

CARDIOLOGY



PRESENTATION

Cardiovascular diseases constitute a leading cause of morbidity and mortality in industrialized countries. Cardiac abnormalities are also one of the major causes of withdrawal of drugs or restriction in their labelling.

The aim of the following techniques, used to characterize mouse cardiovascular system, is to be able to identify phenotypic traits that represent the main human cardiovascular troubles:

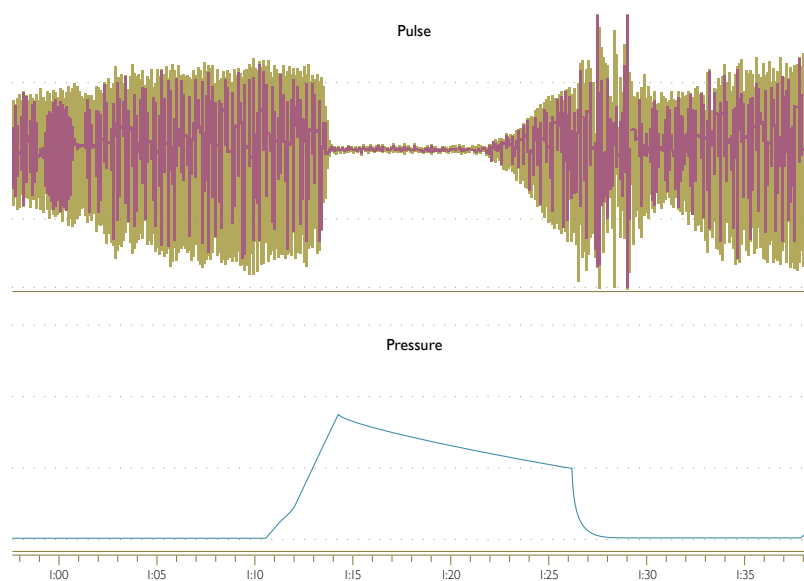
- Hypertension,
- Cardiac electrical abnormalities that could lead to conducting troubles or arrhythmias,
- Cardiac remodelling and failure.

For this purpose, various techniques and models are standardized.

I. BLOOD PRESSURE (BP)

a. Non-invasive blood pressure (NIBP) measurement is a way to record blood pressure in the caudal artery of conscious but restrained mice. The pulse (alternative blood flow) is recorded by a photoelectric detector and the systolic arterial pressure is obtained through the recording of the pressure in a tail cuff. Two parameters are obtained: the systolic arterial pressure and the heart rate.

Example of a classical non-invasive blood pressure in mice



Usual values in current strains

Systolic blood pressure (SBP) and heart rate (HR) values obtained in C57BL/6j and 129S2/SvPas male mice (12 week-old).

	C57BL/6j		129S2/SvPas	
	Mean	S.D	Mean	S.D
SBP (mmHg)	93	3	88	7
HR (BPM)	540	39	454	27

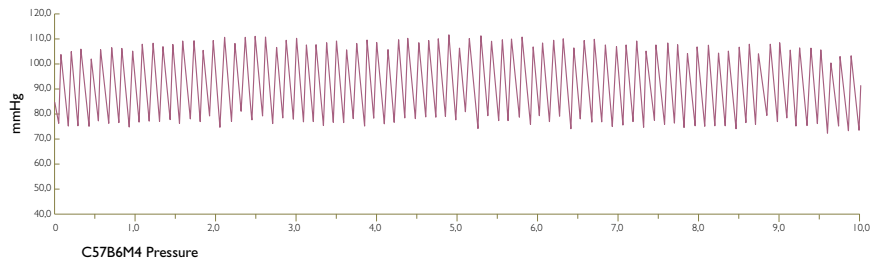
b. Telemetric recording of the blood pressure

The long-term monitoring of BP and HR in mice has been facilitated by the development of radiotelemetry, which gives simple, reliable and accurate chronic measurements of pulsatile blood pressure (systolic and diastolic) and heart rate in conscious and freely moving animals. A miniature device implanted in the carotid artery of each animal measures and transmits the digitalized data via radio frequency signals to a nearby receiver. Interestingly, this method is a reliable way to make pharmacological studies in conscious animals by the simultaneous administration of drugs (intraperitoneally, intravenously, by osmotic minipumps ...).

