Non-invasive cardiac output monitoring in pharmacology: a plethysmographic solution in rats.

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***Abstract*—Cardiovascular monitoring is of great importance in pharmacology but there is a lack of convenient non-invasive alternatives. Hence, we aim to evaluate the relevance of inductive plethysmography (IP) in preclinical cardiac studies. An IP system was specifically designed for rat. Its evaluation carried out using a mechanical test bench has shown appropriate instrumental performances for cardiac monitoring in rats. Measurements were also performed during a volume overload hemodynamic challenge in vivo in rats. The cardiac output variation has similar kinetic and amplitude when compared to results of previous studies. This suggests that our system is suitable for cardiac output monitoring in rat.**

I. Introduction

As one of the three vital functions with respiratory and central nervous system, cardiovascular system is of great importance in pharmacology. The evaluation of pharmaceutical deleterious effects on the cardiac function is mandatory in safety preclinical assays according to the ICH guideline S7 [1]. Additionally, in pathophysiology, cardiovascular diseases are a major field of research in which rat is a widely used model [2]. In this context, gold standard methods used to monitor cardiac output, including implanted telemetry or ultrasonic flow probe, are very invasive. Alternative methods such as tomography, magnetic resonance imaging, echocardiography, or pulsed doppler remain complex to carry out and require heavy equipment [3], [4]. This limits their utilization on pathophysiological use cases. Indeed, these situations are characterized by the apparition of fragile and evolving phenotypes, for which new investigation methods as less obtrusive as possible have to be developed. Moreover, beyond the scientific interest of non-invasiveness, ethics is also a matter in preclinical research as legislative and public pressure steadily increases.

Inductive plethysmography (IP) is a wearable non-invasive measurement technique historically used for respiratory rate and ventilation monitoring in humans [5]. IP enables measurements of instantaneous trunk volume variations caused by respiratory and cardiac fluid exchanges. Exploitation of the cardiac component of the plethysmographic signal has been studied for many years. Works on thoracocardiography have shown the interest of this technique for qualitative cardiac function monitoring [6], [7]. More recent works have proven that calibration of cardiac component can be done using a classical respiratory calibration procedure [8]. As considered by Aliverti *et al.* [9], the cardiac component corresponds to blood exchanges between the trunk and the periphery of the body. In humans, correlation between blood shifted out of the trunk (ΔVtr_c) and stroke volume (SV) has been demonstrated [10]. All these results suggest potential relevance of IP for non-invasive cardiac output monitoring.

Commercial wearable IP systems are available for preclinical respiratory monitoring on medium mammals. But to the best of our knowledge no relevant use for this technique has been described in rodents. The challenge is to revisit IP to make it suitable for very small physiological volume variations in rats. The aim of the current work is to present preliminary evaluation of the technology as a non-invasive tool dedicated to cardiac output monitoring in preclinical studies. In that purpose, performances of the apparatus were first estimated on a test bench. Secondly, cardiac output evolution was monitored during a physiological challenge induced in anesthetized rats.

II. Material and Methods

A. Inductive Plethysmography in rats

IP transducer is a conductor that surrounds the section to measure. When the electromagnetic field is uniform and its orientation is stable this coil has a self-inductance that linearly depends on the surface surrounded. Relative variations of the transducer inductance are proportional to section variations.

The technological challenge mainly comes from scale differences between rats and humans: rat's physiological stroke volume (SV) is likely to range from 100–500 µL [11], [12]) depending on the weight of the animal, while normal SV for adult human is generally 70 ml. As inductance is proportional to section, the inductance of the coil and its relative variations will drop accordingly when transposing to rats; the order of magnitude of inductance in rats is a few hundred nanohenries.

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Existing IP systems have not been designed to monitor these tiny inductance variations. Hence, they do not have proper characteristics as resolution, noise levels or bandwidth. Recent integrated circuits as the Texas Instruments LDC family were put aside because they are not suitable with our coils. We have adapted classical inductance measurement schemes used in IP to ensure proper digital resolution, noise levels and bandwidth in order to create a high-resolution inductance to digital converter dedicated to cardiac monitoring.

The apparatus specifically designed is made of a tubular jacket with a transducer positioned on the thorax. The transducer wire is sewn in a wavy pattern to enable elasticity. The coil is connected to the high-resolution inductance digitizer converter. Samples are read by a MCU and sent to an acquisition PC through a RS232 serial link.

