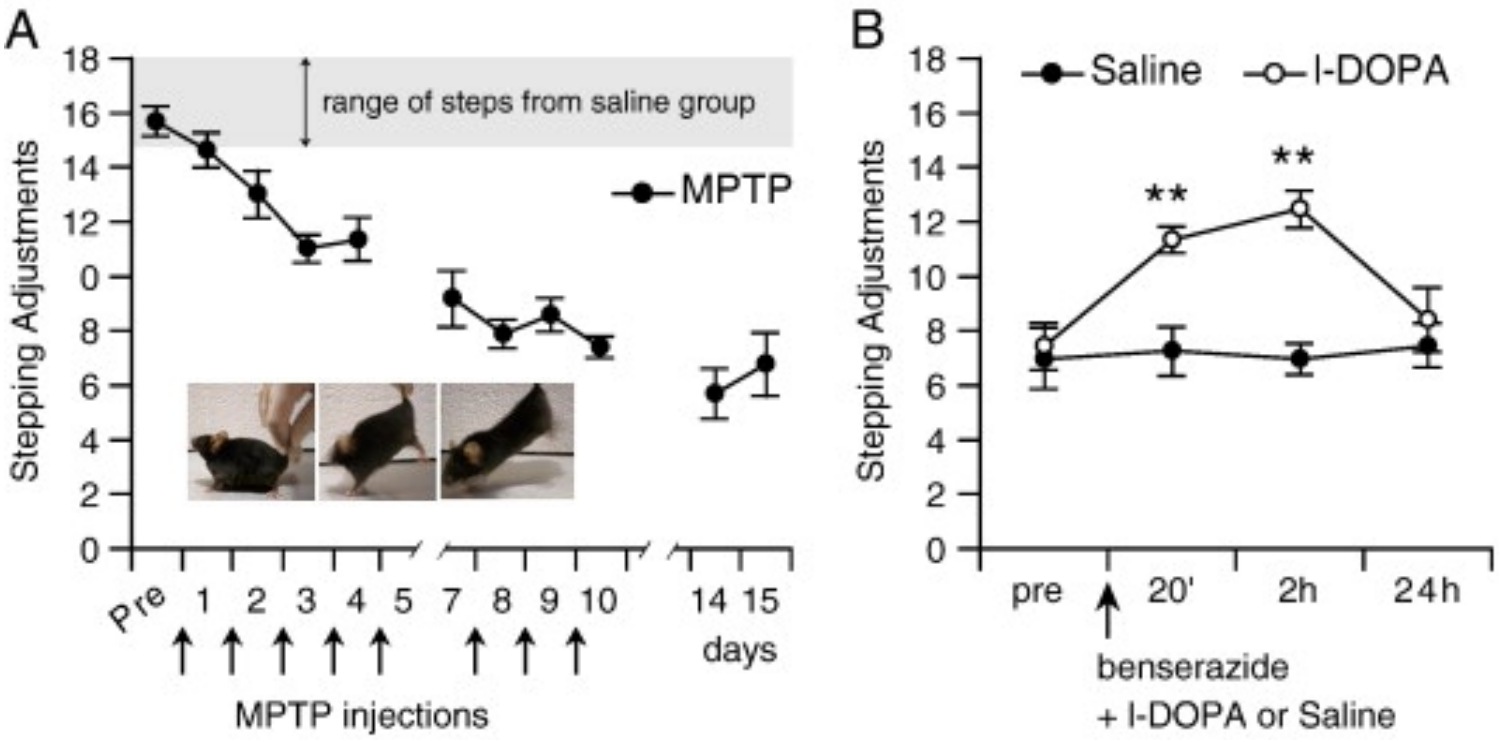
Toxin models		Genetic Models		
6-OHDA	MPTP	PARKIN-KO	PINK1-KO	TG – AAV Alpha-synuclein (mutated or human form)

Variation between models regarding:
DAnergic damage
Mitochondrial respiratory deficit
Oxydative stress
Alpha-synuclein and lewy bodies aggregates
Locomotor and non-motor deficit

No perfect model

Selection dependent on the question/hypothesis

EXERCISE 3 : MPTP and stepping deficit

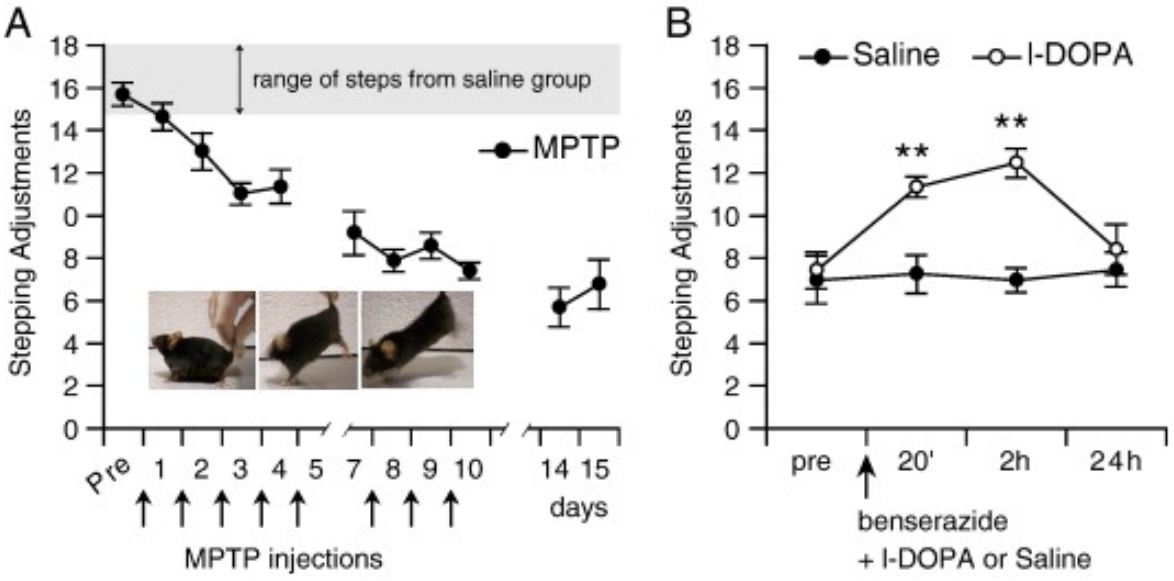


1) Described the animal model used.

2) What type of behavioral test was used in this experiment? What does it allow to quantify?

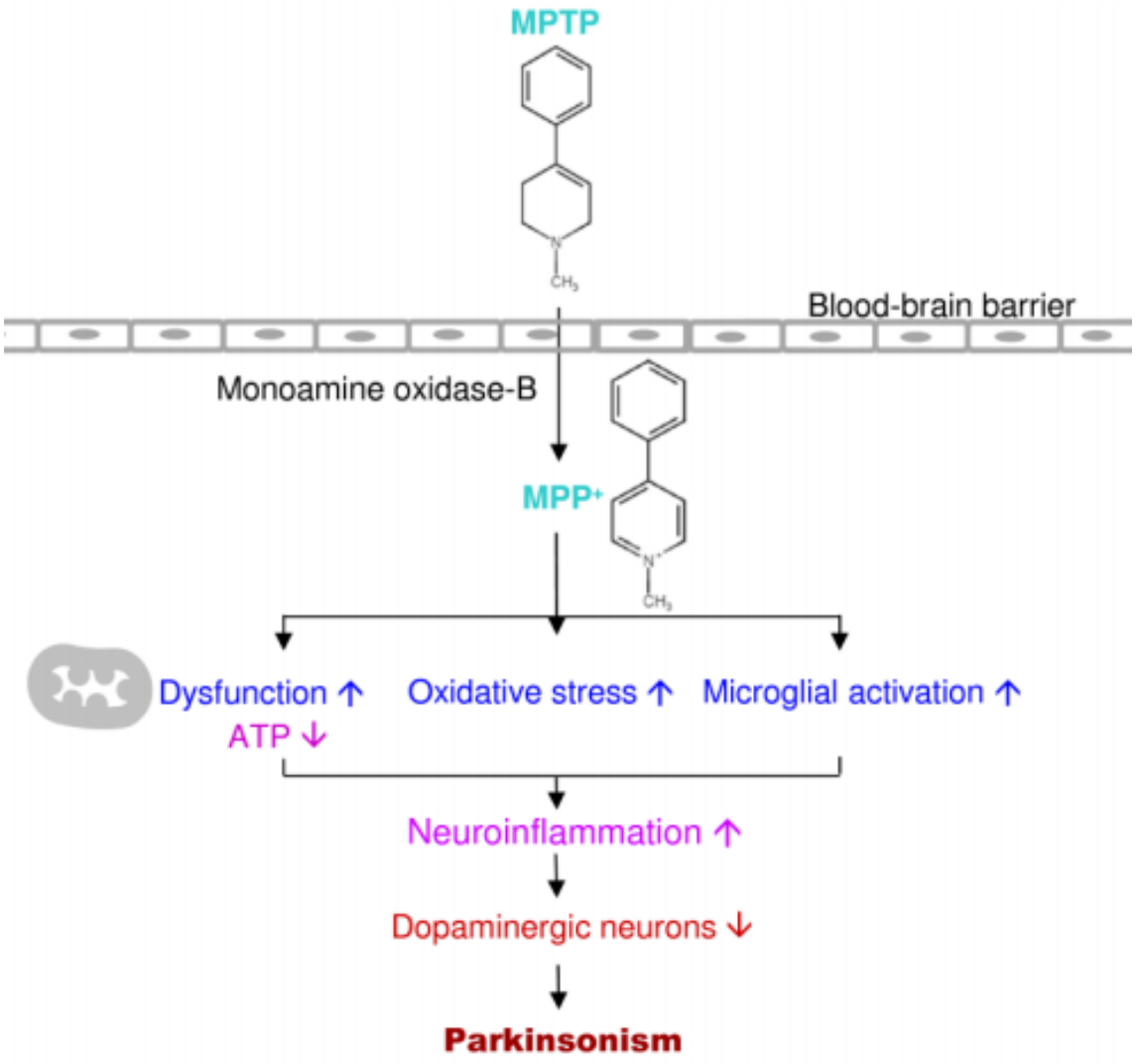
3) From those results can we conclude that the stepping test is a relevant behavioral model of Parkinson disease akinesia in mice? Explain

