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Research article

# Social-housing and use of double-decker cages in rat telemetry studies

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ABSTRACT

Rat telemetry is widely used for biomedical research purposes and is used routinely in early pre-clinical drug development to screen for the potential cardiovascular risk of candidate drugs. Historically, these studies have been conducted in individually housed conditions which can impact significantly on an animal's welfare. Here we present data from a survey of pharmaceutical companies and contract research organisations to define current industry practices relating to the housing of rats during telemetry studies and to expand and complement a similar project in non-rodents. Results of the survey showed that 75% of respondents socially house rats on non-recording days of telemetry studies, whereas on recording days only 46% of respondents socially house the animals. When social housing is used on rat telemetry studies, rats are usually housed with an unrecorded companion animal. We also present and compare data from a telemetry study in standard individually ventilated cages (IVCs) with a study using new double-decker IVCs, both conducted using a companion animal approach. Telemetry signals were successfully collected from the double-decker IVCs without a loss of signal quality whilst offering a more spacious environment that allowed the animals to exhibit natural behaviours including full upright posture. Cardiovascular responses following pharmacological intervention with verapamil were similar when assessed in the standard and double-decker cages. Power analysis was conducted on pooled data from the studies in socially housed animals with preliminary results showing the power of detection of drug-induced effects is equivalent to previously published data in individually housed rats. This illustrates that telemetry recordings can be made from rats in socially housed conditions within standard or larger double-decker cages for the for the collection of cardiovascular telemetry data.

## 1. Introduction

Rats are used routinely during the preclinical phases of drug development to determine potential adverse effects of new medicines on vital organ function; primarily the central nervous system and respiratory systems but also for early screening of cardiovascular risk. Despite some differences in anatomy and in the currents responsible for cardiomyocyte repolarisation, the basic underlying physiology of the heart is the same in rats and humans and rats show comparable effects to many cardiotoxic drugs (Farraj, Hazari, & Cascio, 2011). This makes rats a suitable species for assessing the effects of drugs on cardiovascular parameters such as blood pressure, heart rate, left ventricular function and ECG (for hERG-unrelated changes) (Accardi et al., 2016; Fryer et al., 2012; Ericson, Kågström, Laumola, Martinsson, & Eriksson, 2012). It is preferable for preclinical cardiovascular safety assessments to be made in conscious freely-moving animals which can be achieved using fully implantable telemetry (Kurtz, Griffin, Bidani, Davisson, & Hall, 2005) and rat telemetry is now used routinely across the pharmaceutical industry (Accardi et al., 2016; Bhatt et al., 2016; Segreti, Polakowski, Blomme, & King, 2016). Low compound requirements associated with the small size of the animals facilitates use of this model at an early stage of development contributing to decision-making and compound selection. Rat telemetry is also used widely in academia providing useful in vivo data for many biomedical research programmes.

It is expected that animals used in scientific research are socially housed in a manner appropriate to the species, as outlined within international guidelines for animal welfare (2010/63/EU, 2010; ILAR, 2011). However, the housing of animals used in telemetry studies has often been deemed a justifiable exception. This is due, historically, to

*Abbreviations:* ECG, Electrocardiogram; ICH, International Conference on Harmonisation; IVC, Individually ventilated cage

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the inability of some telemetry system hardware to record from multiple animals within the same cage. Refinements for animals used in telemetry studies have been widely promoted. In particular, social housing by the inclusion of a companion animal in the same cage which can be either uninstrumented or instrumented and recorded on a separate day (Hawkins, 2014; Hawkins et al., 2004). Nevertheless, a review of recent literature describing rat cardiovascular telemetry studies indicates that individual housing is still a common practice (Accardi et al., 2016; Segreti et al., 2016; Tang et al., 2016).

Recent technological innovations have led to a wider availability of systems which transmit on multiple wavelengths and should facilitate the adoption of social housing during recordings in any species. The social housing of non-rodents (dogs, minipigs and non-human primates) used for cardiovascular telemetry studies has been a recent focus for the NC3Rs and the safety pharmacology community (Prior et al., 2016). Smaller implants appropriate for recording from multiple socially housed rodents have recently become available (Data Sciences International, 2018; EMKA technologies, 2018; KAHA Sciences, 2018; Kotańska, Śniecikowska, Jastrzębska-Więsek, Kolaczkowski, & Pytka, 2017; TSE system, 2018) and the principles behind adoption of social housing in large animals should be applicable to rodent telemetry also.