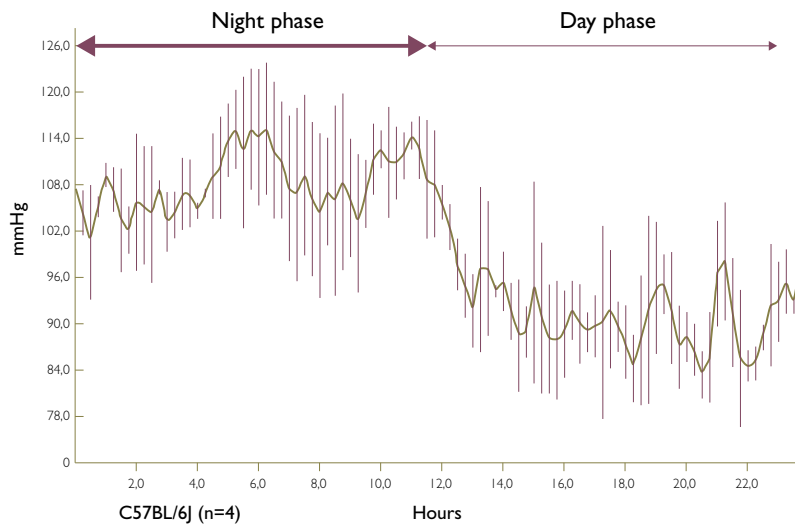
The above mentioned parameters can be monitored for several weeks, either in naïve mice, or upon administration of drugs.

Observations

A) Typical recording of blood pressure in a conscious mouse.



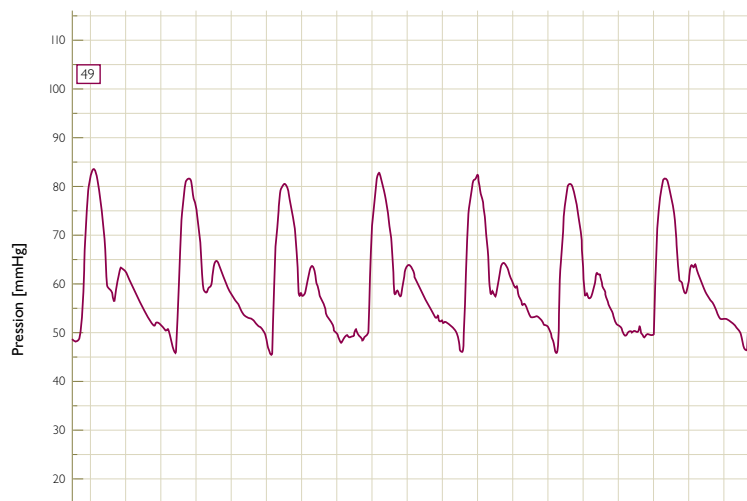
B) 24 hours monitoring of blood pressure in conscious mice.



c. Blood pressure measurements by invasive catheterization under anaesthesia

This test is used to confirm blood pressure phenotypes detected by the tail-cuff method and to perform acute pharmacological testing. A high-fidelity catheter is introduced into a carotid or femoral artery under anaesthesia (pentobarbital or isoflurane). The digitized signal is then transferred to a data acquisition, analysis and storage system. All pressure parameters together with the heart rate are obtained. A typical recording is presented below.

Aortic blood pressure tracing



2. CARDIAC ELECTRICAL ACTIVITY: SURFACE ELECTROCARDIOGRAM (ECG)

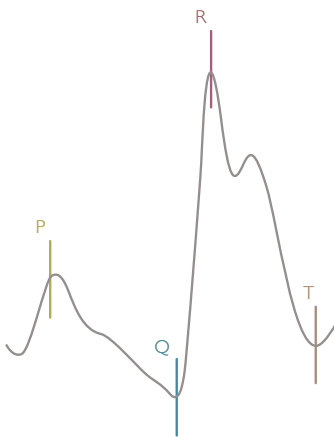
The electrocardiogram (ECG) is the surface electrical activity recording of the heart. It is used to measure the rate and regularity of beats, to detect cardiac arrhythmias and troubles of intracardiac conduction as well as the size and position of the chambers.