In a matter of characterization of the device, we have designed a mechanical test bench to apply controlled volume variations (Fig. 1a and 1b). The bench geometry is a 1 degree of freedom model of the trunk of the rat: the distance between two parallels half-cylinders surrounded by the jacket is controlled with a micrometric translation stage. Volume variations seen by the plethysmographic system (V_{IP}) correspond to the volume of the parallelepiped that separates the half-cylinders. In this configuration, sections are equivalent to volume variations and are directly proportional to the variation of the distance between cylinders. Bench geometry aims to represent geometrical features of the trunk of a 350 g rat with a radius of 2 cm and a height of 3 cm.

V_{IP} variations were recorded while repeated controlled step volume variations of 58 µL were applied using the test bench. V_{IP} has been calibrated by averaging the absolute peak-to-peak amplitude of these steps on 20 cycles. Then a correcting factor was applied to equalize it with the mean amplitude applied by the test bench. The amplitude of each step was then measured on the calibrated V_{IP}. The precision achievable with the device was estimated by calculating the standard deviation on 20 samples.

Noise level has been estimated on a constant volume level. Signal to Noise Ratio (SNR) has been calculated from the ratio between expected peak-to-peak amplitude of physiological cardiac volume and the noise level previously obtained.

B. Animal preparation

Experiments were conducted in accordance with the Directive L276-33 2010/63/EU on the protection of animals used for scientific purposes and the recommendations from the Declaration of Helsinki.

Six healthy four-month aged male OFA rats (Charles River) weighing 380-430g were used. The animals were first anesthetized with an injection of ketamine (50 to 75 mg/kg) and xylazine (10 to 15 mg/kg) and then dressed with our plethysmographic garment. A cannula was inserted in the jugular vein in prevision of later injection of NaCl for the volume overload. A tracheotomy was used to intubate and mechanically ventilate the rats (tidal volume: 2.5 ml and rate: 60 strokes per minute) with a Harvard Model 683 Small Animal Ventilator (Harvard Apparatus, Holliston, Massachusetts USA). To maintain and control anesthesia level during the experiment, a mix of air (78-79%), O_2 (20%) and isoflurane (1–2%) was used. Finally, the animal was equipped with standard human ECG diagnostic electrodes (Red Dot, 3M, St Paul Minnesota USA) placed on paws in a D2 configuration (left and right arms, right leg) as shown on the Fig. 1c.

The protocol used to induce an increase in heart pumping capability is described in Fig. 2 NaCl infusion (15 ml/kg/min) into the jugular vein in 1 minute. This “volume overload” is known to increase the venous return and thus significantly raise the stroke volume. Immediately at the end of the overload a second cardiac sample was taken (T1+1). Then samples of cardiac activity were acquired at T1+2, T1+5, T1+10 and T1+15. For cardiac activity measurements along the protocol, 10 seconds apneas were imposed at each sample time.

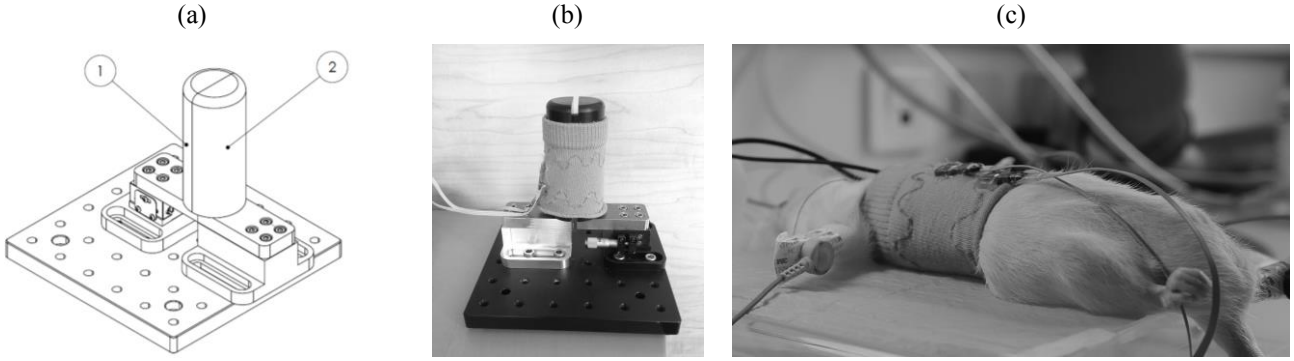

Figure 1. (a) Test bench. Volume surrounded by DECRO inductance plethysmography jacket is proportional to the distance separating the two half cylinders (1 and 2) that is controlled with a micrometric translation stage. (b) The DECRO inductance plethysmography jacket on the test bench. (c) Rat equipped with DECRO inductance plethysmography jacket device, mechanically ventilated and connected to ECG acquisition system.

