Research Paper

Comparison of blood pressure and sympathetic activity of rabbits in their home cage and the laboratory environment

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Methodological improvements in measuring cardiovascular parameters have meant that data can be collected from freely moving animals in their home cage. However, experiments in rabbits still often require them to be restrained in a laboratory setting. The aim of this study was to determine whether measurements collected when rabbits were placed in a holding box in the laboratory are representative of values obtained in freely moving conscious rabbits. Nine New Zealand White rabbits received two radiotelemetry implants to monitor mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA). The MAP measured in the laboratory (71 ± 1 mmHg) was similar to that in the home cage (69 ± 1 mmHg), but there was less MAP variability. The RSNA was also similar in both environments. In contrast, laboratory heart rate (HR) was 7% lower than home cage HR (181 ± 4 beats min^{-1}, $P < 0.001$), but HR variability was similar. Baroreflex gain, assessed by spectral analysis, was 19% higher in the laboratory than in the home cage due to lower MAP mid-frequency variability in the laboratory. Home cage circadian patterns of MAP and HR were strongly influenced by feeding and activity. Nevertheless, MAP and RSNA laboratory measurements were the same as average 24 h values and remained similar over several weeks. We conclude that while HR is generally lower in the laboratory, a valid representation of MAP and RSNA can be given by laboratory measurements.

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The advent of radiotelemetric devices in recent years has allowed collection of data from animals moving freely in the home cage and has meant that many cardiovascular parameters can be measured without disturbance to the animal or with the need for restraint. Multiple recordings can be made around the clock and over extended periods of time. In particular, circadian variation and the long-term effect of treatments, for example the progression of disease or effect of drugs, can be assessed in the home cage. Rabbits have been used in laboratories for cardiovascular research for more than half a century, with experiments conducted initially in anaesthetized, then in conscious animals (Korner, 1954). More recently, blood pressure and sympathetic nerve activity have been measured in freely moving rabbits (Van den Buuse & Malpas, 1997; Barrett *et al.* 2001; Burke *et al.* 2010), but not all procedures can be conducted in the home cage. Acute administration of drugs, acute exposure to stimuli such as hypoxia, and calibration of the sympathetic nerve signal (Burke & Head, 2003) are examples of procedures that still require the rabbit to be restrained at least temporarily. Furthermore, radiotelemetry recording requires a substantial economic investment, and there is also the need for surgery to implant the device, neither of which may be warranted for limited measurements (Kurtz *et al.* 2005). Thus, experiments in conscious rabbits in a laboratory setting continue to be of value, as evidenced by a number of recent publications (Kimotsuki *et al.* 2010; Korner *et al.* 2010; Clayton *et al.* 2011; Roatta *et al.* 2011; Guild *et al.* 2012).

One of the main reasons for the continued use of rabbits is that they are easily handled and able to sit quietly for extended periods in a holding box in the laboratory (Burke *et al.* 2010). However, to date there has been very limited comparison of cardiovascular parameters measured in rabbits restrained in a laboratory

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compared with those measured in the home cage. We have therefore examined resting cardiovascular parameters, renal sympathetic nerve activity (RSNA), as well as baroreflex gain and blood pressure variability, in rabbits confined to a holding box in the laboratory and compared them with those collected over 24 h from the same rabbits in the home cage. Importantly, we determined whether the relationship between the two environments changed over several weeks.

Methods

Ethical approval

Experiments were conducted in nine male New Zealand White rabbits (body weight 2.6–3.2 kg) and were approved by the Alfred Medical Research Education Precinct Animal Ethics Committee and conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Rabbits were housed in controlled conditions of light (lights on 06.00–18.00 h), temperature and humidity in individual cages (height, 54 cm; width, 66 cm; and depth, 67 cm). Water was available *ad libitum*, and rabbits were fed a normal pelleted diet at 12.00 h, supplemented with vegetables. At the end of the experimental period, rabbits were killed by intravenous administration of pentobarbitone (160 mg kg^{-1}).

