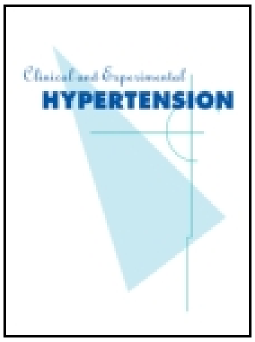

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Blood Pressure Measurement in Freely Moving Rats by the Tail Cuff Method

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Abstract

Inconsistency in consecutive blood pressure values is one of the most frequently observed problems in tail cuff method. The aim of this study was to measure blood pressure using the tail cuff method in rats without heating, anesthesia, and movement restriction. In this study, it has been shown that blood pressure measurement could be obtained without problem using the tail cuff method in freely moving rats in their cage environment. Also, the reliability of consecutive blood pressure values obtained from freely moving rats was higher than ether anesthesia and restricted groups.

Keywords: blood pressure measurement, freely moving rat, ether, restrainer, tail cuff

INTRODUCTION

Blood pressure measurement (BPM) can be carried out in two ways: direct and indirect. There are several advantages and disadvantages to direct and indirect BPM methods. Among the direct measuring techniques, the telemetry method does not require anesthesia or movement restriction during measurement (1–3), and it allows for long-term, continuous BPM (1,4,5). However, compared with the other methods, the telemetry method is more expensive (1,3). Moreover, in this technique, the experimental animal is exposed to a surgical procedure and anesthesia before measurement (1,3,6). Also, potential infection can be added to these disadvantages (1). From the direct BPM methods, the arterial catheter method allows for continuous and reliable BPM. At the same time, this method is not as expensive as the telemetry system. However, this method has disadvantages similar to the telemetry method. Exposure of experimental animals to surgery, anesthesia for the placement of a catheter, catheter occlusion, infection, and restriction of the animal are among the disadvantages of this method (1,3).

The tail cuff method, which is an indirect BPM method, is frequently used (7,8). In previous studies, a strong correlation was shown between direct measurement methods and the tail cuff method (9–13). Compared with direct methods, the tail cuff method is much easier, because it does not require surgical intervention or manual dexterity. Moreover, multiple recordings can be made from different experimental animals using the same probe (1,3,9). For this reason, it is appropriate for measurement of systolic BP changes in a large number of animals. However, the impossibility of continuous measurement is a limitation of this method (1). Also, heating, anesthesia, and restriction are the other disadvantages of this method. Although tail cuff method is a recommended method in the measurement of substantial changes in systolic blood pressure (SBP), it is not recommended in the measurement of diastolic BP or pulse pressure of conscious rodents and observation of the relationship between BP and other variables (1,3).

Some researchers reported inconsistent values in consecutive measurements by the tail cuff method (14). Possible reasons for this include heating, anesthesia, and restriction; in previous studies, heating (15), anesthesia (16,17), and movement restriction (18–20) were shown to change BP values. For this reason, it was aimed to carry out more consistent and reliable BPM with tail cuff method by eliminating some of its disadvantages.

MATERIALS AND METHODS

Animals and Study Design

All experimental protocols conducted on animals were consistent with the National Institutes of Health

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Guidelines for the Care and Use of Laboratory Animals (NIH Publication No. 85-23) and approved by the Pamukkale University Ethics Committee of Animal Care and Usage. In this study, 28 six-month-old, Sprague–Dawley male rats were used, weighing an average of 250 ± 18 g. The rats were housed in a room with controlled temperature (23 ± 1°C) and relative humidity (50 ± 5%), and they were kept in transparent plastic cages (42 × 26 × 15 cm), each containing three to four rats, exposed to a 12:12 light/dark cycle. The food and water intake of the rats was not limited. The rats were randomly divided into four groups ($n = 7$). In the non-heated and freely moving (NHFM) group, the rats were not exposed to heat, anesthesia, or movement restriction. In the heated and freely moving (HFM) group, the rats were exposed to heat, without anesthesia and movement restriction. In the heated and ether (HE) group, the rats were exposed to heat and ether anesthesia. Finally, in the heated and restricted (HR) group, the rats were exposed to heat and movement restriction using the restrainer.