EXERCISE 3

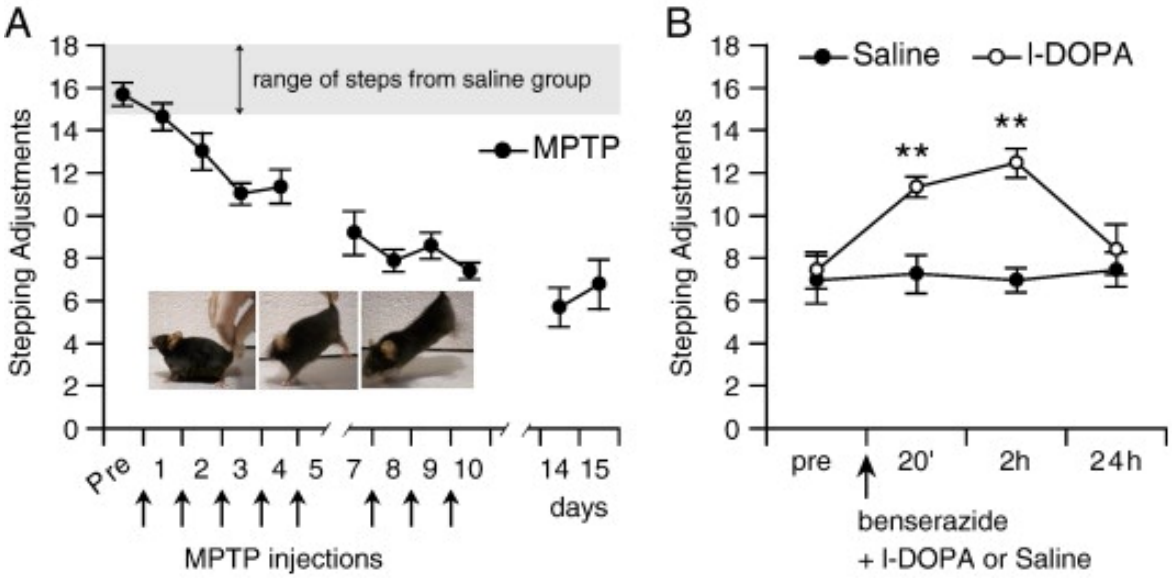


1) **Described the animal model used.** MPTP is a neurotoxin-induced model of Parkinson disease inducing akinesia and rigidity

MPTP mechanism of action



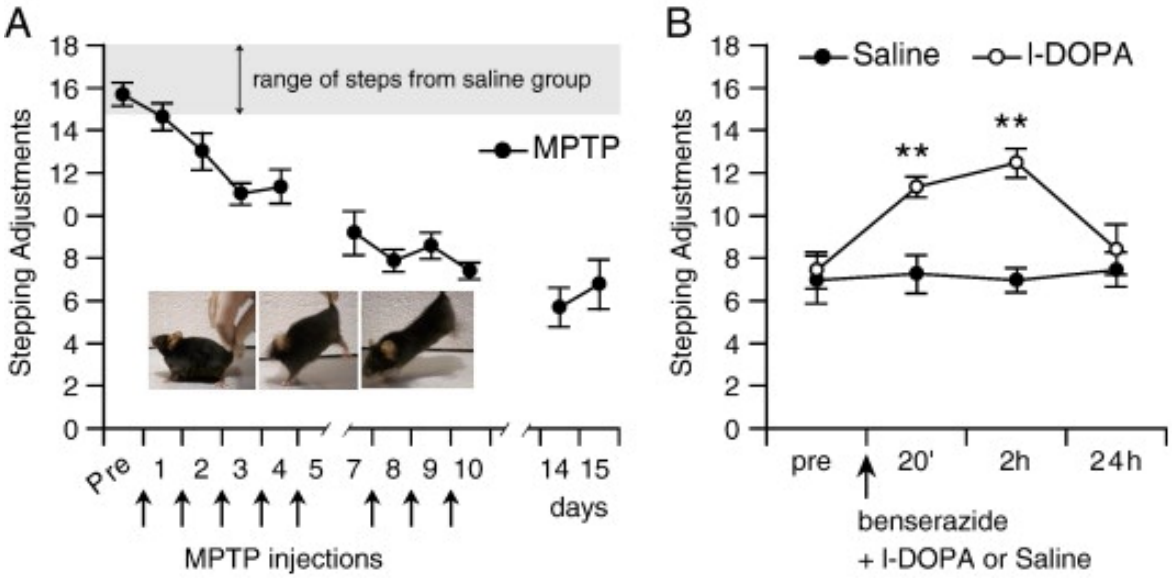
EXERCISE 3



1) **Described the animal model used.** MPTP is neurotoxin-induced model of Parkinson disease inducing akinesia and rigidity

2) **What type of behavioral test was used in this experiment? What does it allow to quantify?**

EXERCISE 3



1) **Described the animal model used.** MPTP is neurotoxin-induced model of Parkinson disease inducing akinesia and rigidity

2) **What type of behavioral test was used in this experiment? What does it allow to quantify?** Stepping test is a behavioral test to study limb akinesia and gait problems seen in patients with Parkinson disease.

3) **From those results can we conclude that the stepping test is a relevant behavioral model of Parkinson disease akinesia in mice? Explain**

EXERCISE 3

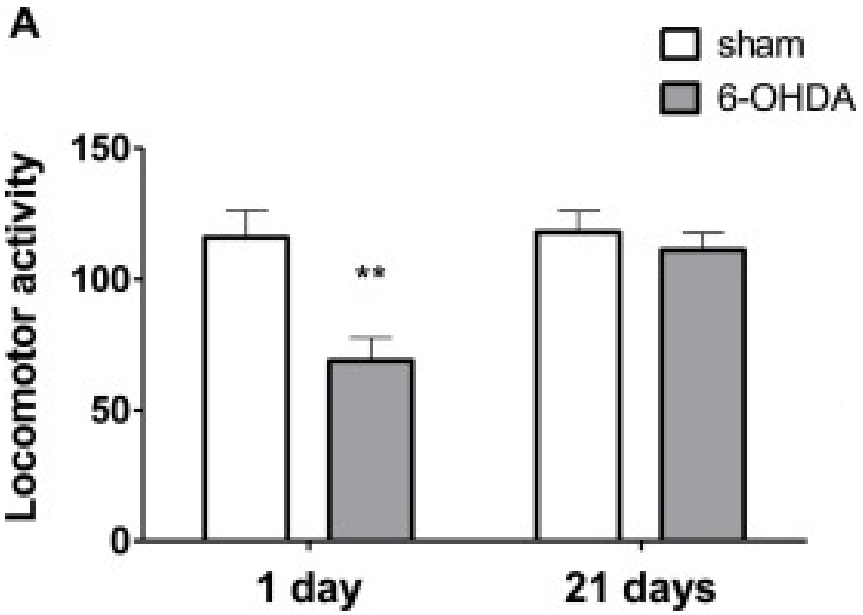
3) From those results can we conclude that the stepping test is a relevant behavioral model of Parkinson disease akinesia in mice? Explain

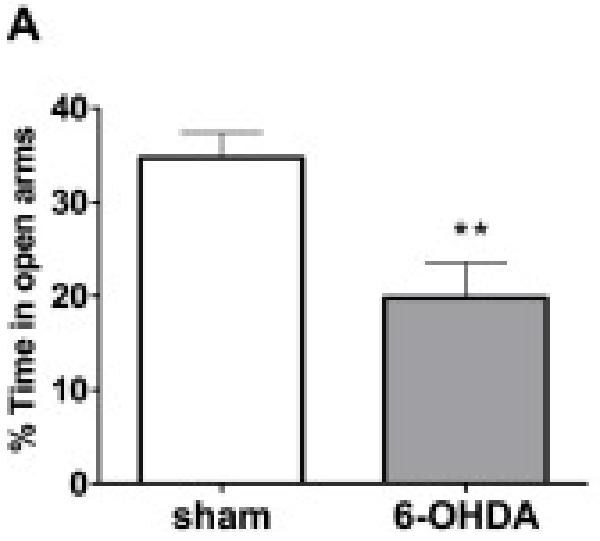
MPTP-treatment is able to decrease stepping performance over time, so limb akinesia as in Parkinson disease. L-DOPA treatment improved the stepping performance of MPTP-treated mice (at 20 min, 2h post-L-DOPA administration).

The data demonstrates that stepping test in mice seems to be a reliable and sensitive behavioral measure for assessing forelimb akinesia of translational value for Parkinson disease.

EXERCISE 4

The 6-hydroxydopamine (6-OHDA) lesion model of parkinsonism in the rat has provided an invaluable tool for investigating the pathophysiology of dopamine (DA) denervation and for evaluating novel therapeutical options.



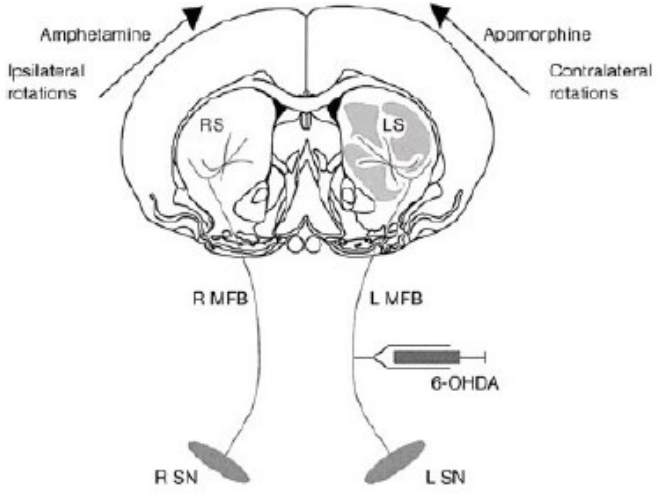


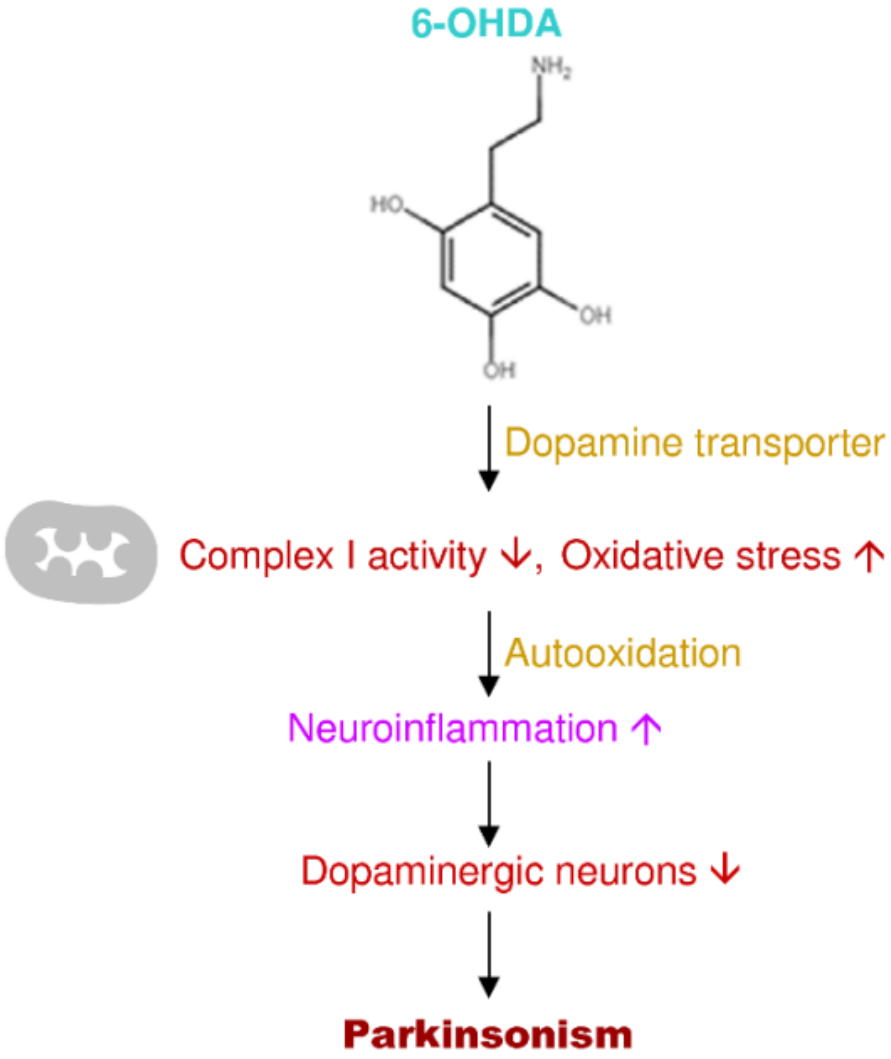
1) **Described the animal model used. What are the benefits and limitation of the model. Validity of the model?**
2) **Which conclusion can you make from those results**

6-OHDA mechanism of action

Injection of 6-OHDA into the MFB (median forebrain bundle = tract of DA fibers of the nigrostriotic pathway)

Verification of the lesion by administration of apomorphine (non-selective D1-2 agonist) which causes contralateral rotations to the lesion site.