Preliminary operations

Rabbits underwent two preliminary surgical operations under isoflurane anaesthesia after induction with propofol (10 mg kg^{-1} I.V.; Fresenius Kabi, Pymble, Australia). Anaesthesia was maintained with the inhalant isoflurane (3–4%, Abbot, Botany, NSW, Australia) that was delivered with oxygen (1 l/min) via an endotracheal tube using an open circuit inhalation anaesthetic apparatus (Komesaroff, Medical Developments Springvale, Victoria, Australia). A radiotelemetry transmitter (model TA11PA-D70; Data Sciences International, St Paul, MN, USA) and catheter (150 mm long with 0.7 mm diameter tip) was implanted in the aorta via a small branch of the left iliac artery. Two weeks later, the rabbit received a radiotelemetry implant for measurement of RSNA (model TR76S or TR46S; Telemetry Research, Auckland, New Zealand), with the battery and transmitter pack placed under the skin of the rabbit's flank (Dorward *et al.* 1985; Barrett *et al.* 2003). Carprofen (3 mg kg^{-1}, I.V.; Pfizer, West Ryde, NSW, Australia) was given before, 24 and 48 h after surgery for analgesia. The first experiment was conducted 1 week later.

Experimental procedures

Home cage measurements. The telemetric arterial blood pressure was obtained via a receiver (model RLA1020; Data Sciences International) positioned on the door inside each cage (Fig. 1). An index of locomotor activity was obtained by monitoring changes in the received signal strength that occurred during movement of the animal. For detection of activity, the transmitter had to move so that slight movements occurring during grooming or eating were not registered as activity. The telemetric RSNA signal was detected by a receiver/amplifier (model TR162; Telemetry Research) situated within 2 m of each cage. The signal was filtered between 50 and 5000 Hz, rectified and integrated using a low-pass filter with a 20 ms time constant. Mean arterial pressure (MAP) and heart rate (HR) data were collected continuously over each 24 h period, and RSNA data was collected for 15 min every 2 h, a regimen which gives a close representation of the underlying average (Guild *et al.* 2008; Fig. 1).

Laboratory measurements. Rabbits were confined to a single rabbit holding box (height, 20 cm; width, 16 cm; and length, 39 cm), which was partly covered to provide a light level similar to the rear of the home cage. Before the first experiment, rabbits were acclimatised to the box and laboratory by sitting in the box undisturbed for 2 h. Four experiments were conducted in the laboratory once a week. Pulsatile arterial pressure was measured by telemetry and also via a catheter, inserted in the ear artery under local anaesthesia, with a Statham P23ID strain gauge pressure transducer (Statham, Hato Rey, Puerto Rico; Fig. 1). One hour for recovery from handling was allowed before the experiment began. On the first laboratory experiment day (week 0), the telemetry signal was adjusted to match the ear artery signal, and this adjustment was applied to MAP measured in the home cage. In subsequent weeks, the telemetry and ear artery signals were compared to ensure that no systematic measurement errors occurred. Telemetry probes were later checked for consistency in measuring a known pressure in laboratory and home cage environments. The RSNA was detected by telemetry using the same system as in the home cage (Fig. 1). The RSNA response to smoke was also measured in the laboratory at weekly intervals to calibrate the electrode (Burke & Head, 2003).

Data analysis

Mean arterial pressure, HR, derived from the pressure pulse, and RSNA were digitized at 500 Hz using an analog-to-digital data acquisition card (National Instruments 6024E, Austin, TX, USA) and averaged over 2 s. The RSNA was normalized to the maximal RSNA recorded during the nasopharyngeal response evoked by smoke, and taken to be 100 normalized units (nu; Burke & Head, 2003). Resting levels of MAP, HR and RSNA were measured in the laboratory over 30 min between 10.00 and 11.00 h on days 0, 7, 14 and 21 (weeks 0, 1, 2 and 3). The home cage data for comparison with laboratory data were averaged

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over 1 h between 10.00 and 11.00 h, the quiet period before feeding, on days 1, 6, 13 and 20. One hour averages from the home cage were also made over 24 h on the same days. Values are expressed as means ± SEM. Data were analysed by repeated-measures analysis of variance that allowed for within-animal contrasts. For comparisons of laboratory data with average 24 h data, Student's paired *t* test was used. Type 1 error was controlled using Bonferroni and Greenhouse–Geisser corrections (Ludbrook, 1994). A probability of $P < 0.05$ was considered significant.

Spectral analysis. The beat-to-beat signals of instantaneous MAP corresponding to the 30 min resting period in the laboratory and the 60 min period between 10.00 and 11.00 h in the home cage were submitted to spectral analysis (Head *et al.* 2001). Baroreflex slope was included if the coherence between MAP and HR across several overlapping segments in the analysed frequency band was >0.4. The average power spectrum was calculated in the following three frequency ranges: low (0.075–0.2 Hz), mid (0.2–0.4 Hz) and high (0.4–1.6 Hz). The mid-frequency range in rabbits contains the peak sympathetic response, and there is close correspondence between baroreflex gain measured in this frequency and that estimated using other methods (Head *et al.* 2001).

