ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 8, Issue 2, 2015

ISSN - 0974-2441

Research Article

# THE PRESENCE OF A LITTERMATE DURING MEASUREMENT OF BLOOD PRESSURE REDUCES THE ACCLIMATIZATION TIME IN CONSCIOUS NAÏVE RATS

**BRINNELL CASZO[1], ZANARIAH AWANG[2], JUSTIN GNANOU[3], HARBINDARJEET SINGH[2]***

**[1]Department of Physiology, Faculty of Medicine and Defence Health, Universiti Pertahanan Nasional Malaysia, Kem Sungai Besi, 57000, Kuala Lumpur, Malaysia. [2]Department of Physiology, Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh, Selangor, Malaysia. [3]Department of Biochemistry, Faculty of Medicine and Defence Health, Universiti Pertahanan Nasional Malaysia, Kem Sungai Besi, 57000, Kuala Lumpur, Malaysia. Email: hjsingh@salam.uitm.edu.my.**

***Received: 24 November 2014, Revised and Accepted: 29 December 2014***

**ABSTRACT**

**Objective:** Familiarizing an animal to the procedure is necessary for accurate measurement of blood pressure (BP). However, this requirement considerably prolongs the procedure, which in some instances could add to spurious results. There is, therefore, a need to establish ways that will help reduce stress and minimize the duration required for BP measurements in conscious animals. This study therefore examined the effect of the presence of a littermate on the duration to achieve the first acceptable blood BP measurement using tail-cuff plethysmography in naïve conscious rats.

**Methods:** BP was measured in 12-weeks old, in-house naïve male Sprague-Dawley rats, either without prior familiarization and without a littermate or after familiarization for 15 minutes, but without a littermate or after familiarization and with a naïve male littermate or without familiarization but with a naïve male littermate or without familiarization but with a naïve female littermate. Time to the first accepted measurement was recorded. Data were analyzed using one-way ANOVA and *post-hoc* analysis.

**Results:** The duration to the first acceptable BP measurement in familiarized and naïve rats was significantly shorter in the presence of a male littermate ($p<0.001$), but not with a female littermate when compared with that in naïve rats without acclimatization or the presence of a littermate.

**Conclusion:** It appears that the presence of a male littermate when measuring BP in naïve male rats significantly decreases the duration required for the measurement of BP. This might improve the efficiency of the procedure and even produce better data.

**Keywords:** Familiarization, Littermate, Tail-cuff rat, Blood pressure.

## INTRODUCTION

Adequate familiarization or acclimatization is essential for reliable measurement of blood pressure (BP) in laboratory animals [1]. Studies which look at the efficacy of anti-hypertensive agents (herbal and non-herbal) require accurate monitoring of BP in experimentally-induced hypertensive rats [2,3] require despite the advent of telemetric measurement of BP in conscious rodents, tail-cuff plethysmography still remains widely used for non-invasive measurement of BP in conscious rats. When measuring BP non-invasively in conscious rats, tail-cuff methods based on either photoelectric sensors or Doppler ultrasonic flow meters and volume-pressure recordings are commonly used [4,5]. However, these methods are affected by numerous experimental conditions, including prior exposure of the animal to the procedure, state of the animal and even ambient temperature [6-8]. Poor attention to these often contributes to labile or variable measurements. Accurate measurement of BP requires the animal to be adequately familiarized and as stress free as possible during the procedure.

Measurement of BP in the conscious rat often also requires the animal to be restrained over the duration of measurement, which could last between several minutes to an hour. An unnecessarily prolonged duration for the measurement of BP can be stressful to the animal, and is often the major cause of spurious BP recordings, as restraint has been shown to increase heart rate and BP in rats [9].

While factors affecting the measurement of BP in rats have been extensively examined, there is little data documenting the effect of the presence of a littermate, whether naïve or otherwise, on the duration of measurement of BP in rats. We hypothesized that the presence of a naive littermate alongside during the measurement of BP could help create a more familiar ambience and reduce stress, consequently reducing the time required for familiarization and measurement of BP, and at the same time providing a less variable and more accurate BP reading. This study therefore examined the effect of the presence of a naive male littermate on the time required to record BP by tail cuff plethysmography in unfamiliar or naive male Sprague-Dawley rats. In addition, it also examined the effect of the presence of a naive female littermate either in diestrus or proestrus on the measurement of BP in order to study the effect of gender of the littermate.