Handling and Training Period

To decrease sensitivity in the tails of the rats and habituate them to the experimental environment and researcher, the rats were exposed to handling and training every day for 1 week, before the BPM. Since the BPM procedure of each rat was predicted to be a total of 25 minutes (environment acclimation for 5 min, heating for approximately 10 min, and measurements for approximately 10 min) every day, each rat was exposed to 25 minutes of handling and training in the room where measurements were to be taken. On day 1, handling was done. On days 2–3 of the training period, handling was conducted; in addition, desensitization of the tails of rats was attempted by attaching soft clips with stiffness that did not disturb blood circulation. Furthermore, throughout the training period, an infrared heat source was also turned on to acclimate the HFM, HR, and HE groups to the heat source. On days 4–7 of the training period, rats were taken to the environment where measurements were to be made and the tails of the rats were cleaned. In order for the rats to be able to move when they wanted, after hanging the tail cuff and temperature probe cables onto a mobile device, the tail cuff probe (15 mm diameter, 30 mm length, MLT125/R, ADInstruments Co., Sydney, Australia) and the skin temperature probe (MLT422/D, ADInstruments Co.) were placed on the tail of the rat (Figure 1). After waiting for 5 minutes to acclimate the rat to the environment, the infrared heat source was turned on to heat the tail. After the temperature of the tail reached 37°C, the cuff was inflated 10 times at 1-minute intervals to acclimate the rat to the cuff pressure. While the cuff was being inflated, some rats attempted to remove the probe. As a deterrent to this behavior, a beep sound at approximately 50 db was given to the rats, preventing them from removing the probe.

Measurements and Analyses

After the 7-day handling and training period, rats were individually taken to the test environment without the application of any anesthetic materials (except the HE group) and their tails were cleaned. After waiting for 5 minutes for the rat to acclimate to the environment, an infrared heat source directed at the tail region was turned on. When the position of the rat changed, the heat source was readjusted. When the tail temperature reached 37°C, 200 mm Hg pressure was applied to the tail by inflating the cuff; then, the SBP was measured. SBP was measured 10 times at 1-minute intervals, and before each measurement, the tail temperature was checked to make sure it was 37°C. While the SBP was being recorded, three of the rats (one rat from the NHFM group and two rats from the HFM group) occasionally

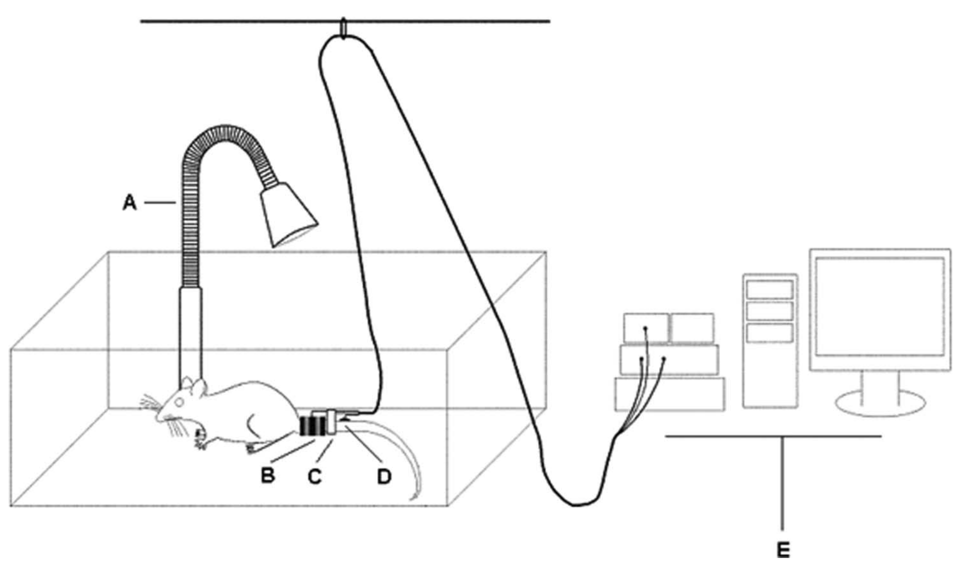

Figure 1. Blood pressure recording setup for heated and freely moving rats. **(A)** infrared lamp, **(B)** tail cuff, **(C)** tail cuff probe, **(D)** temperature probe, and **(E)** data acquisition system with computer. (This figure was drawn using a Microsoft Paint 5.1 program.)