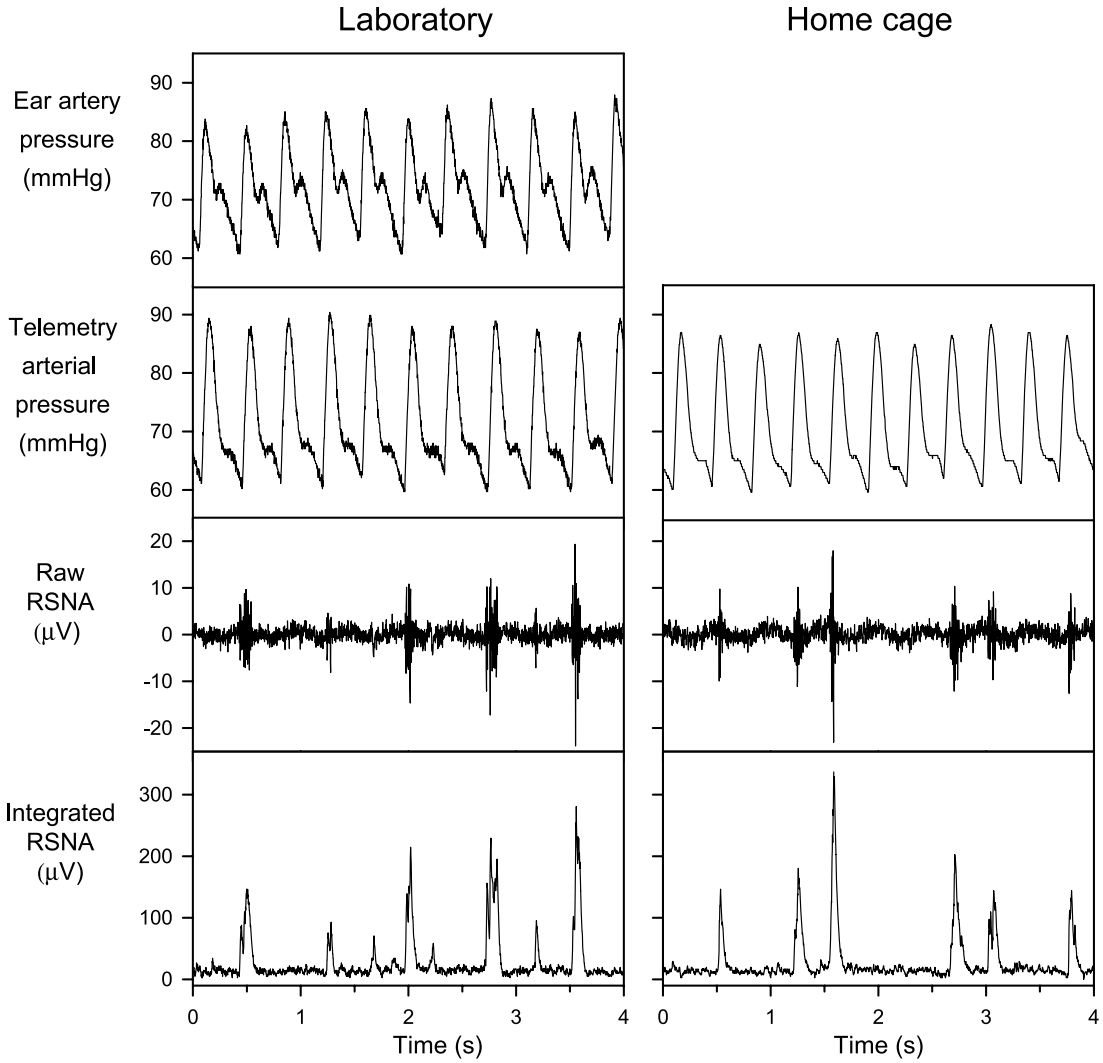

Figure 1. Traces of arterial pressure measured directly from the ear artery and by telemetry from the aorta, and raw and integrated renal sympathetic nerve activity (RSNA) from a single rabbit in the laboratory and in the home cage
Data were collected at approximately 10.00 h on day 6 from the home cage and day 7 in the laboratory.