Heart rate (HR), interval between atrial contraction and ventricular depolarization (P-R interval) and duration of repolarization (QT interval) are analyzed.

a. ECG in anaesthetized conditions

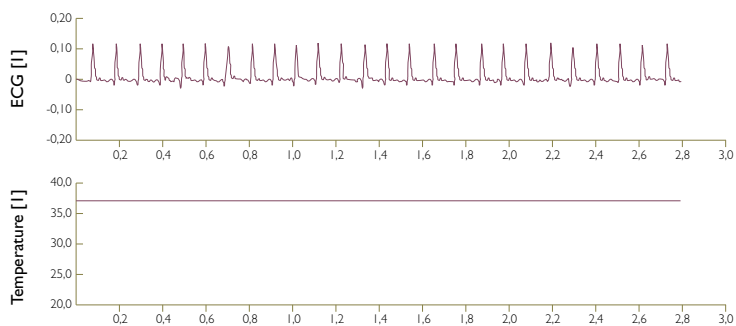
A rapid examination can be performed under a short term tribromoethanol anaesthesia. This anaesthetic regimen was selected because it does not affect the heart rate in great extents.

Example of a typical ECG signal recorded in a C57BL/6J mouse



b. ECG in conscious mice by telemetry

To catch cardiac arrhythmias due to mutations or drugs, a continuous ECG monitoring (similar to the one performed in humans) can be performed by telemetry in mice. The central body temperature is simultaneously obtained.



3. CARDIAC INVESTIGATIONS

To explore cardiac function two methods are used: echocardiography which is used as a non-invasive test to obtain a complete evaluation of the cardiac function and anatomy and left-ventricular catheterization for cardiac haemodynamics.

a. Echocardiography

The examination is performed in anaesthetized mice. Two aspects are considered: cardiac anatomy and function (systolic and diastolic).

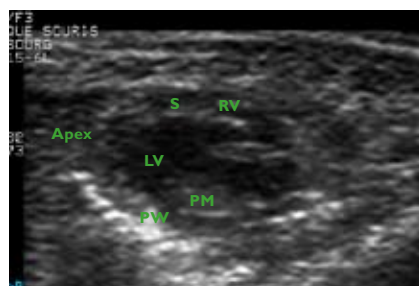
The following parameters are measured:

Cardiac output (CO), Left Ventricular End-Diastolic Diameter (LVEDD), Left Ventricular End-Systolic Diameter (LVESD), Left Ventricular Mass (LVM), Shortening Fraction (SF), Ejection Fraction (EF), Posterior Wall Thickness (PWT), Septum Thickness (ST), E Wave, A Wave, E/A Ratio, Time of the E Wave Deceleration (DT), Aortic Root Dimension (ARD), Isovolumetric Contraction Time (IVCT), Isovolumetric Relaxation Time (IVRT), A Wave Duration (A dur).

Normal values

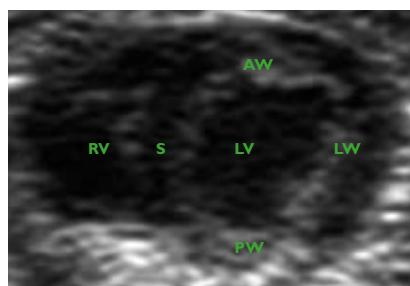
The values below represent a complete echographic evaluation obtained in C57BL/6J and I29S2/SvPas male and female mice (12 week-old).

		I29S2/SvPAS		C57BL/6J	
		males	females	males	females
Weight	g	24.6	22.1	27.0	21.3
Aortic diameter	mm	1.12	1.13	1.40	1.29
LV end-diastolic diameter	mm	3.42	3.22	3.68	3.33
LV end-systolic diameter	mm	2.47	2.16	2.46	2.26
Shortening fraction	%	32.3	32.8	33.8	33
Ejection fraction	%	67.0	68.2	69.0	66.5
Posterior wall thickness	mm	0.63	0.62	0.63	0.65
Septum thickness	mm	0.74	0.71	0.73	0.75
Time/Speed Integration	cm	7.63	7.89	7.07	6.77
E Wave	cm/s	96.2	86.3	104	91.2
A Wave	cm/s	55.5	44.8	62.7	59.8
Ratio E/A		1.77	1.90	1.74	1.59
Times of the E Wave deceleration	ms	38.5	43.7	46.3	48.0
Isovolumetric Contraction Time	ms	23.3	20.8	17.3	21.5
Isovolumetric Relaxation Time	ms	25.8	27.2	26.5	27.3
A Wave duration	ms	36.8	35.4	42.8	41.6