EXERCISE 4 : Animal model used

Benefits of the model
Decreased striatal dopamine levels (0.25pt), Loss of TH positive fibers (0.25pt), Loss of positive TH Snc neurons (0.25pt), Akinesia / bradykinesia contralateral to the lesion (0.5pt)

Limitations of the model
Acute damage of the DAnergic system, unilateral effects, intracerebral injection

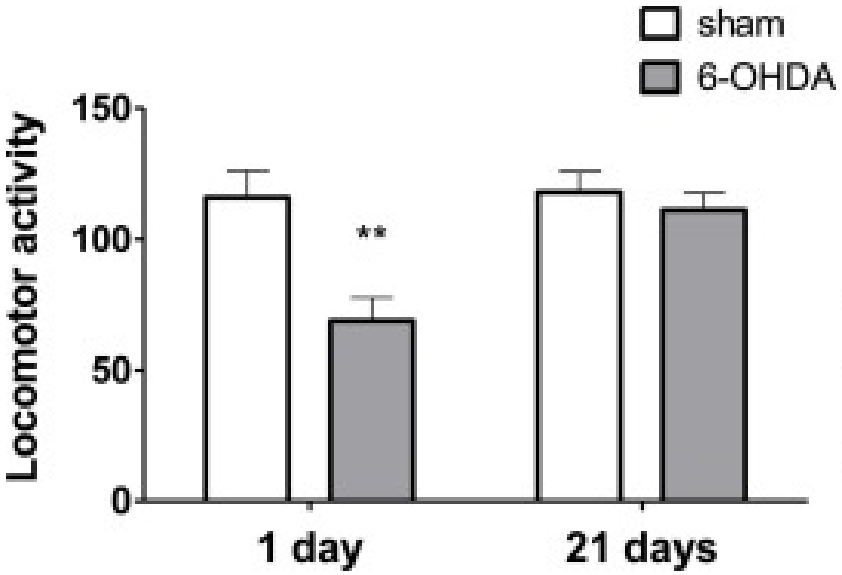
Descriptive validity: Same symptoms as the human disease : in psychiatry . partial, half-model (0.5 pts)

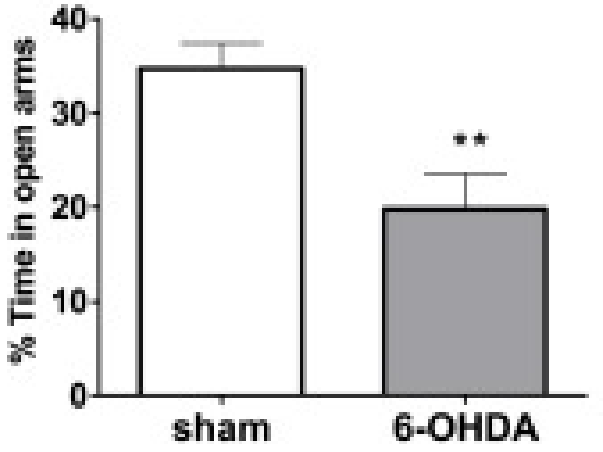
Theoretical validity: Involvement of the same mechanisms between the model and the human pathology. partial: acute model, (0.5 pts)

Predictive validity: The Treatment's answer of the model should be similar to the ones observed in the human illness. good, especially for dyskinesias / (0.5 pts)

EXERCISE 4

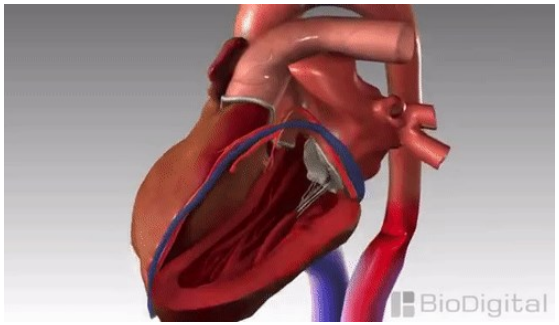
The 6-hydroxydopamine (6-OHDA) lesion model of parkinsonism in the rat has provided an invaluable tool for investigating the pathophysiology of dopamine (DA) denervation and for evaluating novel therapeutical options. Bellow are described the effects of intranigral injection of 6-OHDA.





2) Which conclusion can you make from those results. A significant decrease in locomotor activity was found in the 6-OHDA group 1 day after surgery compared with the sham group Twenty-one days after neurotoxin exposure, no differences were found between the neurotoxin and sham groups. 6-OHDA-lesioned animals spent less time in the open arms of the EPM compared with the sham group. 6-OHDA intranigral injection is able to produce anxiety-like behavior

Animal models of cardio-vascular disease



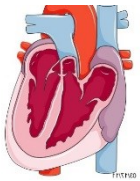
Cardiovascular System

$$\Delta P_A = Q_c \cdot PVR$$
$$Q_c = HR \times V_s$$

Autonomous nervous system

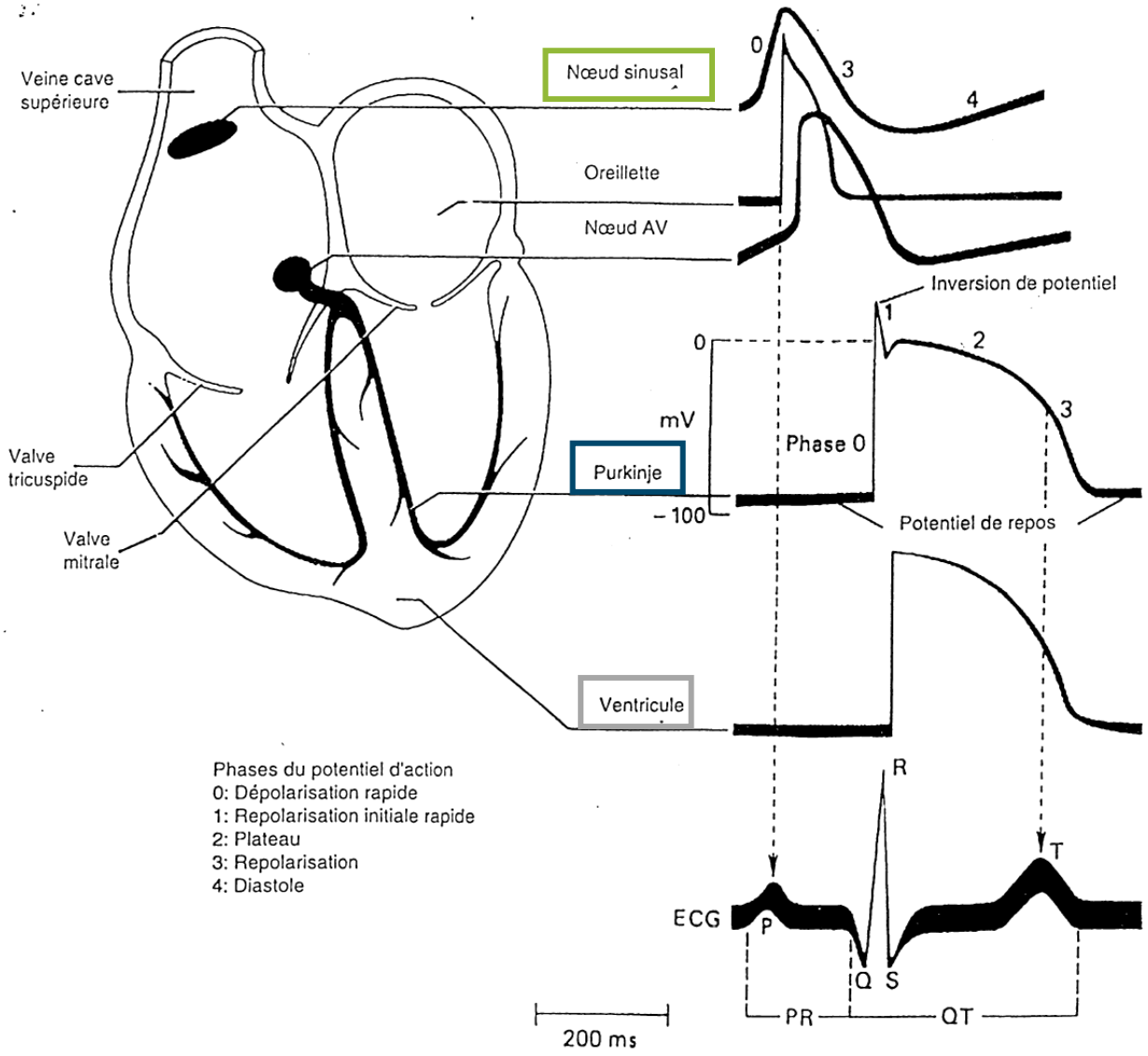
↘ PA → Baro reflex → ⊕ sympathetic / ⊖ parasympathetic

sympathetic:

- **NA** →  Vasoconstriction → ↗ **Perivascular resistance**
- **Noradrenaline (NA)** →  inotrope, chronotrope, lusitrope, dromotrope } positive → ↗ **Cardiac output**
- **NA** →  *Renin secretion* → **Angiotensin I** → *Enzyme de conversion (ACE)* → **Angiotensin II** → Récepteur AT_1 → **Aldosterone** → ↘ **Diuresis**

↗ **Blood pressure** ← *hypervolémia* ← ↘ **Diuresis**

Cardiac electrical activity

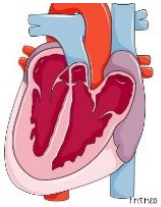
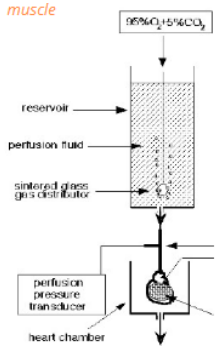
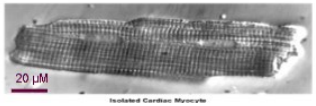
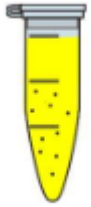
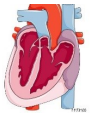
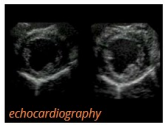
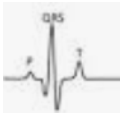
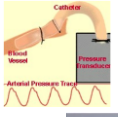