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Results

Circadian pattern of haemodynamic variables, RSNA and locomotor activity in home cage

All parameters averaged hourly over 24 h in the home cage showed a circadian pattern that was associated with feeding and not the light–dark cycle. The MAP, HR and RSNA averaged over 24 h and over 4 weeks were 72 ± 1 mmHg ($n = 9$), 199 ± 4 beats min^{-1} and 8.3 ± 1.2 nu ($n = 6$), respectively (Fig. 2). The MAP and RSNA values averaged over the 12 h when lights were on were similar to values averaged over the 12 h of darkness, but HR was 6% higher during the daytime hours than the night-time ($P < 0.001$; Fig. 2). The MAP and HR showed a rapid increase when food was presented, but RSNA and locomotor activity increased more slowly to a peak period of activity in the hours around lights off (Fig. 2).

Resting levels of cardiovascular variables in home cage *versus* laboratory

Mean arterial pressure, HR and RSNA were measured in the home cage and laboratory between 10.00 and 11.00 h four times over a 4 week period. Average MAP measured in the home cage was 69 ± 1 mmHg, which was similar to MAP measured in the laboratory at the same time (Table 1

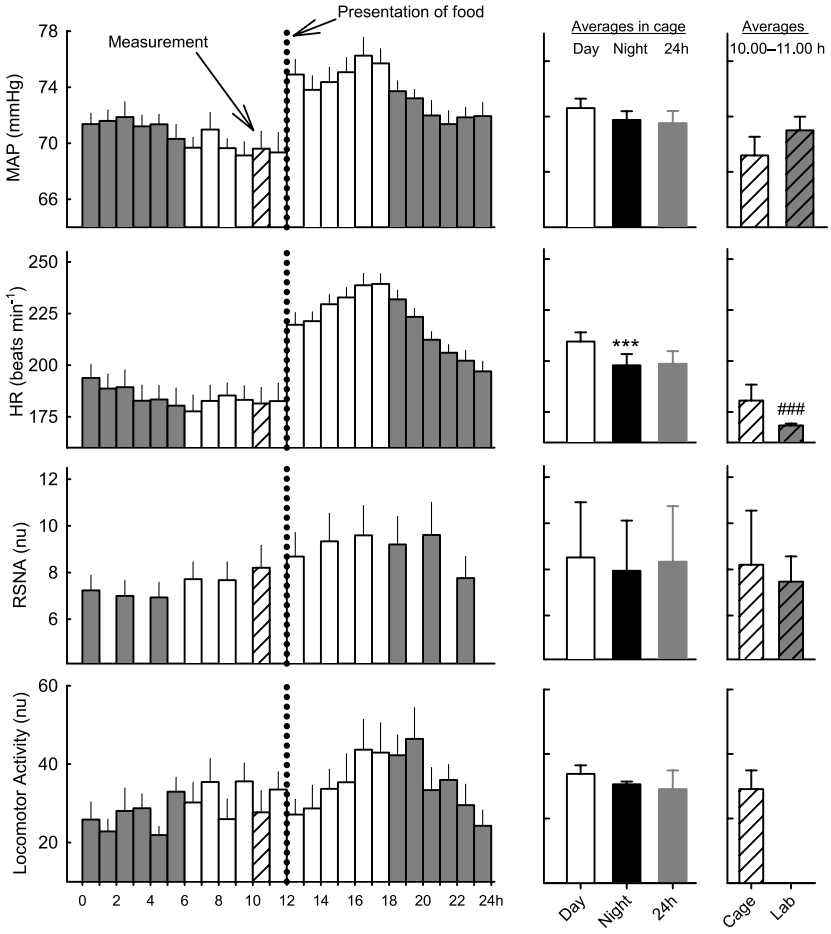

Figure 2. Variables measured in the home cage over 24 hours and averages of measurements in cage and laboratory.
Left panels show mean arterial pressure (MAP), heart rate (HR) and locomotor activity in the home cage over 24 h and averaged over 1 h periods in nine rabbits over 4 weeks. Renal sympathetic nerve activity [RSNA; in normalized units (nu)] was averaged over 2 h periods in six rabbits. Food was presented at 12.00 h (vertical dotted line), and lights were on from 06.00 to 18.00 h (open bars) and off from 18.00 to 06.00 h (grey bars). Hatched bar between 10.00 and 11.00 h is the 1 h period during which home cage and laboratory data were compared. Right panels show MAP, HR, RSNA and locomotor activity averaged over the day (06.00–18.00 h; open bars), night (18.00–06.00 h; filled bars) and 24 h (grey bars). Values collected between 10.00 and 11.00 h (hatched bar in left panel) in the home cage (open hatched bars) and the laboratory (grey hatched bars) were compared. ***$P < 0.001$ for night *versus* day; ###$P < 0.001$ for cage *versus* laboratory at 10.00–11.00 h. Error bars are SEM indicating between-animal variance.