## METHODS

36, 12-weeks old in-house naïve male Sprague-Dawley rats were housed in standard cages at room temperature with a 12/12 hrs light/dark cycle and access *ad libitum* to water and food. They were divided into six groups with six rats per group. Each group of rats had its BP recorded as follows: (i) Without prior familiarization or presence of a littermate, (ii) with 15 minutes of familiarization but without a littermate, (iii) after familiarization for 15 minutes and in the presence of a naïve male littermate, and (iv) without prior familiarisation but in the presence of a naïve male littermate. The last two groups had their BP recorded after familiarization and in the presence of female littermates either in estrus or diestrus. During familiarization, animals were placed in appropriately sized cylindrical restrainers on a heated platform (Kent Scientific, USA) at 37°C with a deflated cuff fitted to the tail for 15 minutes prior to the measurement of BP as recommended by the manufacturers (CODA, Kent Scientific, USA). For the non-familiarized rats, BP measurement was commenced immediately following the application of the cuff. All littermates were held in similar restrainers that were placed next to but about 3.5 cm apart, from the animal whose BP was being measured. A group of male and female rats from the various litters delivered at about the same time by sister dams and had no previous exposure to BP measurement acted as naive

littermates. Male littermates were housed along with the experimental animals for at least 24 hrs in the same cage prior to the measurement. Female littermates though derived from the same colony or litter were maintained in separate cages after weaning. All BP recordings were made at the same time of the day. The estrus status of female littermates was confirmed through a vaginal smear done about an hour before the measurement of BP.

The restrainers used during the measurement of BP were cylindrical in shape and made of transparent acrylic with fitted darkened nose cones. Two sizes were used depending on the weight of the animal. A medium sized restrainer (diameter and length of 6.5 and 21.5 cm respectively) was used for animals weighing 200-300 g and a large sized restrainer (diameter and length of 7.5 and 22.5 cm respectively) was used for animals weighing between 300 and 350 g. The ambient room temperature was maintained at 22°C and the body temperature of the rats was measured frequently during the procedure using an infra-red thermometer (Thermoworks, China) to ensure that the animals were kept sufficiently warm. BP was recorded using tail-cuff plethysmography where the occlusion cuff maximum pressure was set at 250 mmHg. According to the recommendations of the manufacturer, a set of 25 measurements of BP was considered as a single cycle. Cycles were repeated if necessary, up to a maximum of two cycles. As per the setting, all animals went through 10 trial measurements of BP before the data was collected for analysis by the instrument. A computer algorithm provided in the software of the instrument selected the data points based on the minimum tail blood volume, duration between systolic and diastolic BP measurements, and the overall shape of the pressure trace to determine the acceptable measurement. The time taken to reach the first acceptable measurement, the total time required for measurement of BP, number of acceptable measurements, systolic and diastolic pressures, mean arterial pressure, heart rate and tail blood volume were recorded. Data are presented as mean±standard error mean and were analyzed using one-way ANOVA with a *post-hoc* bonferroni test. This study was approved by the Animal Users and Care Committee of Faculty of Medicine, Universiti Teknologi MARA, Malaysia.

## RESULTS

Data on the duration to the first acceptable measurement and the number of acceptable measurements are provided in Table 1.

Compared to naïve animals without the presence of a littermate, the duration required to reach the first acceptable BP measurement was significantly shorter in familiarized animals either with ($p<0.01$) or without a littermate ($p<0.05$). The duration was also significantly shorter in naïve rats whose BP was measured in the presence of a male littermate when compared with that in naïve rats without the presence of a littermate ($p<0.05$). No significant differences were evident in the duration required to reach the first acceptable measurement between naïve controls without a littermate and those naïve rats with a female littermate.

The presence of a male littermate during measurement, irrespective of whether the animal was familiarized or not, significantly increased the number of acceptable measurements recorded during a single cycle of 25 measurements compared with that in naïve animals without the presence of littermates (Table 1). No significant differences were evident in body weight, systolic, diastolic and mean arterial pressures between the six groups (Table 2). Similarly, heart rate, tail blood volume was also not different between the six groups (Table 2).

## DISCUSSION

The main observations of note from this small study are: (i) Prior familiarization to the procedure significantly reduces the duration required to reach the first acceptable or stable measurement of BP (Table 1), (ii) the presence of a male littermate during measurement either in familiarized or naïve rats significantly reduces the duration required for measurement of BP (Table 1), (iii) this effect is less or absent in the presence of a female littermate (Table 1), (iv) the number of acceptable measurements in a single cycle increases with familiarization and in the presence of a male littermate (Table 1), and (v) the presence of a littermate does not affect the eventual BP reading, heart rate and tail blood volume (Table 2).

Although the final BP recording was not affected significantly, the presence of a male littermate during measurement reduced considerably the overall time required for the measurement of BP in naïve male rats. In addition, measurements recorded in the presence of a male littermate in familiarized rats also showed less variation (Table 1). The immediate implications of the findings is that they could reduce considerably the overall time required for measurement of BP in rats, as the number of measurements in a cycle could be reduced and hence the duration of each cycle. In addition, the duration of restraint required could also be significantly reduced. Overall it could improve the reliability and quality of the BP data.