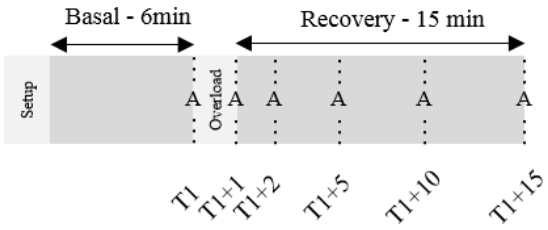

Figure 2. Experimental protocol: after 6 minutes in basal conditions a 15 ml/kg volume overload is performed (T1) in 1 minute. Recovery of the animal is monitored for 15 min with regular samples of cardiac activity measured in apnea (indicated as A).

C. Recorded signals and treatments

Trunk volume variation signal ($Vtr(t)$) was acquired at 100 Hz with the inductive plethysmography system developed in the laboratory (DECRO, TIMC-IMAG Lab, France). ECG was conditioned with BioAmp and acquired at 500 Hz with a PowerLab acquisition system and Chart Software (ADInstruments Pty Ltd). Synchronization between systems was ensured by connecting a trigger signal to one of the inputs of the Powerlab and using acquisition start trigger feature.

Using Chart software, a 50 Hz notch filter was applied on ECG signals to remove powerline interferences. The heart rate (HR) calculation algorithm integrated in the software provided a convenient solution to monitor the animal during experiments.

Signal post processing was performed using Matlab software (Matworks, Massachusetts, USA). A numeric band pass FIR filter (F_{CL} = 2 Hz, F_{CH} = 40 Hz, Order = 100) was applied to ECG. HR was calculated from R-R interval detection on ECG signal. $Vtr(t)$ signal was filtered with a numeric low pass FIR filter (F_C = 15 Hz, Order = 100). Calibration of $Vtr(t)$ was performed with a method derived from [6], [7], [13] and similar to the one used with the test bench. Respiratory minima and maxima were detected on $Vtr(t)$ on 10 respiratory cycles. The height of each cycle is expected to correspond to the tidal volume. Therefore, a correcting factor was applied in order to equalize the amplitude of this arbitrary volume with the tidal volume of the ventilator.

$Vtr(t)$ contains both respiratory (Vtr_r (t)) and cardiac ($Vtr\_c(t)$) components. A break of the ventilator (apnea) totally removes the respiratory component of the signal. Blood volume shifted (ΔVtr_c) was calculated from the difference between maxima and minima of each cardiac cycle in $Vtr\_c(t)$. ΔVtr_c was averaged on all cardiac cycles of each apnea.

An image of the cardiac output CO was estimated from the product of the heart rate (HR) and ΔVtr_c.

$$CO = HR * \Delta Vtr\_c. \quad (1)$$

III. Results

A. Quantitative test-bench characterization

The precision of the test bench is first evaluated: for 20 repetitions of a 58 µL volume variation applied with the test bench, the standard deviation measured is 7 µL. Once calibrated, the precision of V_{IP} is then estimated: for 20 steps of 58µL the standard deviation of V_{IP} is 5 µL. Fig. 3 shows V_{IP} obtained on the test bench. The numeric resolution of the system visible on the last part of the curve is 2 µL. Noise level is equal to quantization noise (4 µL). Considering a cardiac signal of 100 µL peak-to-peak, the SNR of the system is greater than 26 dB.

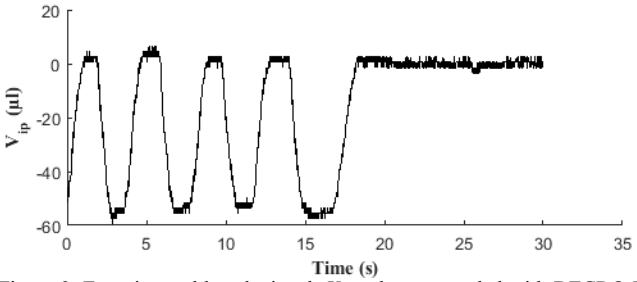

Figure 3. Experimental bench signal: V_{IP} volume recorded with DECRO IP jacket placed on the mechanical test-bench with repeated volume variations of 58µL. Last part of the signal (from 18 s) shows the noise level and quantization noise.