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Table 1. Cardiovascular variables measured between 10.00 and 11.00 h in the home cage and in the laboratory, and in the home cage over 24 h

Cardiovascular variable	Cage 10.00–11.00 h	Laboratory 10.00–11.00 h	*P*1	Cage 24 h	*P*2
Mean arterial pressure (mmHg)	69 ± 1	71 ± 1	0.164	72 ± 1	0.63
Heart rate (beats min^{-1})	181 ± 4	168 ± 4	0.0004	199 ± 4	0.00002
Renal sympathetic nerve activity (normalized units)	8.1 ± 1.3	7.5 ± 0.8	0.57	8.2 ± 1.2	0.61

Values are means ± SEM, averaged over 4 weeks. *P*1 is probability for comparison between home cage and laboratory data measured from 10.00 to 11.00 h; *P*2 is probability for comparison between laboratory data measured from 10.00 to 11.00 h and home cage data averaged over 24 h.

and Figs 2 and 3). The RSNA was also similar in both environments (Table 1 and Figs 2 and 3). Both MAP and RSNA measured in the laboratory were within the range of values observed over 24 h (Table 1 and Fig. 2). In contrast, HR measured in the laboratory (168 ± 4 beats min^{-1}) was 12 ± 3 beats min^{-1} lower than that measured in the home cage over the same period ($P < 0.001$; Table 1 and Figs 2 and 3). This level of HR was also markedly lower than the HR observed during the lights-off period in the home cage (198 ± 6 beats min^{-1}, $P < 0.001$; Fig. 2). There was no significant effect of time in either the cage or laboratory for any of the variables (Fig. 3). In the home cage, there was a strong correlation between both MAP ($r = 0.83$) and HR ($r = 0.90$) and locomotor activity ($P < 0.01$; Fig. 4). The slope of the relationship between MAP and activity was 5.5 mmHg (log unit activity)$^{-1}$ and between HR and activity 46.4 beats min^{-1} (log unit activity)$^{-1}$ (Fig. 4).

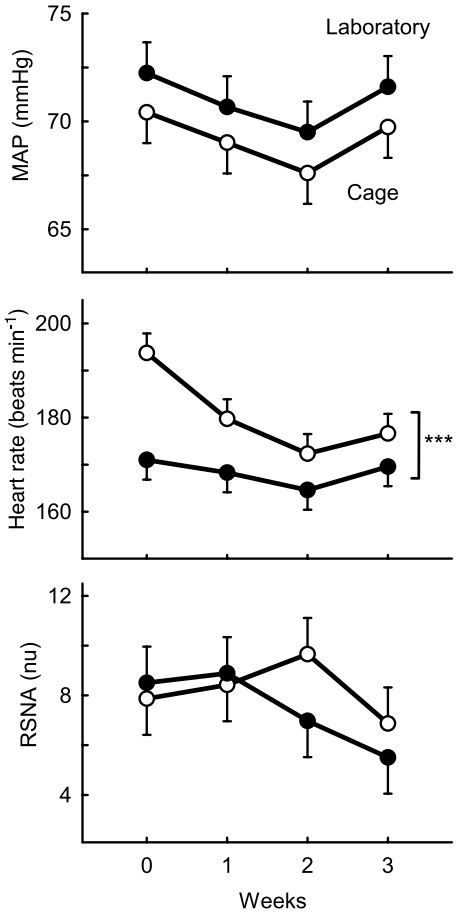

Figure 3. Average MAP, HR and RSNA (in normalized units) measured weekly over 4 weeks in rabbits confined in a holding box in the laboratory (filled circles) or in the home cage (open circles)
Data were collected between 10.00 and 11.00 h. Error bars are SEM indicating within-animal variance; ***$P < 0.001$ for comparison between home cage and laboratory.