moved. The recordings were obtained approximately 10–20 seconds after the end of the rats' movement. SBP measurement was made for 3 days, after the handling and training period. Training procedures and measurements of the rats in the NHFM and HFM groups were performed in the same cage where they lived. The same handling and training were performed on the rats in HE and HR groups. On the day of the measurement, the rats in the HE group were anaesthetized using diethyl ether (Fluka Chemical Corp., Hauppauge, NY, USA). Also, the rats in the HR group were placed in a plastic restrainer (Kursunluoglu Metal Co., Denizli, Turkey) during the training period and measurements. During all measurements, the ambient temperature was 23 ± 1°C and the tail temperature of the rats in the NHFM group was approximately 31 ± 1°C. The handling and training of the groups were initiated with 10-day intervals, and at the end of the 7-day period, the measurements were taken by the same researcher, at the same time of the day for 3 days (between 8.00 AM and 12.00 AM). SBP, heart rate, and tail temperature were recorded by a Powerlab 8SP data acquisition system (ADInstruments Co.) and Chart 5 software program (ADInstruments Co.). The average value of measurements obtained consecutively from each rat was considered to be that rat's SBP value. SBP and heart rate values of the HFM, HR, and HE groups were compared using one-way analysis of variance and post hoc Tukey test. A $P < .05$ was considered significant. The reliability of consecutive SBP values obtained from each of the rats was evaluated using the intraclass correlation coefficient (21). Values between 0.70 and 1.0 were considered to have high reliability.

RESULTS

The tail temperature of the rats of the NHFM group was approximately 31 ± 1°C during measurements. The blood flow pulsations of the tails of the rats at this temperature level could not be sufficiently recorded, so an accurate measurement could not be performed in the NHFM group. On the other hand, problem-free measurements were obtained from the HFM, HR, and HE groups' rats (Figure 2). Compared with the HR group, the SBP values of the HFM and HE groups were determined to be significantly lower. Also, the SBP values of the HE group were lower than those in the HFM group (Table 1). Moreover, the consecutive SBP values of the HFM, HR, and HE groups were found to be highly reliable. However, the reliability of the SBP values of the HFM group was higher than that for the HR and HE groups (Table 1). Also, the heart rate values of the HR group were significantly higher when compared with the values obtained from the HFM group (Table 1).

DISCUSSION

In this study, it has been shown that BPM could be obtained without problem using the tail cuff method in freely moving rats in their cage environment. Moreover, consecutive SBP values obtained from these rats were reliable. Also, the reliability of SBP values obtained from freely moving rats was higher than that obtained from the groups that were exposed to ether anesthesia or restriction. A possible cause for the more reliable measurements from freely moving rats is that these rats were exposed to good handling and training but anesthesia and restrictive techniques were not used. Because consecutive SBP values obtained from HFM rats were quite consistent, tail cuff method can be recommended for the measurement of not only substantial changes in BP values but also small changes in BP values.

However, since pulsations of tail blood flow of NHFM group rats were not recorded sufficiently, accurate BPM could not be done. Similarly, in our another unpublished study, accurate SBP measurements could not be done on rats that were placed on restrainer but not applied heating. Heating dilates tail artery and increases tail blood

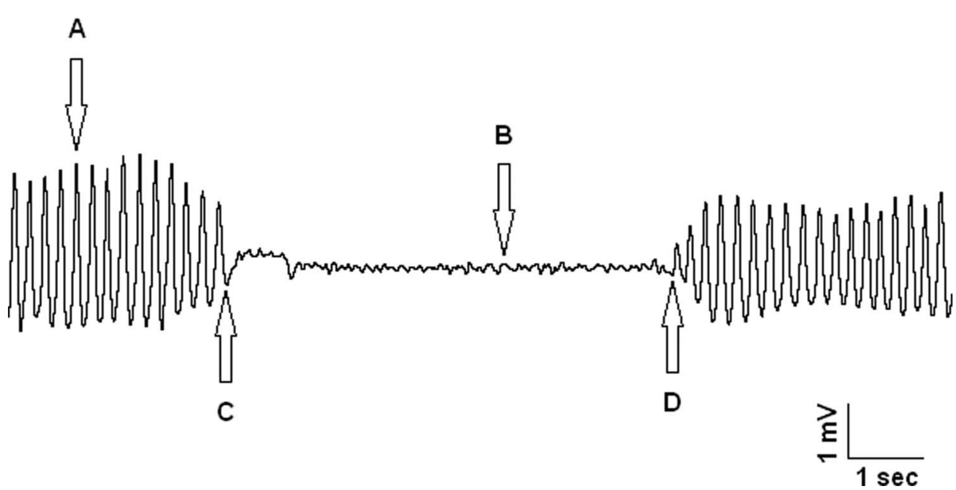

Figure 2. A sample of systolic blood pressure record from a rat from heated and freely moving group. **(A)** tail blood flow pulsations, **(B)** basal noise, **(C)** stopping point of blood flow, and **(D)** starting point of blood flow.