Pace-maker cells

Conductive cells

Contractile cells

To study cardiovascular system in pathology

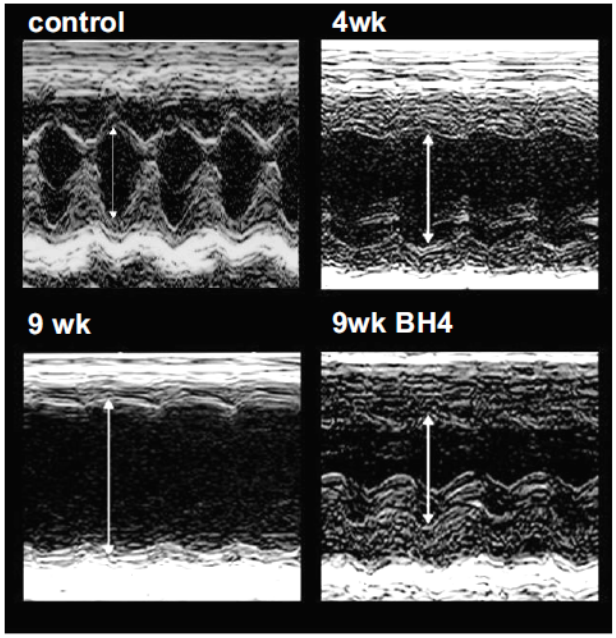
In vivo	Ex vivo	Ex vivo In vitro	In vitro
Animal model	**Isolated organs**	**Cellular model**	**Molecular model**
	 	Primary cells Cell lines h-iPSC Engineered tissu	Homogenates Kinase/enzyme activity etc…
Genetic selection Genetic manipulation Drug administration Surgery			
Cardiac function **Cardiac morphology**	 		
  			
Blood pressure			
   			

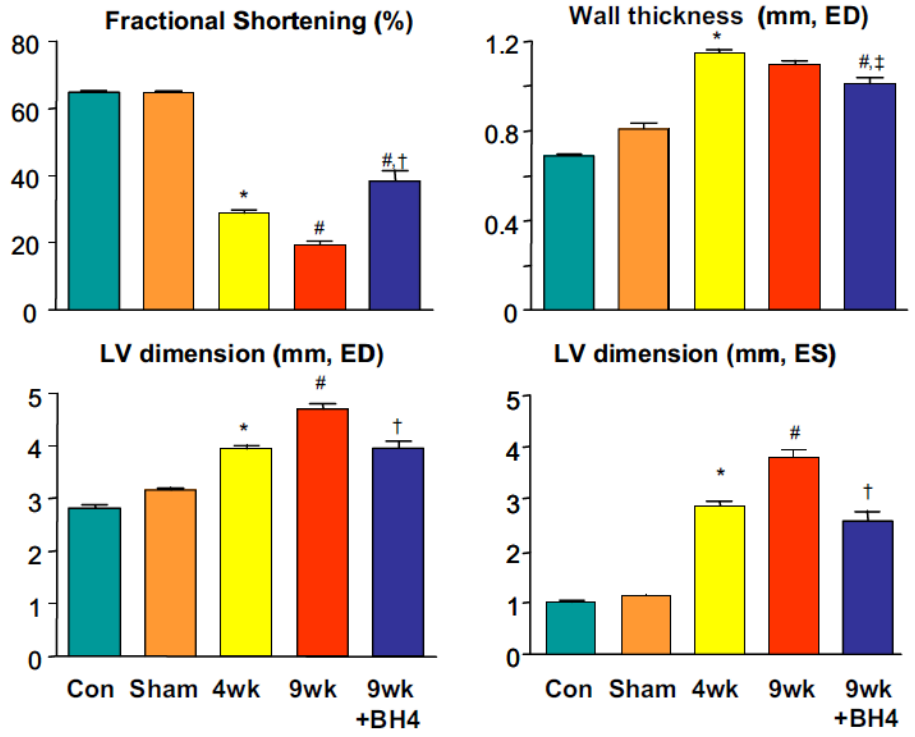
Cardiovascular readouts

Function	
Hemodynamics	• **BP**
Cardiac function and morphology	• **ECG : HR, ECG anomalies, arrhythmia (QT interval, …)** • **Echocardiography** (LV systolic and diastolic function, chamber dimensions) • **Cardiac hypertrophy** : LV weight… • **Ischaemia : infarct area in ischemia models** • Plasma natiuretic peptides (BNP)
Vascular function and morphology	• **Endothelial dysfunction** (e.g. alteration of endothelium-dependent vasorelaxation) • **Vessel wall remodelling and thickening**: SMC hypertrophy, hyperplasia (histology)

EXERCISE 5 : Functional exploration of cardiac function

An hemodynamic stress was induced in mice by transverse aortic constriction leading to pressure overload in the left ventricle. Cardiac function was assessed at different time points and the effect of pharmacological treatment using BH4 was tested.





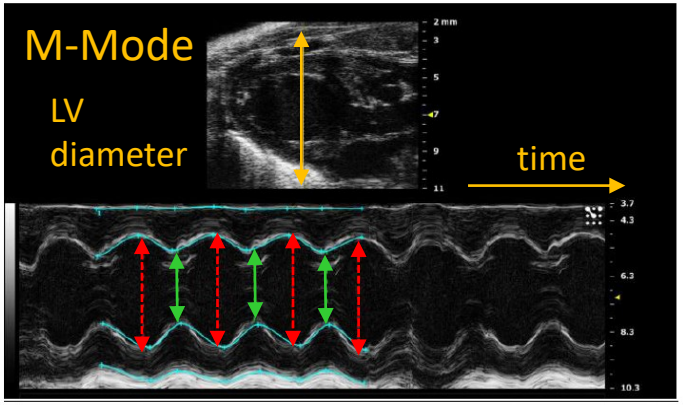
1- Define and describe the test used in this experiment?
2- What does it allow to quantify? Interpret the results.

Moens et al., et al. 2008 Circulation

EXERCISE 5

1- Define and describe the test used in this experiment?

Transducer (probe)



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

LV internal diameter in systole (LVIDs)

LV internal diameter in diastole (LVIDd)

Echocardiography: 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.

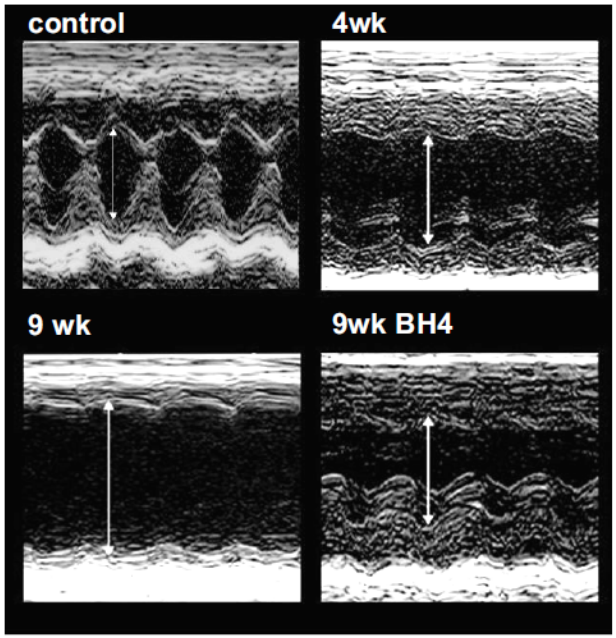
The probe receives back the echoes, which are translated in electrical signal and amplified.
Used for morphological exploration of the heart chambers and large vessels
Conducted on anaesthetized, unconscious animals (typically using isoflurane)

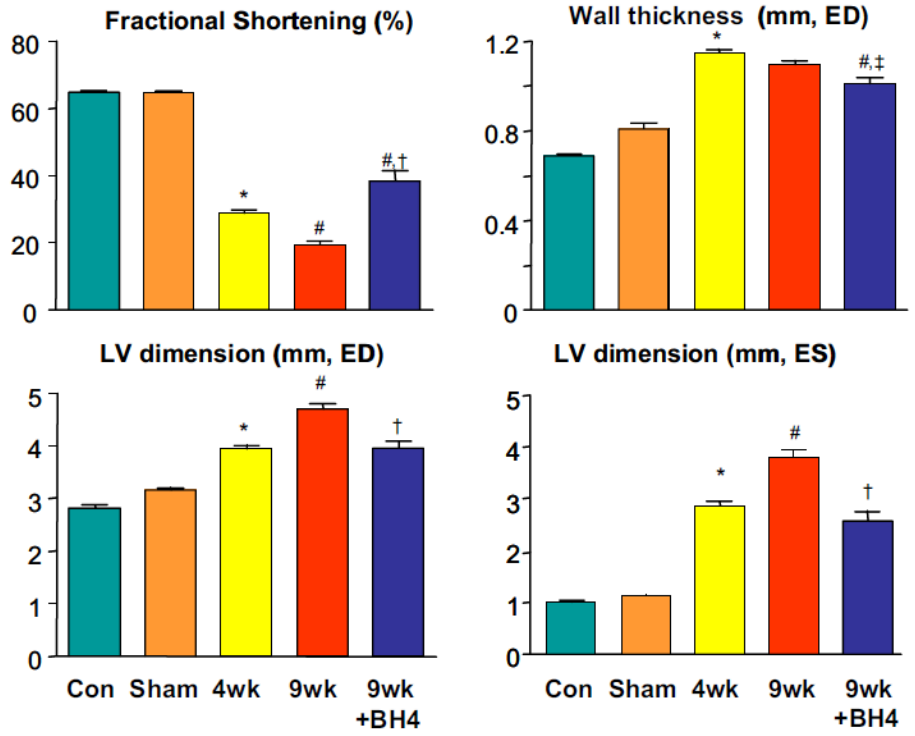
2- What does it allow to quantify? Interpret the results.

- **Morphology :** LV end systolic volume (LVESV), LV end diastolic volume (LVEDV), LV mass.
- **Function :** Stroke volume (SV), ejection fraction (EF), Cardiac output

Moens et al., et al. 2008 Circulation

EXERCISE 5





3- Interpretation of the results :

Fractional shortening is significantly reduced at 4 and 9 weeks → systolic dysfunction
Increase left ventricle wall thickness and increased dilation → hypertrophy and dilatation
The treatment allows to reduce the cardiac function and remodelling.

Moens et al., et al. 2008 Circulation

EXERCISE 6

Hypertension is recognized as one of the leading risk factors for human morbidity and mortality.

Angiotensin II (Ang II) is a vasoconstrictive peptide hormone formed within the renin–angiotensin system (RAS) which plays an important role in regulating cardiovascular homeostasis and blood pressure.

In this study, six compounds (1a, 1b, 1c, 2, 3 and 4), all potential blockers of AGII receptors were designed and synthesized.

How will you test the anti-hypertensive effect of those compounds in animal (animal model, methodology and experimental conditions)?

EXERCISE 6

How will you test the anti-hypertensive effect of those compounds in animal (animal model and methodology)?