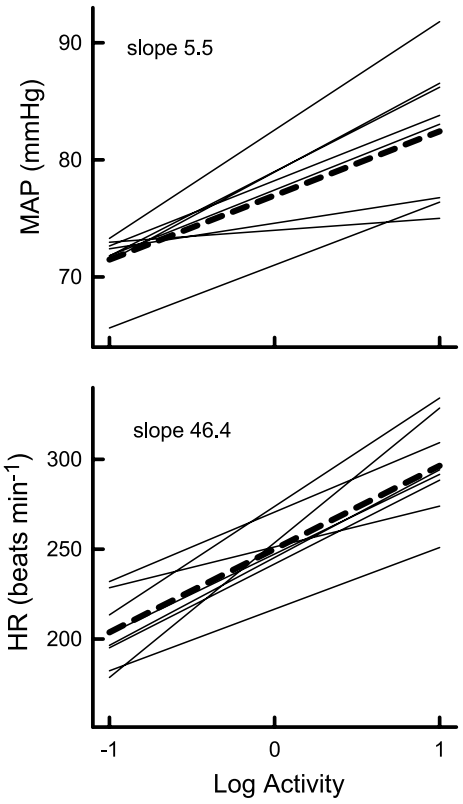

Figure 4. Relationships between MAP, HR and log locomotor activity for each minute over 6 days in the home cage in eight rabbits
Thin lines represent individual regression lines and thick dashed lines represent average regression with $r = 0.83$ for MAP and $r = 0.90$ for HR.

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Cardiovascular variability and baroreflex sensitivity in home cage and laboratory

Spectral analysis was used to determine MAP and HR variability and baroreflex gain in eight rabbits in both the home cage and the laboratory. The MAP variability was lower in the laboratory than in the home cage in all frequency ranges (Fig. 5). In the autonomic mid-frequency range, MAP variability (averaged over 4 weeks) was 37% lower in the laboratory than in the home cage, but there was no trend associated with time (Table 2 and Figs 5 and 6). By contrast, HR variability in the laboratory was similar to that in the home cage in the mid- and high-frequency ranges, but was lower in the laboratory in the low-frequency range (Table 2 and Figs 5 and 6). Baroreflex gain in the autonomic mid-frequency range, averaged over 4 weeks, was 21% higher in the laboratory than in the home cage ($P < 0.001$; Table 2 and Fig. 6). Coherence was likewise 19% greater in the laboratory than in the home cage ($P < 0.001$; Table 2 and Fig. 6). None of the parameters changed significantly over the 4 week measurement period (Table 2).

Discussion

Our results show that blood pressure and RSNA measured in rabbits confined to a dark box in the laboratory closely reflect those values observed when the rabbits are given limited freedom to move about in their home cage. Surprisingly, despite having an indwelling catheter in the ear artery, inserted under local anaesthetic, MAP measured in the laboratory was closely similar to that measured in the home cage. Furthermore, both MAP and RSNA recorded in the laboratory were well within the range of those variables recorded over a 24 h period in the home cage, including the peak elevation during feeding. The importance of these findings is that experimental procedures that can only be performed in a laboratory environment would not be affected by changes in baseline blood pressure or sympathetic activity, because they are similar to those in a freely moving animal in its home cage environment.

We observed lesser MAP variability in the laboratory, but across all frequencies, suggesting that this was probably not related to altered autonomic function or sympathetic activity. Indeed, RSNA was similar in the two environments. Blood pressure variability measurements include a 'non-specific' variability, as well as the autonomic influences such as sympathetic activity, which appears to influence most readily in the 0.2–0.4 Hz band in rabbits (Head *et al.* 2001). Coherence between MAP and HR, an indicator of the strength of the relationship between HR and MAP, was higher in the laboratory, suggesting that a greater proportion of the signal is autonomic, which is consistent with the view that the non-specific variability is less. One explanation is that this is due to reduced movement in the laboratory environment. Studies in mice have reported that MAP variability is greater in active compared with inactive mice (Davern *et al.* 2009; Palma-Rigo *et al.* 2011), and Kuo & Yang (2005) showed that MAP variability was elevated in awake rats compared with those in quiet sleep.

Baroreflex gain was higher in the laboratory than in the home cage and was related to the lower MAP variability and, as mentioned above, may be simply due to the reduced non-specific variability in the autonomic band.