Table 1. Systolic blood pressure, heart rate, and reliability value of consecutive measurement of systolic blood pressure throughout experiment for 3 days

	Day 1			Day 2			Day 3		
Group	SBP (mm Hg)	r	HR (beats min^{-1})	SBP (mm Hg)	r	HR (beats min^{-1})	SBP (mm Hg)	r	HR (beats min^{-1})
HFM	112.4 ± 1.5	0.95	308 ± 9	113.2 ± 1.1	0.98	304 ± 11	115.1 ± 1.2	0.97	311 ± 7
HE	105.9 ± 2.7*	0.82	324 ± 12	102.5 ± 3.3*	0.76	331 ± 8	106.2 ± 3.1*	0.79	319 ± 13
HR	125.3 ± 2.2*,**	0.88	355 ± 16***	127.1 ± 1.9*,**	0.91	349 ± 12***	121.9 ± 2.5*,**	0.85	342 ± 15***

Note: $n = 7$ for each group.
Abbreviations: SBP – systolic blood pressure; HR – heart rate; r – reliability of consecutive systolic blood pressure values; HFM – heated and freely moving; HE – heated and ether; HR – heated and restricted.
*$P < .001$ versus HFM; **$P < .001$ versus HE; ***$P < .01$ versus HFM.

flow. Thus, it contributes to easy and correct measurements of BP (22). For this reason, some researchers have exposed the rats to heat in the heating chamber before the BPM with the tail cuff method (11,23,24). Another group of researchers has taken measurements using a heating chamber (11,25) or heating lamp (14). During the measurement of the HFM, HR, and HE groups, like many researchers, we applied heating. We believe that the heating method used in this study is more appropriate compared with keeping the rats in the heating chamber for a certain amount of time, because all the recordings in this study were obtained when the tail temperature of each rat was exactly 37°C. On the other hand, the tail temperature of rats that are heated in the heating chamber and let out may be different at the time of the measurement or in each of the consecutive measurements, and this difference can affect recorded BP values.

Diethyl ether is an anesthetic agent that is preferred for BPM in many studies (26). In this study, ether anesthesia caused a decrease in BP values and an increase in the heart rate. The effect of ether on BP and other cardiovascular parameters has been reported in previous studies (16,17). According to the results of this study, the reliability of the SBP values obtained from freely moving rats was higher than that obtained from the ether group. This may be due to the effect of diethyl ether on BP and a different response of rats to ether anesthesia.

In this study, the superiority of the method used in HFM group is that it eliminates some disadvantages of tail cuff method by way of doing measurements without restriction or anesthesia. However, in studies where measurements are done using the tail cuff method in awake rats, the movements of the rats have been restricted through methods such as the placement of rats in plastic restrainers (14,23,24), placement of rats on a platform higher than the ground, and fixation of the tail (27). Previous studies have shown that restriction of rats' movements causes stress and increases BP values (18–20). Also in this study, it was shown that restriction caused an increase in BP and heart rate values. On the other hand, the placement of the probe on the tail of an awake rat may also create stress. Due to these reasons, to reduce stress in rats and allow them to acclimate to the method, a handling and training program was prepared and performed. On the fifth day of the acclimation process, tail blood flow pulsations could be observed, and SBP values could be obtained from rats in the HFM group. However, these consecutive SBP values were not consistent and higher than the SBP values obtained from the same group after training period (data not shown). At the end of the 7-day acclimation period, reliable BP measurements could be obtained without any problems. Previous studies have shown that the handling decreases stress and affects BP and heart rate values (19,28). In this study, measurement has been facilitated by handling and training. So, at least one more week of additional time and effort is necessary.

In conclusion, in this study, BPM method used for HFM group can be recommended to research fields in which tail cuff method has been recommended.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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