Here we present the data from a recent survey of the safety pharmacology community, in pharmaceutical companies and contract research organisations, to better understand current rat telemetry housing practices (Prior, Gellatly, & Jackson, 2018). We also present evidence that telemetry data can be reliably generated in socially housed animals in two different cage systems. The original cage system employed by Vivonics Preclinical Ltd. was standard single-storey individually ventilated cages (IVC) in which telemeterised rats were socially housed with uninstrumented companions in groups of two. This set-up allowed the animals to be in close proximity to the telemetry receiver, positioned directly beneath the cage, which decreases the likelihood of signal drop-out. Use of IVC cages is now a common practice within animal research facilities to limit the exposure of staff to potential allergens and is compatible with telemetry recordings (Krohn, Hansen, & Dragsted, 2003). More recently, double-decker rodent IVCs have become available which offer a more spacious environment allowing animals to exhibit natural behaviours including upright posture, shown to be an important component of a rat's welfare (Buttner, 1993; Makowska & Weary, 2017). Housing of rats in this multilevel caging system has also been shown to induce a positive affective state and to improve overall wellbeing (Wheeler, Swan, & Hickman, 2015). We assessed whether it would be feasible to make telemetry recordings from rats socially housed in double-decker cages and whether the use of double-decker cages would affect the ability to detect drug-induced changes in cardiovascular parameters compared to housing in standard cages.

## 2. Methods

### 2.1. Rodent telemetry housing survey

Questionnaires were sent to targeted individuals with responsibility for either running telemetry studies in-house or for outsourcing this work. The questions asked were:

1) Do you run telemetry studies in rats?
2) Are the animals socially-housed on studies? Available answers were a) No - they are individually-housed at all times, b) Yes - only on days between recordings, c) Yes - we include unrecorded companion (s) in the same cage and d) Yes - multiple animals are recorded from the same cage.
3) What is the purpose of these telemetry studies?

Demographic information elsewhere within the survey allowed assessment of regional information.

### 2.2. Telemetry recording in standard and double-decker cages

Animal care and experimental procedures were performed under the authority of a valid Home Office project licence and conformed to the UK Animals (Scientific procedures) Act, 1986. All studies involving animals are reported in accordance with the ARRIVE guidelines (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010). A total of 7 animals were used in the experiments. Rats were maintained in a 12 h light:dark cycle and were given full access to a standard rat RM1E (Special Diet Services) diet and drinking water at all times.

Briefly, male Han Wistar rats were implanted with C50-PXT transmitters (Data Sciences International, St. Paul, MN USA) under isoflurane anaesthesia at Charles River Laboratories (Margate, UK). The pressure sensor was inserted in the abdominal aorta so the tip lay distal to the renal arteries and the implant body was secured to the peritoneal wall. Biopotential leads were positioned to record lead II ECG according to a previously published method (Sgoifo et al., 1996). Animals were transferred to the University of Nottingham facility at around 2–3 months old. The animals were socially housed with a non-instrumented companion upon arrival at the facility and remained with the same companion throughout the study. The telemetry transmitters operate on a single frequency precluding the option of pair housing two telemetered animals for simultaneous recordings. Cages were prepared with bedding material and enrichment (chew stick and tubes). Animals were 397-669 g at the time of study.

Phase 1 - Rats were socially-housed in standard IVCs, used for their normal housing and for telemetry recording (Techniplast, GR900, internal height 20 cm, Fig. 1a). One telemetry receiver (RPC-1 Datasciences Inc) was placed beneath each cage to detect the telemetry signals. Rats received either vehicle (water for injection) or verapamil (10 and 30 mg/kg, SigmaAldrich) on each dosing day in a modified William's square design. Doses were administered by oral gavage in a dose volume of 5 mL/kg at approximately 10 am each day and recordings were made from at least 1 h before dosing to 22 h post-dose.

