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ORIGINAL ARTICLE

Non Invasive Measurement of Systolic Blood Pressure in Rats: A Simple Technique

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Abstract: *Background:* Non invasive, simple and economical instrument to measure blood pressure in r365-ats is important in cardiovascular research. *Methods:* Systolic blood pressure measuring instrument was fabricated using a tail cuff, photoplethysmograph, pressure transducer and PC with Biopac Software for recording. Tail cuff was used to occlude the tail artery, photoplethysmograph picked the blood flow pulses in the rat tail and the pressure transducer measured the cuff pressure and converted it into analog voltage. PPG signals were converted into voltage and amplified by the recording system with two channel amplifiers and in addition it also amplified analog voltage converted by the pressure transducer. *Results:* Calibration of the instrument entailed a simple Bland Altman's plot of pressure recorded as voltage changes against pressure changes in the mercury sphygmomanometer which is considered as gold standard. A regression value of r=0.9 and p=0.00 was obtained. A pilot study was done on ten female rats. Three blood pressure readings were taken on two occasions. Between - animal variation of BP was 123±7 (mean SD), CV=5.9% and within - animal variation of BP was 120 ±7, CV=5.5%. *Conclusion:* The tail cuff and PPG based technique to measure systolic blood pressure in rats is simple, economic, accurate and reliable.

Key words: tail cuff, blood pressure, photoplethysmograph (PPG)

Introduction

Objectives: To develop a non invasive, simple and economic blood pressure measuring system that can be used for physiological experiments in a laboratory and to evaluate the blood pressure against a sphygmomanometer which is considered the gold standard

Background: Measurement of blood pressure (BP) is an important entity in cardiovascular research in animals. Both direct and indirect methods can be used to measure BP in many experimental animals; commonest among them are the rats. Direct method involves recording BP either using indwelling catheters or radio telemetry [1].

Catheter is inserted into one of the major arteries like aorta, carotid or the femoral. The indwelling catheter is then exteriorised and connected to a pressure transducer to measure BP directly. This is the most accurate and a gold standard to validate other methods of BP measurement. This procedure is usually done on anesthetised rats for terminal experiments and it is important to account for the physiological effects of anaesthesia and surgical procedure [2]. Radiotelemetry involves a surgical procedure and implantation of a radiotransmitter in the animals' body and a remote receiver that records the BP. Using these methods, accurate BP can be measured continuously in freely moving animals in experiments over long periods of time [2-3]. Disadvantages include morbidity associated with surgery (for transmitter implantation or replace battery), high cost of setup, transmitter, maintenance, material and human resource [4].

Device to measure blood pressure indirectly/non-invasively in animals is important to researchers. BP can be measured on tail or limb of the animal. Methodology consists of utilizing an external inflatable cuff placed on the tail or limb to occlude the blood flow. An occlusion tail-cuff is used to occlude the caudal artery of the tail and blood flow pulse is sensed by a sensor placed distal to the tail cuff. BP is determined by monitoring the appearance and disappearance of pulse along with the pressure changes during inflation and deflation of the cuff [2].

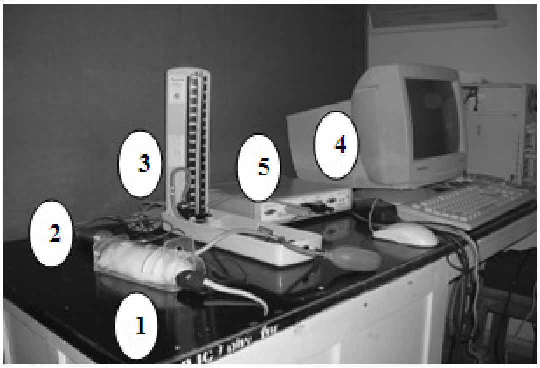
Commonly used non-invasive blood pressure sensor technologies include photoplethysmography (PPG), piezoplethysmography and volume pressure recording. Regardless of the type of sensor used all of these methods share certain advantages and disadvantages [5-7]. Oscillations in blood volume induced by the blood pressure pulses are picked up by the PPG. During blood pressure measurements when pressure in the cuff increases to above the systolic BP the pulses disappear and reappear when the pressure in the cuff decreases below systolic BP. Systolic BP can be determined by measuring the pressure value in the cuff when PPG pulse reappears during deflation. This is a simple technique and does not need calculations or formulas [5]. This non invasive recording system is valuable in experimental hypertension research as repeated measurements can be obtained in conscious animals [8] without preheating [9]. This method can be used to screen large number of animals for systolic hypertension or differences in systolic BP. Disadvantages being, this method is not recommended in animal experiment that intends to capture subtle changes in blood pressure [5] and the high cost of this instrument. Studies have shown that indirect BP measurement is accurate, reliable and when compared to the direct BP measurement is similar [8]. Keeping in view the advantages and disadvantages we set out to build an economic alternative to record systolic blood pressure non-invasively using a tail cuff and PPG. This BP recording device was intended to conduct simple physiological studies to assess the cardiovascular function in rats.