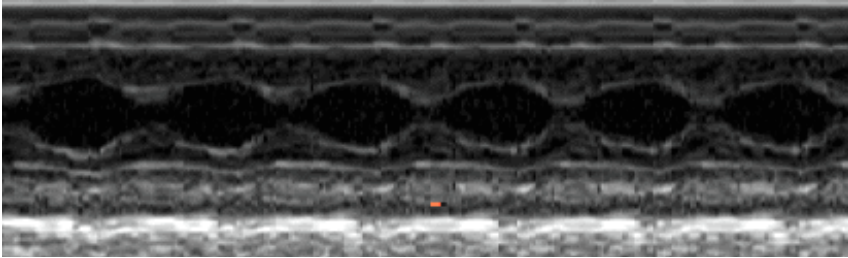
2D Parasternal Long-Axis

LV: left ventricular
PM: papillary muscle
PW: posterior wall
RV: right ventricular
S: septum

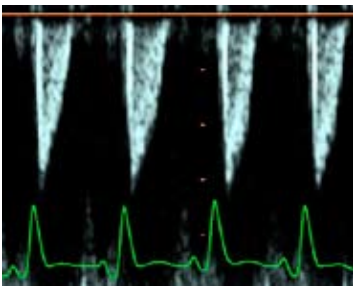


2D Parasternal Short-Axis

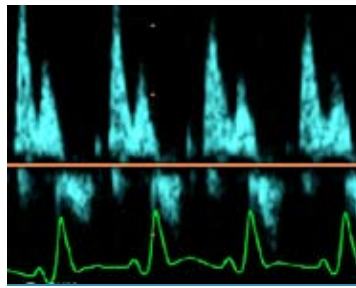
AW: anterior wall
LV: left ventricle
LW: lateral wall
PW: posterior wall
RV: right ventricle
S: septum



M-mode Left Ventricle (short axis)



Doppler Aortic Tract (apical view)

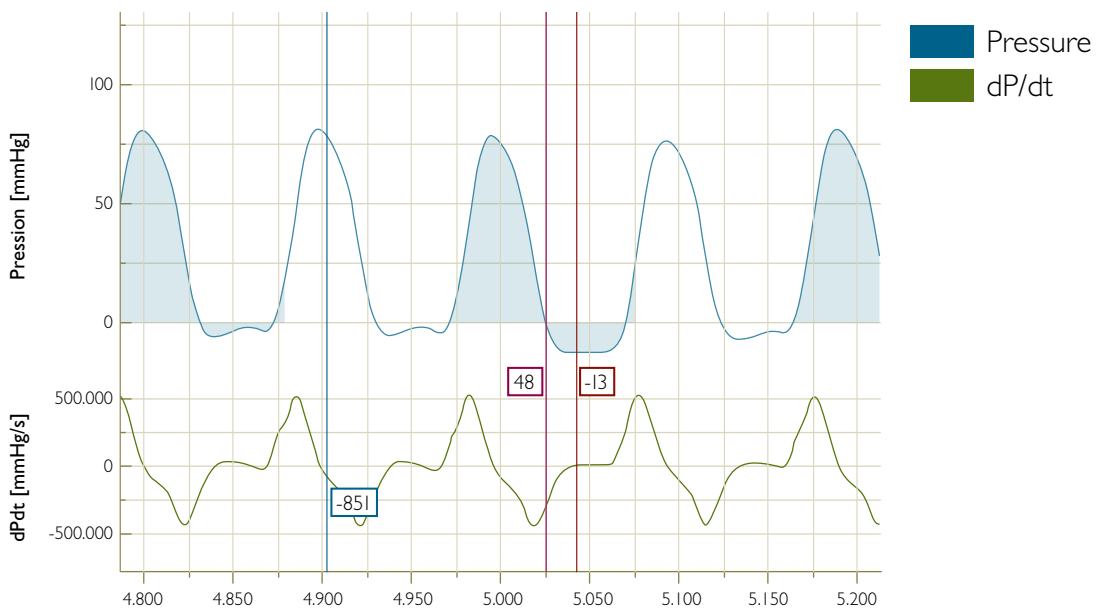


Doppler Mitral Diastolic Filling (apical view)