Phase 2 - Animals were transferred to larger double-decker IVCs (Techniplast GR1800, internal height 38 cm, Fig. 1b) along with their companion. Four RPC-1 receivers were multiplexed together to allow telemetry recordings from each double-decker cage (Fig. 1c). The animals were dosed using the same design as in Phase 1, following a period of 1 week of acclimatisation to the new cages. The dosing in the second phase was conducted 5 weeks after the end of the first phase. A blood sample (approximately 32 μL) was taken from each animal on each dosing day by tail vein microsampling (Prior, Marks, Grant, & South, 2015) at 3 h post-dose in both phases and plasma prepared by centrifugation (1500 *g* 4 °C for 10 min). The samples were stored frozen until it was analysed for determination of verapamil concentration using a LC-MS/MS method (Thermo TSQ Quantiva with Thermo Vanquish UPLC system).

#### 2.2.1. Data recording and sampling

Blood pressure, ECG, activity and temperature signals were recorded continuously at 1000 Hz (50 Hz for temperature) using Notocord HEM software (NOTOCORD Inc., Paris, France) prior to, and for up to 22 h after the start of dosing. Cardiovascular and temperature data were extracted at set timepoints before and after dosing for graphical purposes as an average of 5 min of continuous data taken at each timepoint. Activity data was extracted as the total activity count with each hour. For statistical comparison of drug-induced effects, all data were summarised as large duration averages (superintervals) (Sivarajah et al., 2010; Skinner, Xing, Lu, Ren, & Oldman, 2017) from 0 to 3 h, 4–10 h, 10–16 h and 16–22 h post-dose. Data between 3 and 4 h were not included due to disturbance caused by the blood sample taken at 3 h post-dose. To assess the degree of signal drop-out, data were extracted from all animals on one dosing day in both housing condition as 15 s averages and number of time points affected by signal drop-out were counted.