Material and Methods

Ethical approval was obtained prior to the study from the Animal Ethical Committee, St Johns Medical College, Bangalore

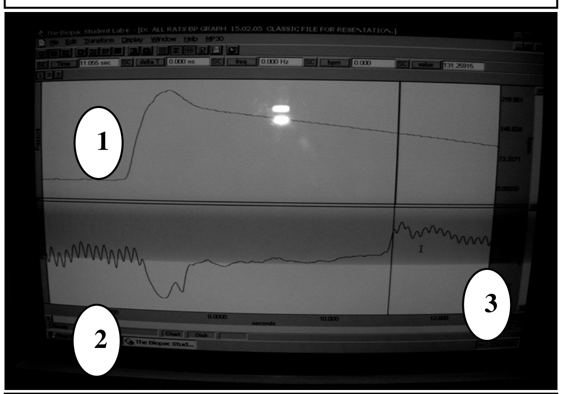
Description of apparatus
Instrumentation: Planned to develop and calibrate a rat-tail cuff in the department of physiology. The components included an inflatable cuff, pressure transducer, mercury sphygmomanometer, photoplethysmograph (PPG) and a recording system, BIOPAC MP 30.

Figure-1: Blood pressure recording set up



1. Rat placed in a restrainer with the tail cuff.
2. Pressure transducer
3. Mercury sphygmomanometer
4. Computer
5. BIOPAC Systems

Figure-2: BIOPAC and Computer with two active channels



Simultaneous recording of
1. Pressure within the tail cuff
2. Pulsations of the tail artery
3. Line placed when pulsations begins during deflation of tail cuff to ascertain systolic blood pressure

Tail cuff construction: This resembled the blood pressure measuring cuff used in human beings. Thin (0.5mm) translucent rubber was used to make the bladder; it measured 1 cm in width and 3 cm in length [10]. This was placed in a cuff made of non stretchable cloth and wrapped on the tail and secured using velcro. A mercury sphygmomanometer was attached to the bladder via a thin non collapsible rubber tube.

Photoplethysmograph (PPG) (BIOPAC System INC. Santa Barbara, CA93117, USA version 3.8.2): We used a photoplethysmographic device that uses infra red light as source of light and infra red light photo sensor for pulse detection. It measures the changes in blood volume. A light source in the transducer transilluminates the tail and a photo detector detects changes in light intensity within the tail caused by pulsatile variations in blood volume. The technique is based on the differential light absorbing characteristics of tissue and blood; living tissue is relatively transparent to light in the infrared range, whereas blood is relatively opaque to light within this frequency range. The PPG consists of infrared (900 nm) light emitting diode as the light source and a photo detector diode; both the source and the detector are placed opposite to each other over the tail held by Velcro. The output of the photo detector IR diode is passed through a notch filter with a notch frequency of 50 Hz, this is followed by an amplifier and a fourth order Butterworth filter having a cutoff frequency of 40 Hz and the resulting filtered output is the required pseudo-blood-pressure signal, which is stored in a PC using a data acquisition system.

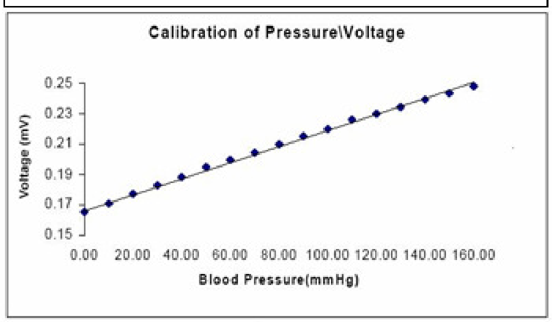
The photoelectric sensor detects pulsation at close to room temperature without stressing animals and these have been in use for more than 2 decades [5,11].

Pressure transducer (17318 Statham P23AA, HATO REY, Puerto Rico): This device consists of four bonded strain gauges forming a bridge connection which measures the strain produced on diaphragm by the pressure applied. The pressure transducer is used to measure the inflation pressure and convert it to analog voltage which is then amplified for recording.

Recording system (BIOPAC System INC. Santa Barbara, CA93117, USA version 3.8.2): Computer with Biopac MP30 software was used as an acquisition system. Two active channels operated with 2-channel amplifier and cables. One channel was connected to the pressure transducer that converted pressure into analog voltage which was then amplified for recording. The second circuitry within the amplifier converted blood flow changes form the tail pulsation into voltage suitable for recording.

Results

Figure 3: Calibration Plotting



Calibration: In clinical measurements comparison of a new measurement technique with an old one is needed to see if they agree sufficiently for the new to replace the old. Blood pressure recorded by the pressure transducer was compared with pressure recorded using mercury sphygmomanometer (Aesculap, Nova-Presameter, Jetter and Scheerer, Germany) which is considered as gold standard for calibration. Pressure sensed by the pressure transducer recorded as voltage changes by the instrument (Biopac) was plotted against the level of mercury observed by controlled inflation for every 10 mm Hg. The results were plotted using Bland Altman's plot [12] and a regression value of r=0.9 and p=0.00 was obtained.

Standardization and Pilot study: A pilot study was done on ten rats using two channels with PPG and pressure transducer. Heart rate and blood pressure was recorded using the tail cuff and acquisition system. Measurements were done on 10 female rats aged between 3-4 months. Three readings of systolic BP was taken with a gap of 2 min between readings. BP was recorded on two occasions with an interval of two days. Between subject variability of BP was Mean SD 123±7, CV=5.9% and within subject variability of BP was 120 ±7, CV=5.5%.

Conclusion

This non invasive technique to measure systolic BP in rats is accurate, reliable simple and economical. It is an ideal instrument for cardiovascular research in physiology laboratories to measure parameters like heart rate and BP in rats.

To ensure that BP recording is accurate it is essential to conduct the procedure in a quiet room with room temperature between 22° C to 26° C, and to get the animals habituated to the research workers and the restrainer.

Acknowledgement

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