1 – Choose/validation of the animal model of hypertension

Genetic model: ***spontaneous hypertensive rat (SHR)*** *vs. Wistar Kyoto (WKY)*
Genetic+environment: *Dahl salt-sensitive rats vs. Normal+high salt diet*
Transgenic rats: *overexpression of gene regulating BP*

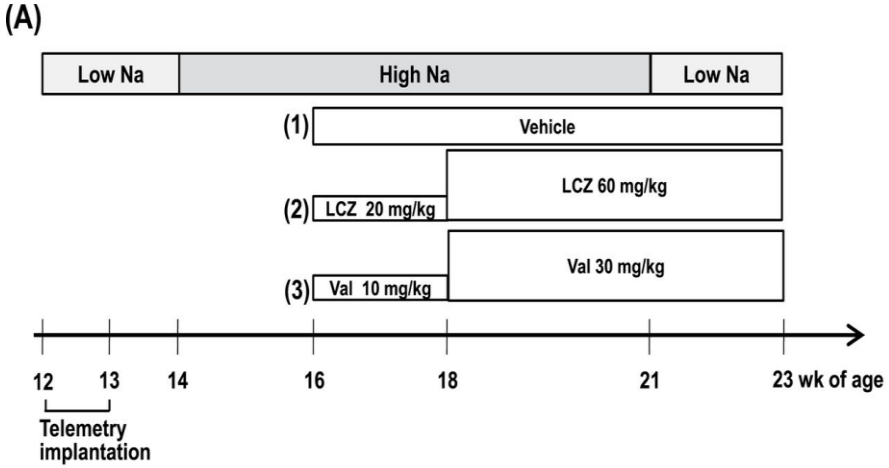
2- Tests to monitor blood pressure

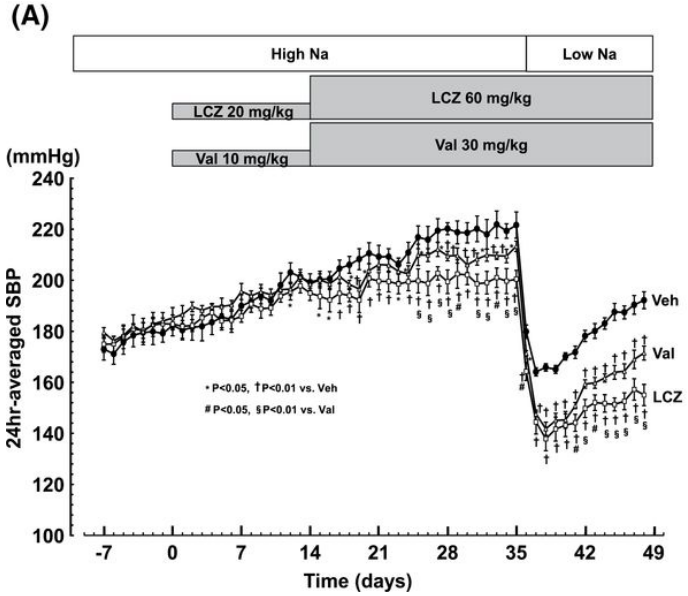
Radiotelemetry (Invasive, continuous monitoring, conscious animal, battery life limited)
Catheterism (direct measurements, blood samples, invasive + gaz anesthetics)
Tail cuff method (vigil animal, adaptation period, snapshot of BP)

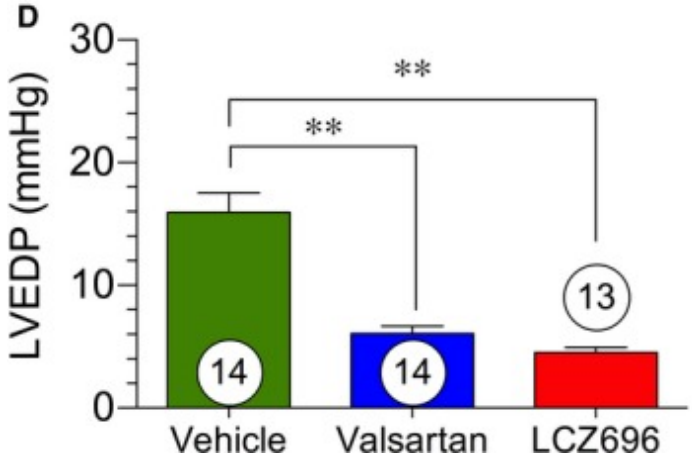
3- Experimental conditions

Dose response, non-selective effects, efficacy vs. known effective drugs (e.g. valsartan, losartan)

EXERCISE 7 : Valsartan regulation of cardiovascular system







1) Described the method used to assess arterial blood pressure.

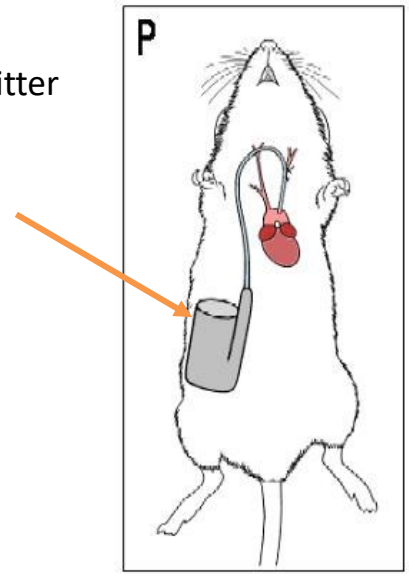
2) Interpret the data.

3) How will you test for the potential arrhythmic effect of those compounds? What else could be assessed to explore the effect of those drugs on the cardiovascular system.

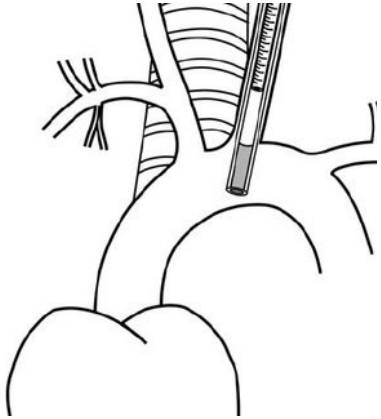
EXERCISE 7 - Method used to assess arterial blood pressure.

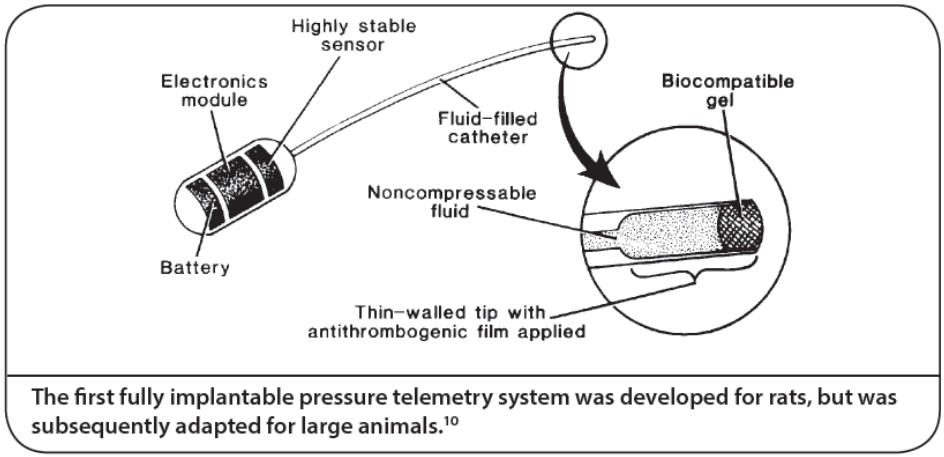
- Implant of a pressure probe in the animal by surgery.
- Direct method : BP could be continuously monitored, resulting in a nuanced picture of BP changes over an extended period of time
- Radio transmitter sends the BP data while the animal is conscious and free
- Adapted to rodents and large animals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

EXERCISE 7 – Explore the potential arrhythmic effect of LCZ and Valsartan

Measurement of electrical activity by ECG recording:

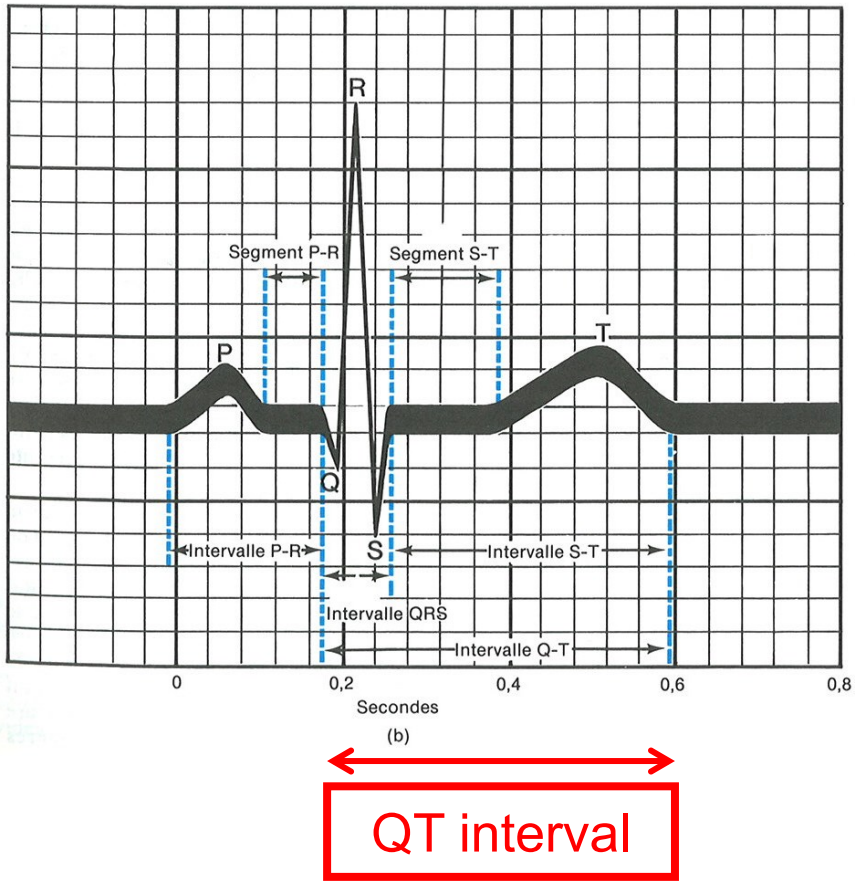
- Heart rate
- ECG anomalies, arrythmia (long QT)

Modalities :

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

Onde T (ventricular repolarisation)

QRS (ventricular depolarisation)

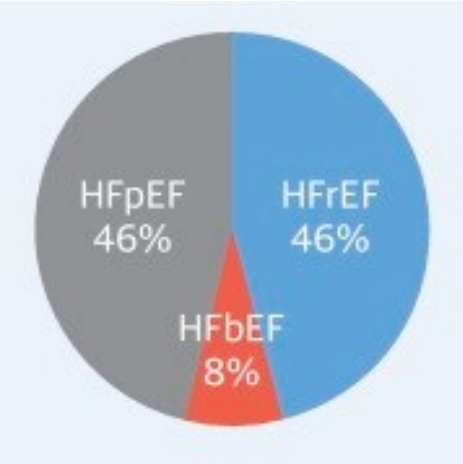


QT interval

EXERCISE 8: Study of heart failure with preserved ejection fraction

Etiology/Prevalence

Hypertension (90%)
Diabetes (52%)
Obesity
Hyperlipidemia
Women > Men



Shah KS et al., JAAC 2017

Treatment Strategy

Symptomatic
Comorbidities
Non specific
Men=Women

Lung congestion, Fibrosis, Diastolic dysfunction, Hypertrophy, higher diastolic Ca^{2+}, SR Ca^{2+} leak and decay TT : Model differences.

A better understanding of HFpEF and gender differences susceptibility is needed to better adapt patient treatment

EXERCISE 8 :
Which model of heart failure with preserved ejection fraction will you choose ?