The precise mechanism by which the presence of a littermate influences the BP measurement in the rat is unclear and was not determined in this study but it might be related to a decrease in stress in the animal whose BP is being measured. It might be due to "social buffering" where a companion helps reduce the stress responses [10]. Though in this study we had not measured any surrogate or markers of stress levels, restraint required during the measurement of BP itself has been shown to increase BP, heart rate, and changes in stress hormone production [11]. This has been attributed to increased activity in the sympatho-adrenal medullary system [12], which can be mitigated by prior familiarization [12,13]. In spite of efforts to reduce the effects of restraint, it is undeniable and widely accepted, as noted in the recommendation of American Heart Association regarding animal BP recording that experimental animals do become stressed and a rise in BP is inevitable [14]. It was however interesting to note in this study that while familiarization to the procedure and equipment itself reduced the time required to obtain the first acceptable BP reading, the presence of a littermate further reduced this time (Table 1). In addition, the variability in the duration to the first acceptable measurement was also less when measurements were made in the presence of an adult male littermate (Table 1). Whether this was due to "social buffering" reducing the stress levels during restraint is uncertain. Since all male animals were housed together for at least 24 hrs before the measurement of BP, it is possible that some degree of "social buffering" could result [10]. Housing durations from as short as 12 hrs have been found to be sufficient to cause buffering in groups of Wistar rats

**Table 1: The duration to first acceptable reading total duration and mean number of acceptable measurements**

| Group | Duration (seconds) to first acceptable measurement | Total duration (seconds) per cycle | Mean number of acceptable measurements |
|---|---|---|---|
| Control | 390.83±272.06 | 801.67±0.15 | 9.5±2.89 |
| With familiarization for 15 minutes | 85.67±87.53* | 799.83±0.24 | 14.67±1.54* |
| With familiarization and littermate | 31.83±11.86** | 803.00±0.41 | 18.66±1.26** |
| Without familiarization but with a male littermate | 45.83±25.99* | 798.50±0.30 | 18±0.77** |
| Without familiarization but with a female littermate in dietrus | 373.50±236.45 | 798.00±0.57 | 11.17±2.39 |
| Without familiarization but with a female littermate in estrus | 241.00±178.10 | 800.17±0.22 | 15±1.24* |

*p<0.05, **p<0.01 compared to control

**Table 2: Body weight, heart rate, BP and tail blood volume in the six groups**

| Group | Body weight (g) | Systolic BP (mmHg) | Diastolic BP (mmHg) | Mean arterial pressure (mmHg) | Heart rate (beats/ minutes) | Tail blood volume (ml) |
|---|---|---|---|---|---|---|
| Control | 309.33±22.15 | 134.88±11.17 | 88.52±3.87 | 103.68±3.93 | 310.04±45.2 | 18.1±2.3 |
| With familiarization for 15 minutes | 294.17±30.65 | 129.50±4.14 | 91.82±2.0 | 104.03±1.74 | 370.98±14.6 | 22.4±1.7 |
| With familiarization and littermate | 289.67±42.42 | 135.01±13.31 | 95.93±5.09 | 108.66±5.20 | 360.85±13.5 | 19.6±1.9 |
| With male littermate | 339.50±30.16 | 146.41±8.43 | 97.58±2.38 | 113.49±2.70 | 330.47±43.5 | 18.9±2.3 |
| With female littermate in diestrus | 349.67±35.45 | 135.01±9.20 | 88.54±3.33 | 103.66±3.35 | 329.83±26.9 | 16.0±2.0 |
| With female littermate in estrus | 296.33±31.83 | 134.88±13.75 | 89.18±4.08 | 106.33±5.16 | 324.92±25.1 | 19.3±1.3 |

BP: Blood pressure

subjected to a conditioned stimulus [15]. While visual signals may cause social buffering in certain species of animals [16], in adolescent rats, however, touch or direct contact is necessary to reduce stress [17]. In addition, it was recently been shown that olfactory signals might also be involved in the mitigation of the fear response to a conditioned stimulus in male Wistar rats [11]. Animals exposed to the scent of a donor animal also expressed a reduced fear response [11] compared to those without a donor. This has been attributed to olfactory signals passing from the olfactory bulb to the posteromedial region of the olfactory peduncle, which is believed to be responsible for "social buffering" [18]. In our study, animals were separated from each other by restrainers with nose cones that were kept about 3.5 cm apart during the measurement of BP, and it is possible that any impact of social buffering might have only occurred through some olfactory signals.