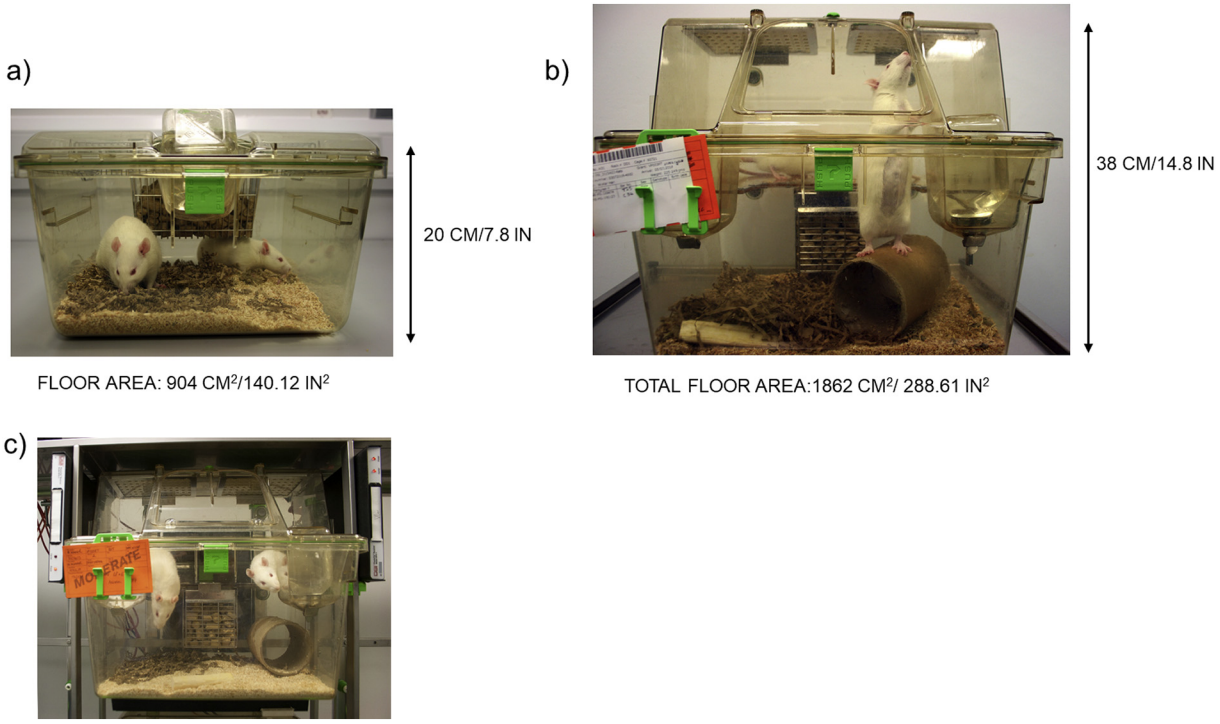


**Fig. 1.** a) Standard IVC cage (Techniplast GR900) used during Phase 1 and b) double-decker cage (Techniplast GR1800) used during Phase 2. Dimensions of cages are shown. c) Double-decker in cage rack during telemetry recording.

*2.2.2. Statistical analysis*

Data was analysed using a repeated measures analysis of covariance with pre-dose measurements used as the covariate. The statistical model used included fixed effects of dose (control, 10 mg/kg or 30 mg/kg of verapamil), time (0-3 h, 4-10 h, 10-16 h and 16-22 h) and cage type (standard single storey or double-decker) and associated interactions. The individual animal and associated interactions were included in the model as random effects.

Comparisons were performed for each timepoint separately. The effect of treatment with verapamil was assessed by comparison of the treated phases of the study (both the 10 and 30 mg/kg dose) with the control for each cage type separately and then the interaction between dose and cage type was assessed. This interaction tests whether the dose effect is the same for each cage type. A significant interaction indicates evidence that the cage type has an impact on the dose effect. All comparisons were conducted using a 2-sided 5% test and accompanied by a 95% confidence range. No multiple comparison adjustments were made.

Activity data was analysed by first transforming using Log(activity +1) and then performing the analysis as described above. Results of this analysis are presented as geometric means and comparisons can be interpreted as fold-changes.

Power analysis was performed for pooled data across the two caging types. The methods used for the power calculation involved fitting the model described in the statistical analysis section, estimating the appropriate within animal pooled standard deviation from this analysis and using this figure to estimate the minimal differences detectable between two treatment groups with 80% power. The variation in detectable differences with changes in group size ($n$ = 4, 6 and 8) were calculated.

## 3. Results

### 3.1. Telemetry housing survey

Twenty-eight responses to the survey were received, from facilities that run rat telemetry studies (13 contract research organisations and 11 pharmaceutical companies) or 4 individuals responsible for outsourcing these studies. Respondents were from the UK, Europe, USA and Canada and answers were combined into Europe or outside-Europe categories. All the respondents from Europe (14/14) socially housed their rats on non-recording days compared with only 50% (7/14) of respondents from outside Europe (Fig. 2). The majority of European respondents (79%, 11/14) retained social housing during the telemetry recording periods - 57% (8/14) with an unrecorded companion, or 21% (3/14) with recordings from multiple rats in the cage. However, only 14% (2/14) of respondents from outside Europe socially house their rats during the telemetry recording periods (by employing an unrecorded companion). The purpose of the telemetry studies were predominantly early screening studies (before the regulatory non-rodent study) for blood pressure and heart rate measurements (89% of respondents) or left ventricular pressure/other function (32%). Other purposes included investigative studies only when required (54%), or

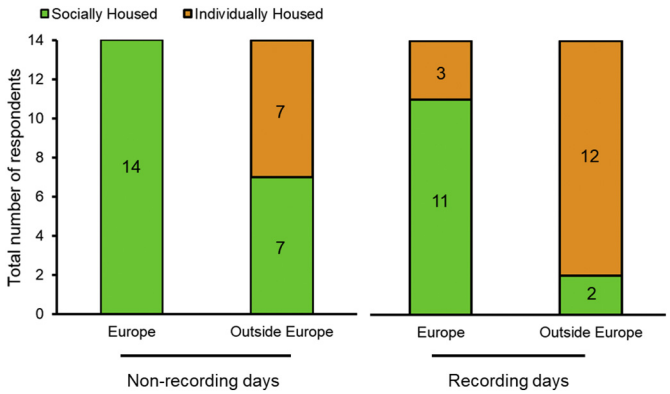


**Fig. 2.** The number of survey respondents housing animals socially or individually on recording and non-recording days of rat safety pharmacology telemetry studies. The numbers within the bars gives the actual number of respondents.