ORIGINAL RESEARCH

Diabetes and Excess Aldosterone Promote Heart Failure With Preserved Ejection Fraction

Bence Hegyi, MD, PhD; Juliana Mira Hernandez, DVM, PhD; Christopher Y. Ko, PhD; Junyoung Hong, PhD; Erin Y. Shen, BS; Emily R. Spencer, BS; Daria Smoliarchuk, BS; Manuel F. Navedo, PhD; Donald M. Bers, PhD; Julie Bossuyt, DVM, PhD

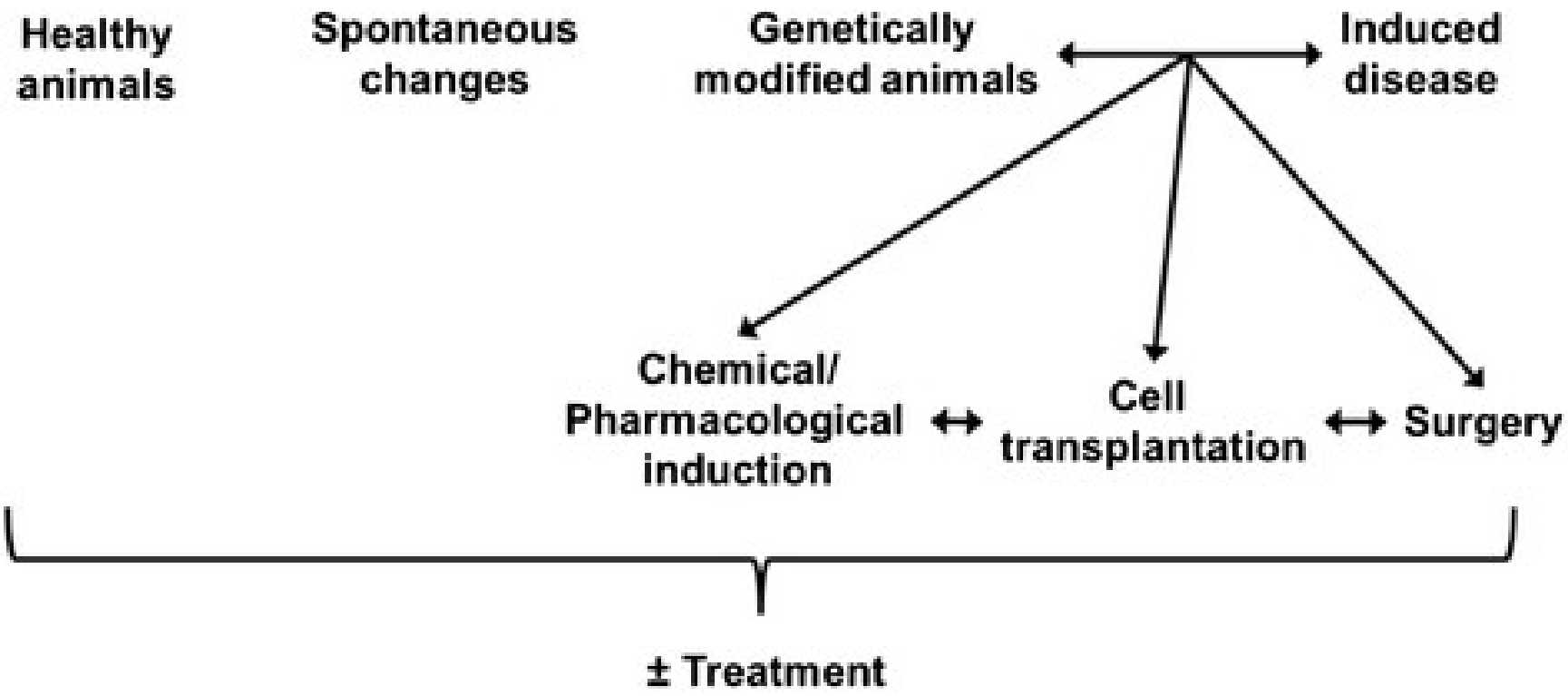
Nitrosative Stress Drives Heart Failure with Preserved Ejection Fraction

Gabriele G. Schiattarella[1,2], Francisco Altamirano[1], Dan Tong[1], Kristin M. French[1], Elisa Villalobos[1], Soo Young Kim[1], Xiang Luo[1], Nan Jiang[1], Herman I. May[1], Zhao V. Wang[1], Theodore M. Hill[1], Pradeep P.A. Mammen[1], Jian Huang[1], Dong Ik Lee[3], Virginia Hahn[3], Kavita Sharma[3], David A. Kass[3], Sergio Lavandero[1,4], Thomas G. Gillette[1], and Joseph A. Hill[1,5]

EXERCISE 8 :
Which model of heart failure with preserved ejection fraction will you choose ?

- Read the articles
- Describe each model of HFpEF
- How did they validate the model ?
- Which model is the most relevant to you ? Explain your choice.
- How could we improve those model ?

Types of animal model



Vanhove C et al. EJNMMI Physics 2015

Types of genetically modified animal

- **Transgenic models :** overexpression of a heterologous gene (not normally present in the mouse genome) or overexpression of a homologous gene (present in the mouse genome).

- **Knock-in model** : replacement of an endogenous gene with a mutant or heterologous gene, but leaves the original promoter region intact. Expression is controlled by normal regulatory mechanisms.

- **Knock-out and conditional knock-out models** remove a gene from the genome, and thus do not increase levels of gene expression.

Modelling symptoms of major depression

Table 1 | **Modelling symptoms of major depression* in mice**

Symptom	How might symptom be modelled in mice?
Markedly diminished interest or pleasure in everyday activities (anhedonia)	Reduced intracranial self-stimulation, progressive ratio responding for positive reward (for example, sucrose) and social withdrawal
Large changes in appetite or weight gain	Abnormal loss in body weight after exposure to chronic stressors
Insomnia or excessive sleeping	Abnormal sleep architecture (measured using electroencephalogy)
Psychomotor agitation or slowness of movement	Difficulty in handling and alterations in various measures of locomotor activity and motor function
Fatigue or loss of energy	Reduced activity in home cage, treadmill/running-wheel activity, nest building and active waking electroencephalogram
Indecisiveness or diminished ability to think or concentrate	Deficits in working and spatial memory and impaired sustained attention
Difficulty performing even minor tasks, leading to poor personal hygiene	Poor coat condition during chronic mild stress
Recurrent thoughts of death or suicide	Cannot be modelled
Feelings of worthlessness or excessive or inappropriate guilt	Cannot be modelled

*Symptoms used in the *Diagnostic and Statistical Manual-IV* diagnosis of major depression.

Modelling symptoms of anxiety

Table 2 | **How symptoms of anxiety disorders* might be modelled in mice**

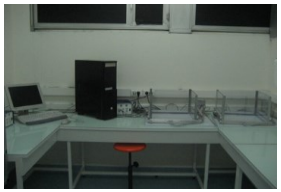
Symptom	How might symptom be modelled in mice?
Avoidance of places from which escape could be difficult (agoraphobia)	Increased avoidance of exposed, well-lit areas
Sudden onset of intense fearfulness, often with respiratory distress and fear of 'going crazy' (panic attack)	Increased flight from a predator
Anxiety provoked by social situations, leading to avoidance behaviour (social phobia)	Low social interaction with unfamiliar conspecific
Anxiety provoked by a specific feared object, leading to avoidance behaviour (specific phobia)	Conditioned taste avoidance
Re-experiencing a traumatic event, leading to increased arousal and avoidance of stimuli associated with the event (post-traumatic stress disorder)	Increased freezing response to fear-conditioned cue or context
Anxiety-provoking obsessions and anxiety-reducing compulsions (obsessive–compulsive disorder)	Increased marble burying and excessive grooming
Difficulty concentrating or mind going blank (generalized anxiety disorder)	Impaired sustained attention
Sleep disturbance/insomnia	Abnormal sleep architecture (measured using electroencephalogy)
Autonomic hyperarousal (tachycardia, blushing, sweating and frequent urination)	Radiotelemetric measurement of heart rate dynamics during anxiety-provocation, such as increased stress-induced hyperthermia
Flashbacks of traumatic events	Impairment in extinction of fear memory
Cognitive bias towards ambiguous or weak threat cues	Increased fear conditioning to partial threat cue
Heightened startle response, particularly in threatening contexts	Increased acoustic startle response and fear-potentiated startle response
Separation anxiety	Increased ultrasonic vocalizations in pups separated from their mother
Feelings of losing control or going crazy during a panic attack	Cannot be modelled

*Symptoms used in the *Diagnostic and Statistical Manual-IV* diagnosis of anxiety disorders.

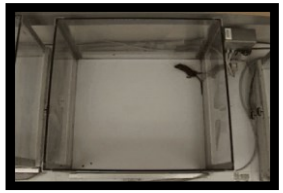
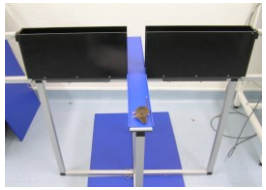
How do we measure anxiety/depression in animals?

Behavioural tests

Anxiolytic activity		Mixed activity	Antidepressive activity
Open Field	Elevated plus maze	Novelty Suppressed Feeding	Forced swim test