The beneficial effect of the presence of a littermate on the duration required to reach an acceptable BP measurement was absent in the presence of a female littermate (Table 1). The reason for this is not apparent. The animals were all sexually matured, and it seems that the presence of an unfamiliar sexually matured female, whether in diestrus or estrus, does not exert the type of calming influence that a male littermate might do. Incidentally, the females were housed separately from the males after weaning and were always kept separate. Whether this had hindered "social buffering" or social regulation to take place with female littermates is unclear. Clearly additional studies are needed to examine this.

In conclusion, the findings of this study seem to suggest that measurement of BP in familiarized or non-familiarized male rats in the presence of a naive male littermate significantly reduces the time required for measurement of BP and variability of the measurement without compromising the quality of experimental data. Since many recording devices are actually designed for high through-put research and record BP from more than one animal at a time, it appears that this practice may actually benefit the researcher by increasing efficiency of the experiment and quality of the emerging data.

**REFERENCES**

1. Gross V, Luft FC. Exercising restraint in measuring blood pressure in conscious mice. Hypertension 2003;41:879-81.
2. Khorshid O, Abdel-Ghaffar E, Mishriki A, Galal A, Hareedy A. Possible cardiovascular protective effect of some PPAR activators in experimentally-induced hypertensive model in rats. Asian J Pharm Clin Res 2012;5(3):67-72.
3. Devi J, Pamba P. Antihypertensive activity of bamboo shoot: A review. Asian J Pharm Clin Res 2015;8(1):46-7.
4. Reddy AK, Taffet GE, Madala S, Michael LH, Entman ML, Hartley CJ. Noninvasive blood pressure measurement in mice using pulsed Doppler ultrasound. Ultrasound Med Biol 2003;29(3):379-85.
5. Feng M, Whitesall S, Zhang Y, Beibel M, D'Alecy L, DiPetrillo K. Validation of volume-pressure recording tail-cuff blood pressure measurements. Am J Hypertens 2008;21(12):1288-91.
6. Buñag RD, Butterfield J. Tail-cuff blood pressure measurement without external preheating in awake rats. Hypertension 1982;4(6):898-903.
7. Williams TD, Chambers JB, Henderson RP, Rashotte ME, Overton JM. Cardiovascular responses to caloric restriction and thermoneutrality in C57BL/6J mice. Am J Physiol Regul Integr Comp Physiol 2002;282(5):R1459-67.
8. Kubota Y, Umegaki K, Kagota S, Tanaka N, Nakamura K, Kunitomo M, *et al.* Evaluation of blood pressure measured by tail-cuff methods (without heating) in spontaneously hypertensive rats. Biol Pharm Bull 2006;29(8):1756-8.
9. Irvine RJ, White J, Chan R. The influence of restraint on blood pressure in the rat. J Pharmacol Toxicol Methods 1997;38(3):157-62.
10. Hennessy MB, Kaiser S, Sachser N. Social buffering of the stress response: Diversity, mechanisms, and functions. Front Neuroendocrinol 2009;30(4):470-82.
11. Stamp J, Herbert J. Corticosterone modulates autonomic responses and adaptation of central immediate-early gene expression to repeated restraint stress. Neuroscience 2001;107(3):465-79.
12. Kvetnansky R, McCarty R, Thoa NB, Lake CR, Kopin IJ. Sympatho-adrenal responses of spontaneously hypertensive rats to immobilization stress. Am J Physiol 1979;236(3):H457-62.
13. Chiueh CC, Kopin IJ. Hyperresponsivitiy of spontaneously hypertensive rat to indirect measurement of blood pressure. Am J Physiol 1978;234(6):H690-5.
14. Kurtz TW, Griffin KA, Bidani AK, Davisson RL, Hall JE. Recommendations for blood pressure measurement in animals: Summary of an AHA Scientific Statement from the Council on High Blood Pressure Research, Professional and Public Education Subcommittee. Arterioscler Thromb Vasc Biol 2005;25:478-9.
15. Kodama Y, Kiyokawa Y, Takeuchi Y, Mori Y. Twelve hours is sufficient for social buffering of conditioned hyperthermia. Physiol Behav 2011;102(2):188-92.
16. da Costa AP, Leigh AE, Man MS, Kendrick KM. Face pictures reduce behavioural, autonomic, endocrine and neural indices of stress and fear in sheep. Proc Biol Sci 2004;271(1552):2077-84.
17. Wilson JH. Prolactin in rats is attenuated by conspecific touch in a novel environment. Cogn Affect Behav Neurosci 2001;1:199-205.
18. Kiyokawa Y, Wakabayashi Y, Takeuchi Y, Mori Y. The neural pathway underlying social buffering of conditioned fear responses in male rats. Eur J Neurosci 2012;36(10):3429-37.