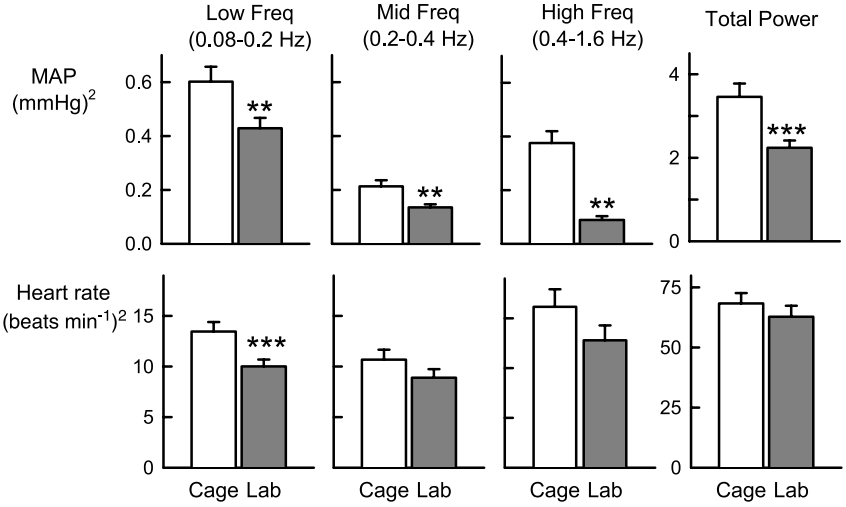

Figure 5. Average MAP and heart rate power at low, mid and high frequency and total power (all frequencies)
Data from the home cage (open bars) or the laboratory (grey bars) were averaged over 4 weeks. Error bars are SEM and indicate between-animal variance. $^{**}P < 0.01$, $^{***}P < 0.001$ for comparison between home cage and laboratory.

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Table 2. Variability, baroreflex gain and coherence in rabbits in the home cage or a laboratory holding box

Parameter	Week 0	Week 1	Week 2	Week 3	Effect of time	Effect of environment
Cage						
MAP mid-frequency power $(mmHg)^2$	0.20 ± 0.03	0.21 ± 0.04	0.22 ± 0.06	0.22 ± 0.05	n.s.	–
HR mid-frequency power $(beats\ min^{-1})^2$	8.83 ± 1.00	10.87 ± 1.09	10.98 ± 2.09	12.00 ± 3.16	n.s.	–
Baroreflex gain $(beats\ min^{-1}\ mmHg^{-1})$	5.27 ± 0.62	6.31 ± 0.94	6.58 ± 1.24	5.78 ± 1.03	n.s.	–
Coherence	0.56 ± 0.04	0.62 ± 0.05	0.61 ± 0.06	0.55 ± 0.07	n.s.	–
Laboratory						
MAP mid-frequency power $(mmHg)^2$	0.16 ± 0.01	0.15 ± 0.02	0.12 ± 0.03	0.11 ± 0.02	n.s.	**
HR mid-frequency power $(beats\ min^{-1})^2$	8.60 ± 1.34	10.02 ± 1.58	9.30 ± 2.40	7.63 ± 1.39	n.s.	n.s.
Baroreflex gain $(beats\ min^{-1}\ mmHg^{-1})$	6.74 ± 0.46	7.39 ± 0.47	8.47 ± 0.68	7.57 ± 0.72	n.s.	***
Coherence	0.73 ± 0.03	0.73 ± 0.02	0.78 ± 0.03	0.65 ± 0.04	n.s.	***

Values are means ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$ for comparison of cage with laboratory data. Abbreviations: HR, heart rate; MAP, mean arterial pressure; and n.s., not significant.

In addition, it may also reflect the quiet state of the rabbits in the holding box. Studies in rabbits, mice and humans report that exposure to stress decreases HR baroreflex sensitivity (Conway *et al.* 1983; Farah *et al.* 2004; Burke & Head, 2009). Conversely, cardiac baroreflex sensitivity increases during the inactive period of the circadian rhythm in mice (Palma-Rigo *et al.* 2010) and during sleep in humans and rats (Conway *et al.* 1983; Kuo & Yang, 2005). The evidence suggests that the rabbits may be in a greater state of relaxation in the laboratory box than in the home cage.

Interestingly, HR was lower in the laboratory than in the home cage, when measured during the same relatively quiet 1 h period before feeding as well as over 24 h. The higher HR measured in the home cage may also be related to the ability of the rabbits to move and exercise in that environment. The difference of 12 beats min^{-1} (7%) was associated with a greater level of locomotor activity of 2.8 log units. However, this higher activity was associated with a rise in MAP of only 1.5 mmHg, approximately 2% based on the resting MAP. Thus, in the rabbit, MAP is less influenced by activity than is HR. The slope of the relationship between MAP and activity was similar to that previously observed in mice (Davern *et al.* 2009) and rats (G. A. Head, unpublished observations).