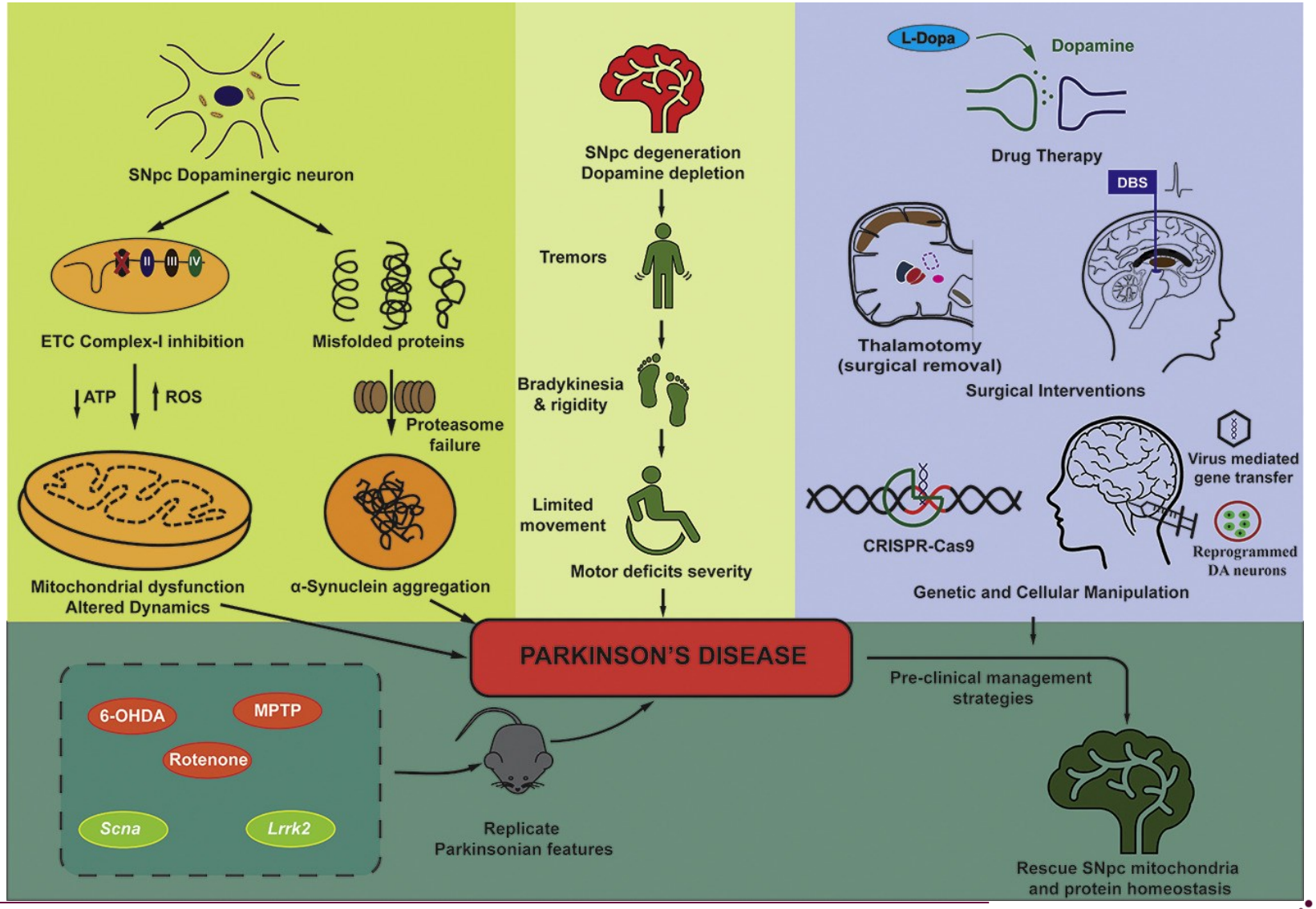





predictivity

Overview of Parkinson Disease



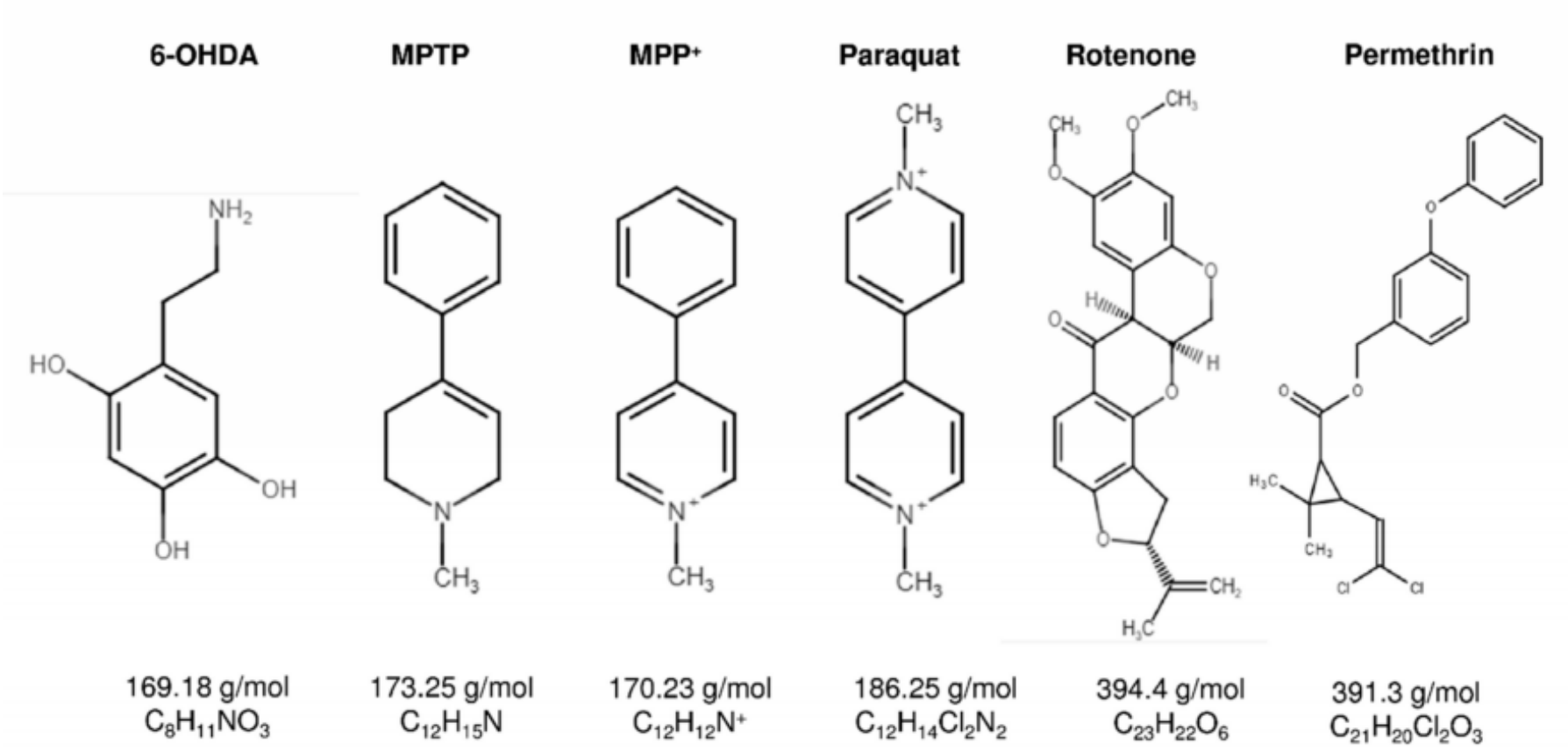
Animal models in Parkinson disease

Toxin models		Genetic Models		
6-OHDA	MPTP	PARKIN-KO	PINK1-KO	TG – AAV Alpha-synuclein (mutated or human form)

Variation between models regarding:
DAnergic damage
Mitochondrial respiratory deficit
Oxydative stress
Alpha-synuclein and lewy bodies aggregates
Locomotor and non-motor deficit

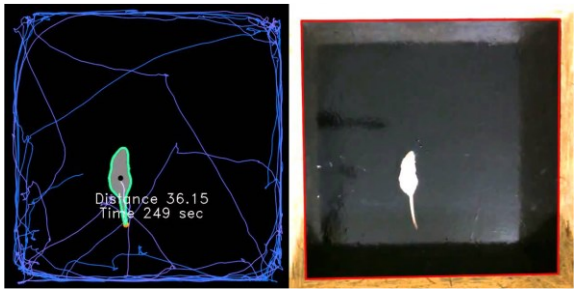
No perfect model
Selection dependent
on the question/hypothesis