b. Left-ventricular catheterization under anaesthesia

This test can be used to confirm cardiac phenotypes detected by echocardiography and to perform acute pharmacological screening. This time-effective method allows to record intracardiac pressures and related haemodynamic parameters ("gold standard" for cardiac haemodynamics).

Aortic blood pressure tracing



4. ATHEROSCLEROSIS

Atherosclerosis is a very important vascular disease affecting many people in industrialized countries. Its evolution over years is the substrate of many clinical conditions such as myocardial ischemia and infarction finally leading to heart failure. After being considered as simple consequence of passive lipid deposit, it is now known to be due to interaction of complex mechanisms involving the adventitial vasa-vasorum and the tunica media. Many genes have been identified as key regulators of atherothrombosis and transgenesis will probably offer the opportunity to identify new factors involved in the progression of this disease as well as for the induction of acute events. In our current scheme of cardiovascular phenotyping, aorta and great vessels are examined ex vivo for plaque formation and rupture.

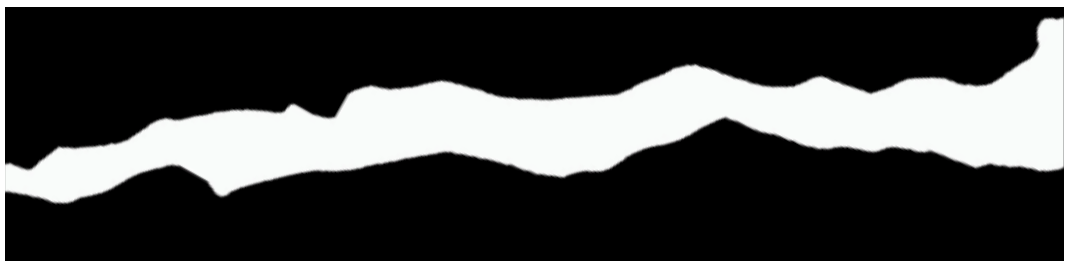
The heart lesion areas and total aortic areas are delimited and calculated using a proprietary software.



En face Sudan-IV-stained aorta



Lesion areas



Total aortic area

5. INDUCTION OF CARDIOVASCULAR PATHOLOGICAL STATES

In genetically engineered mice, many cardiac phenotypes cannot be detected under basal conditions. The animal models for cardiovascular diseases (hypertension, cardiac hypertrophy, heart failure) are very useful tools to challenge the cardiovascular system and to analyze the influence of a drug or a mutation. The cardiovascular follow-up is performed with all methods described above and at the end of the protocol, histological, molecular and biochemical measurements (blood and tissue biomarkers) are also performed.

Four standardized models of pharmacologically induced hypertension and cardiac hypertrophy are available :

- Induction of cardiac hypertrophy by infusion with isoproterenol. This model is currently used to assess cardiac hypertrophy linked with the beta-adrenergic system. This hypertrophy is associated with tachycardia.
- Induction of hypertension associated with cardiac hypertrophy by infusing angiotensin II with osmotic minipumps. This model is currently used to assess cardiac hypertrophy. In this model the hypertrophy is associated with hypertension (+40mmHg) and cardiac fibrosis.
- Induction of hypertension associated with cardiac hypertrophy by administration of L-NAME combined with a high-salt diet. In this model cardiac hypertrophy is associated with hypertension and cardiac fibrosis.
- Induction of hypertension associated with cardiac remodelling by DOCA administration and high-salt diet in unilaterally nephrectomized mice.

Models of myocardial infarction leading to cardiac remodelling and failure :

- Myocardial infarction induced by the ligation of the left main coronary artery.
- Cryoinjury-induced myocardial infarction (highly standardized method to create MI with low peri- and postoperative mortality).