B. Qualitative physiological validation

During apneas, we observe periodic variations of $Vtr(t)$ on all the animals. As shown on Fig. 4, these variations are concomitant to the ECG R-peaks.

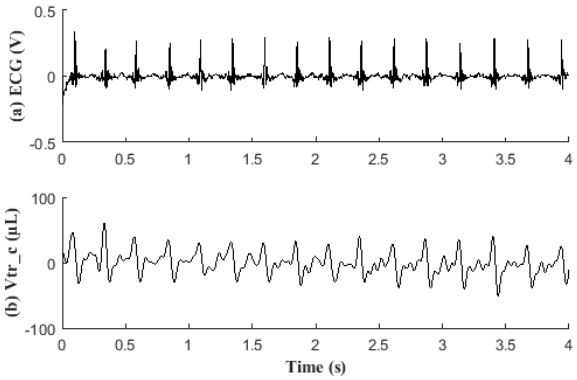

Figure 4. Experimental synchronized signals in rat: (a) Filtered ECG, (b) *Vtr_c (t)* during one apnea.

For each rat, mean cardiac parameters (HR, ΔVtr_c, CO were calculated at each apnea.

TABLE I. CARDIAC PARAMETERS

Time	HR (bpm)	ΔVtr_c (µL)	CO (ml/min)
Basal	226±23	66±14	14±3
T1+1	221±18	98±18	22±4
T1+2	231±21	101±51	22±11
T1+5	225±22	75±32	16±7
T1+10	225±22	67±27	14±6
T1+15	224±25	70±31	15±7

Values are mean ± SEM (n=6 rats)

Volume overload leads to an immediate increase of 48% of the cardiac output from 14 to 22 ml/min. Return to basal activity occurs after ten minutes (Fig. 5).

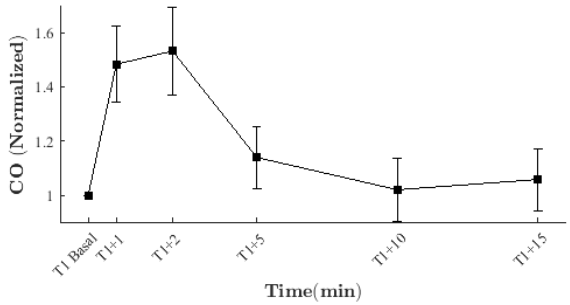

Figure 5. Normalized CO variations (means ± SEM) measured with DECRO IP jacket during volume overload performed at T1 (n=6).

IV. DISCUSSION AND CONCLUSION

Regarding the instrumental challenge, the experiments on the test bench have shown that the precision is estimated to be lower than 7 µL. The SNR of 26 dB may be improved by reducing the bandwidth to physiological frequencies. Noise, resolution and precision values obtained are lower than 10% of the expected cardiac amplitude (≈100µL). This confirms the aptitude of the device to perform physiological measurements of volume variations in rats.

Based on the measurement of cardiac variations of the trunk, it has been possible to qualitatively monitor a hemodynamic challenge. The cardiac activity evolution observed during this challenge has similar kinetic and amplitude (+48% after 1 min and return to baseline in 10 min) when compared to results previously obtained in similar studies (from +60% to +120%), with return to baseline in 10 min [12], [14], [15].

As cardiac frequency is constant during the experiment we can consider CO and ΔVtr_c as equivalent parameters representing cardiac activity. Calibration performed from respiratory yields to CO values that are smaller compared to physiological values [11], [12]. As expected, this observation is in coherence with results in humans [9], [10]. However ΔVtr_c values estimated (66±14 µL) are very close to the values obtained with aortic flow probes on animal with similar weight (81±11 and 73±4 µL) [14], [15] . This suggests the cardiac parameters available with our system are close to those obtained with aortic flow probes, referenced as the gold-standard.

To conclude, our plethysmographic device is suitable for cardiac function monitoring in rats. Future works will concern the explanation of this proximity: a model will be elaborated then validated by simultaneous monitoring with our system and an aortic flow probe. More realistic physiological conditions should also be ensured with proper signal extraction of cardiac component out of the respiratory one. Cardiac signal processing algorithms already validated in humans [10] will be implemented for rat signals.

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