Neurotoxins used to induce Parkinson disease in vivo models



Parkinson disease behavioral tests

Locomotor activity

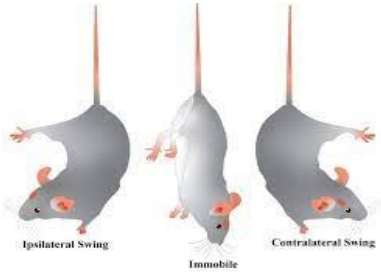


Stepping Test

Limb-Use Test

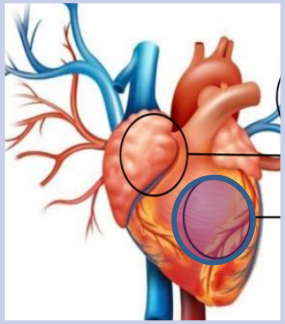
Rotarod

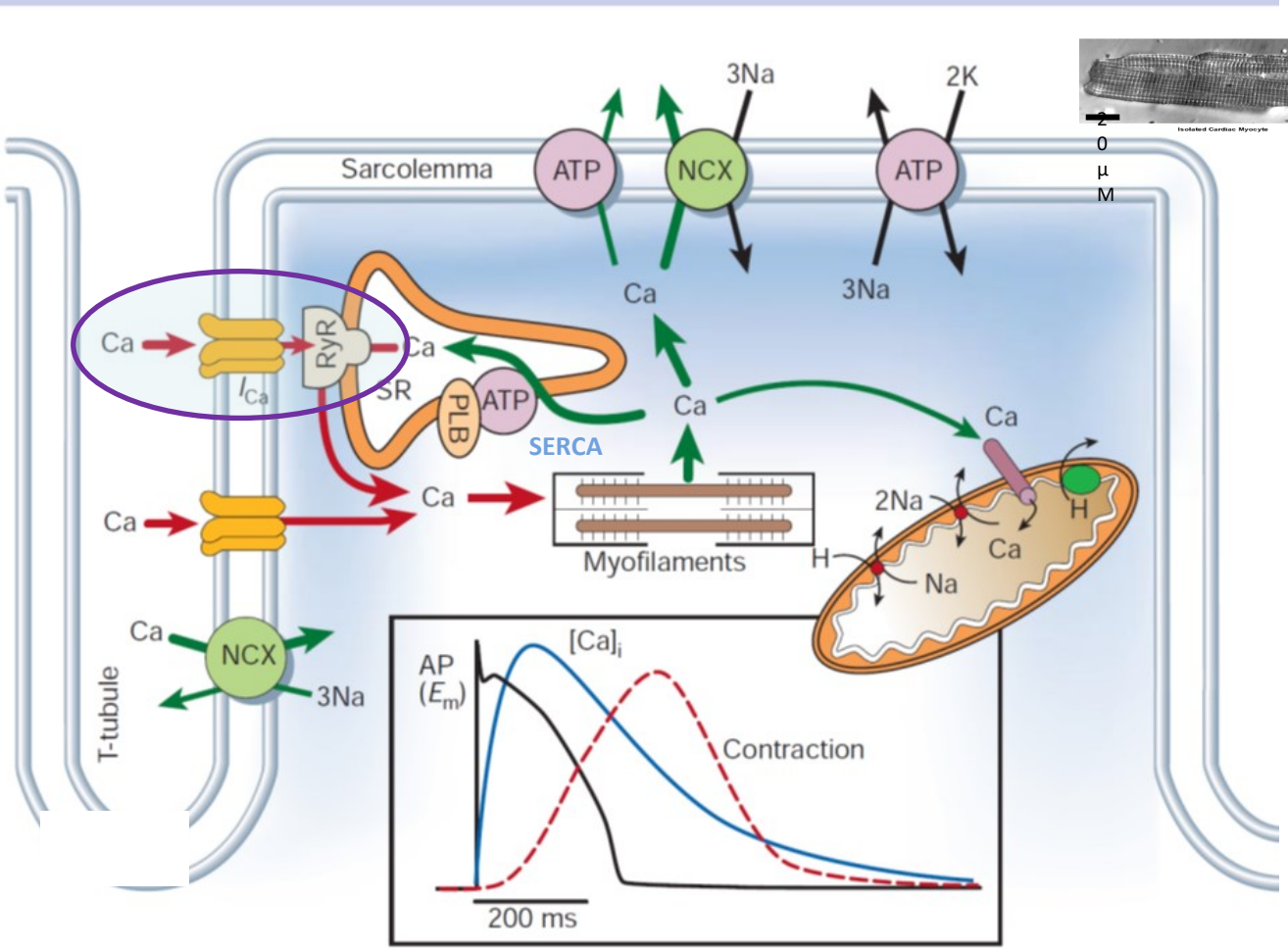
Elevated body swing test



Excitation-contraction coupling and CARDIAC CONTRACTION

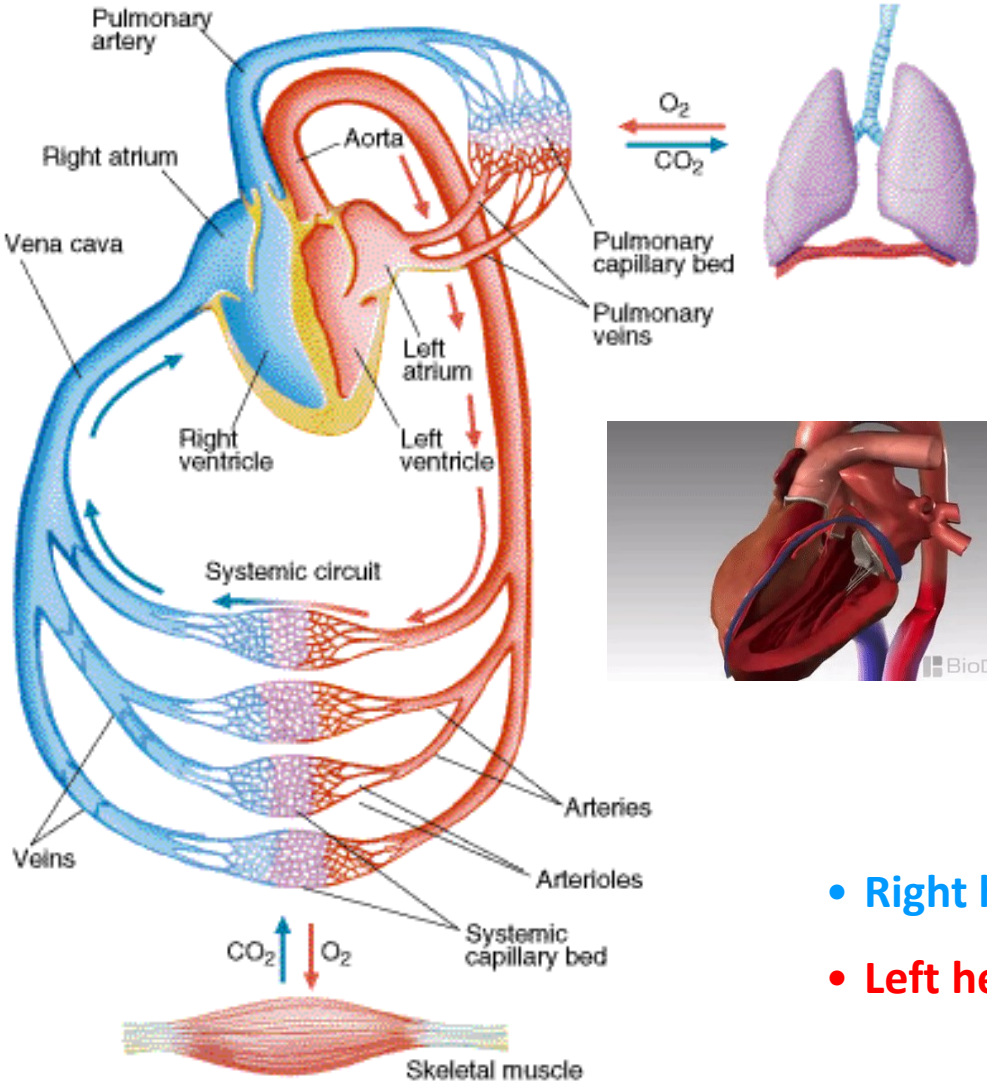
Figure 1 Ca^{2+} transport in ventricular myocytes. Inset shows the time course of an action potential, Ca^{2+} transient and contraction measured in a rabbit ventricular myocyte at 37 °C. NCX, Na^+/Ca^{2+} exchange; ATP, ATPase; PLB, phospholamban; SR, sarcoplasmic reticulum.





Bers et al., 2002 Nature ; Zamponi et al., 2015 Pharmacol Rev

Organ perfusion is driven by ARTERIAL BLOOD PRESSURE (ΔP_A)



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

$$Q_c = HR \times V_s$$

Q_c : cardiac output (L/min)

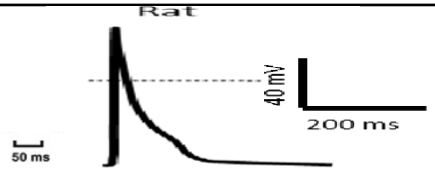
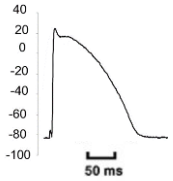
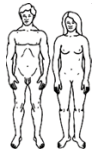
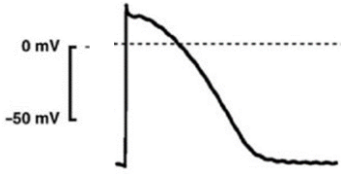
PVR : peripheral vascular resistances

HR : heart rate

V_s: systole ejection volume

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

Cardiovascular features across species

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

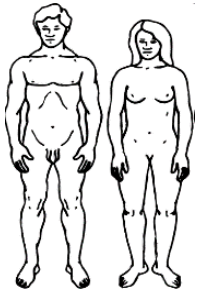
Pulsen et al., 2017 Acta Physiol

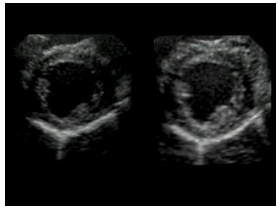
Study methods of cardiac function/contraction

In vivo

Animal model

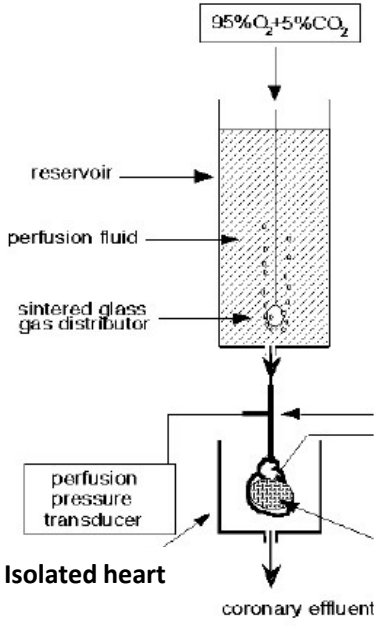
Clinical Study





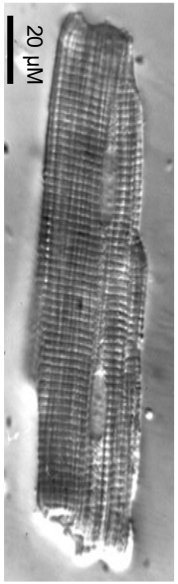
Ex vivo

Isolated organ



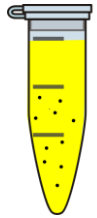
Ex vivo / In vitro

Isolated cardiomyoctes



In vitro

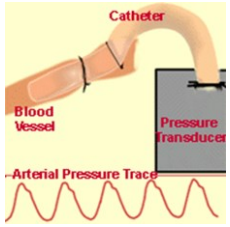
Biochemical study



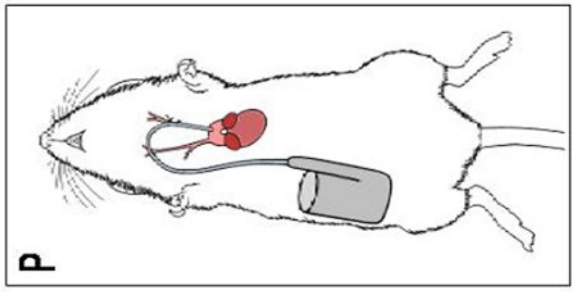
Exploring blood pressure in animal

INVASIVE

Catheterism



Radiotelemetry



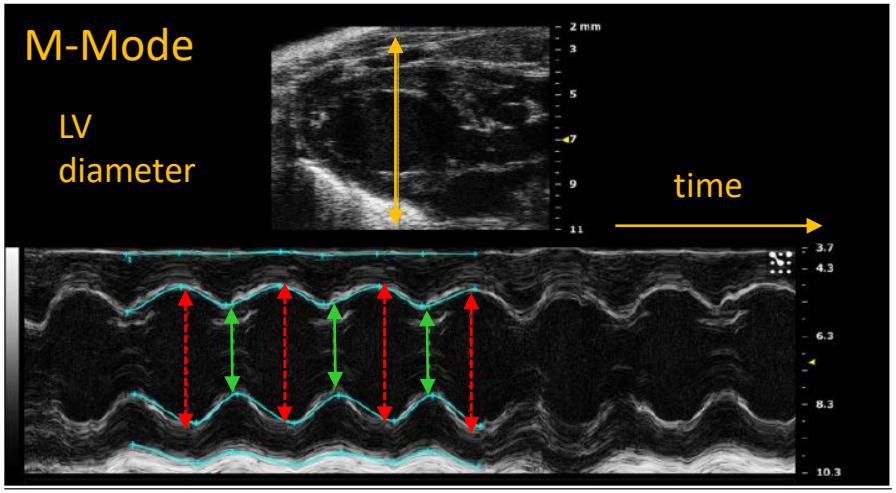
NON INVASIVE

Tail cuff method

Sphygmomanometer

Exploring cardiac function (*in vivo*)

Echocardiography : Non invasive technique to explore cardiac morphology and function using ultra sounds



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

LV internal diameter in diastole (LVIDd)

LV internal diameter in systole (LVIDs)

- **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

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

Exploring electrical activity of the heart (*in vivo*)

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)

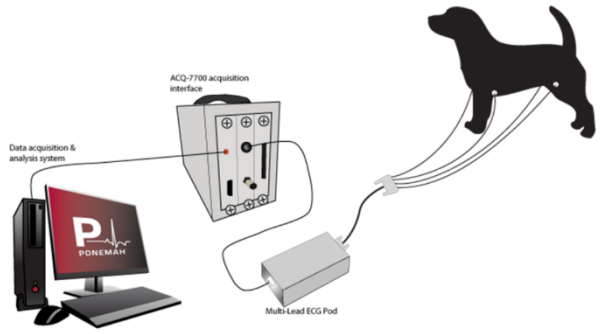


Figure 5: Hardwired ECG System Setup for Large Animals

(B)

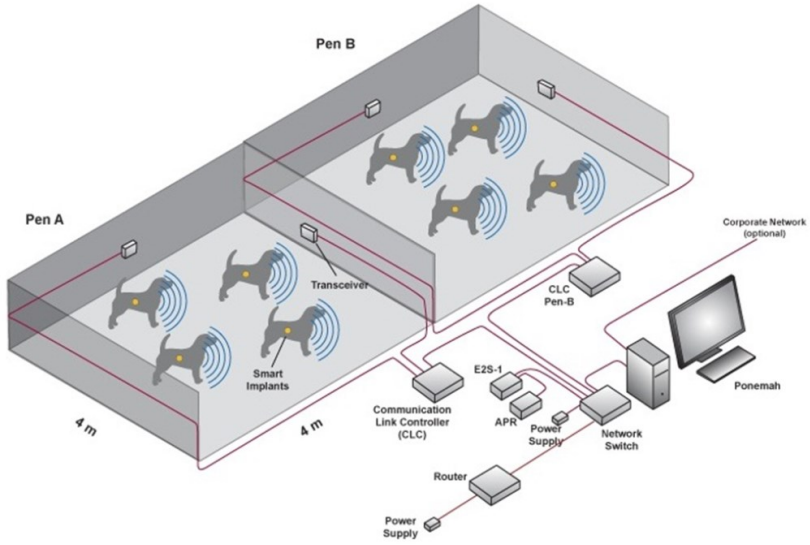


Figure 2: PhysioTel Digital Implantable Telemetry System Setup for Large Animals