One possible effect of confining rabbits to a holding box is that it may be a stressful environment. The cardiovascular effects of stress in rabbits have been well characterized and include increased blood pressure, heart rate and RSNA (Burke *et al.* 1998; Burke & Head, 2009). In the present study, the similarity of MAP and RSNA in the home cage and laboratory and the markedly lower HR in the laboratory suggest that the animals were unlikely to be stressed in the laboratory environment. McBryde and colleagues also noted a lower HR in rabbits placed in a box compared with the home cage, but a greater level of stress (wrapping the animal in a towel) evoked markedly higher levels of HR (McBryde *et al.* 2009). This differs from studies in mice and rats restrained in a similar way, that is, confined in a close space such as a cylinder. Rats restrained for tail-cuff measurements had elevated blood pressure and HR compared with measurements in the home cage (Bazil *et al.* 1993; Irvine *et al.* 1997). Mice confined in a similar manner in a cylinder also had increased blood pressure and HR (Gross & Luft, 2003; Jackson *et al.* 2007). The reason for the difference between rabbits and rodents may be a behavioural one. Rabbits are burrowing animals and a close, dark environment that mimics a burrow may not be a stressful environment.

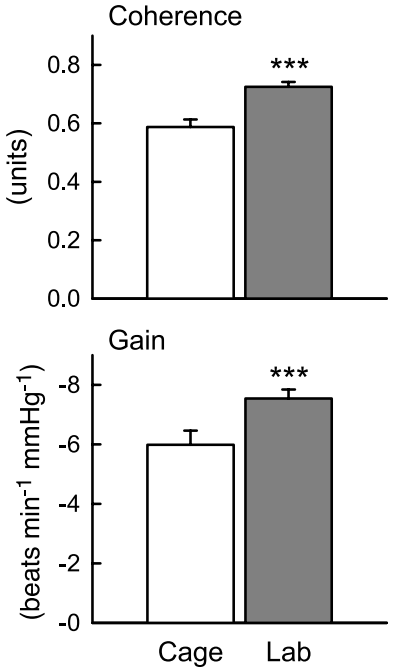

Figure 6. Average coherence and baroreflex gain measured in the mid-frequency range (0.2–0.4 Hz)
Data from the home cage (open bars) and the laboratory (grey bars) were averaged over 4 weeks. Error bars are SEM and indicate between-animal variance. $^{***}P < 0.001$ for comparison between home cage and laboratory.

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The data collected over 24 h show that, in food-restricted animals, the rabbit cardiovascular system appears to be more sensitive to presentation of food than to changes in light (Jilge *et al.* 1987; Van den Buuse & Malpas, 1997; Barrett *et al.* 2001). Nocturnal species such as rats and mice have a circadian rhythm that is more closely linked to the light–dark cycle (Basset *et al.* 2004; Head *et al.* 2004; Davern *et al.* 2009). However, this occurs with unlimited access to food. Restricted feeding in these species alters the circadian patterns of activity, temperature, corticosteroids and other endocrine markers, uncoupling them from the light-dependent rhythm and entraining them to a feeding-related rhythm (Gooley *et al.* 2006; Kaur *et al.* 2008; Wu *et al.* 2008). The present study also included recording of RSNA over 24 h and showed that RSNA follows the same food-related circadian pattern as MAP and HR. The pattern of BP and HR surge with feeding is not fixed by habituation and shifts depending exactly on the feeding time as shown by Van den Buuse & Malpas (1997). However, rabbits fed *ad libitum* more often eat in the dark than in the light (Sanderson & Vanderweele, 1975), and the nocturnal rhythm reflects this, with MAP and HR at their lowest in the daylight hours and highest in the dark (Eijzenbach *et al.* 1986; Vaughan Williams *et al.* 1986).

The development and refinement over recent years of telemetry systems to transmit blood pressure and sympathetic nerve activity in rabbits has meant that there is more emphasis on recording from freely moving animals in the home cage. We have now demonstrated that whilst HR is lower, blood pressure and RSNA measured in rabbits confined in a laboratory setting are a close reflection of those determined in the rabbits' home cage. Importantly, this relationship does not change over several weeks of weekly assessment. Furthermore, our data show that lower levels of blood pressure variability over all frequencies (i.e. non-specific) and the higher coherence between blood pressure and heart rate suggest autonomic influences that may, in fact, be more readily observable in the laboratory environment, particularly where movement is restricted.

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