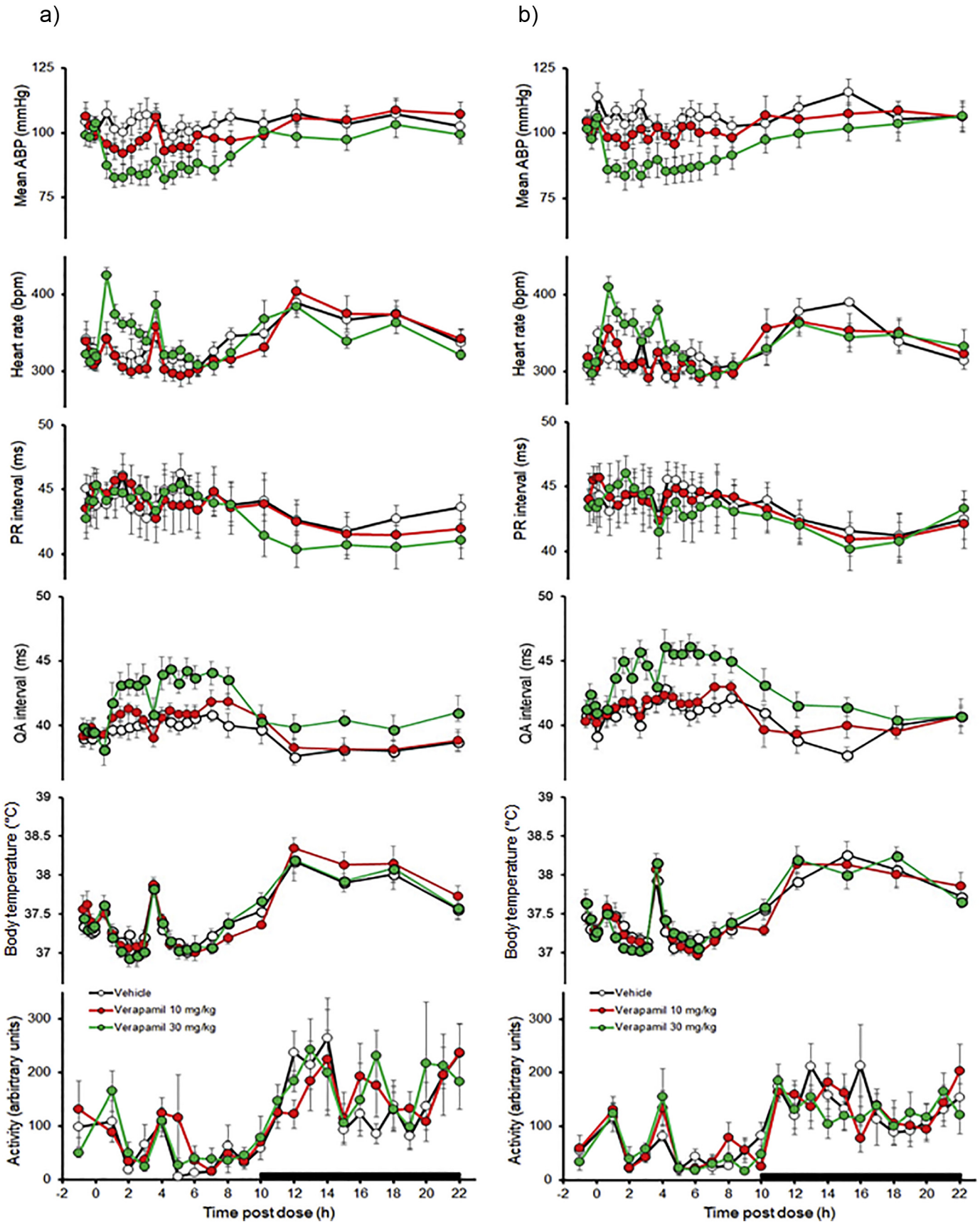


**Fig. 3.** Effects of verapamil in telemetered rats housed in a) single storey and b) double-decker cages (mean ± SE mean). Dosing occurred at 0 h, bar shows night phase.

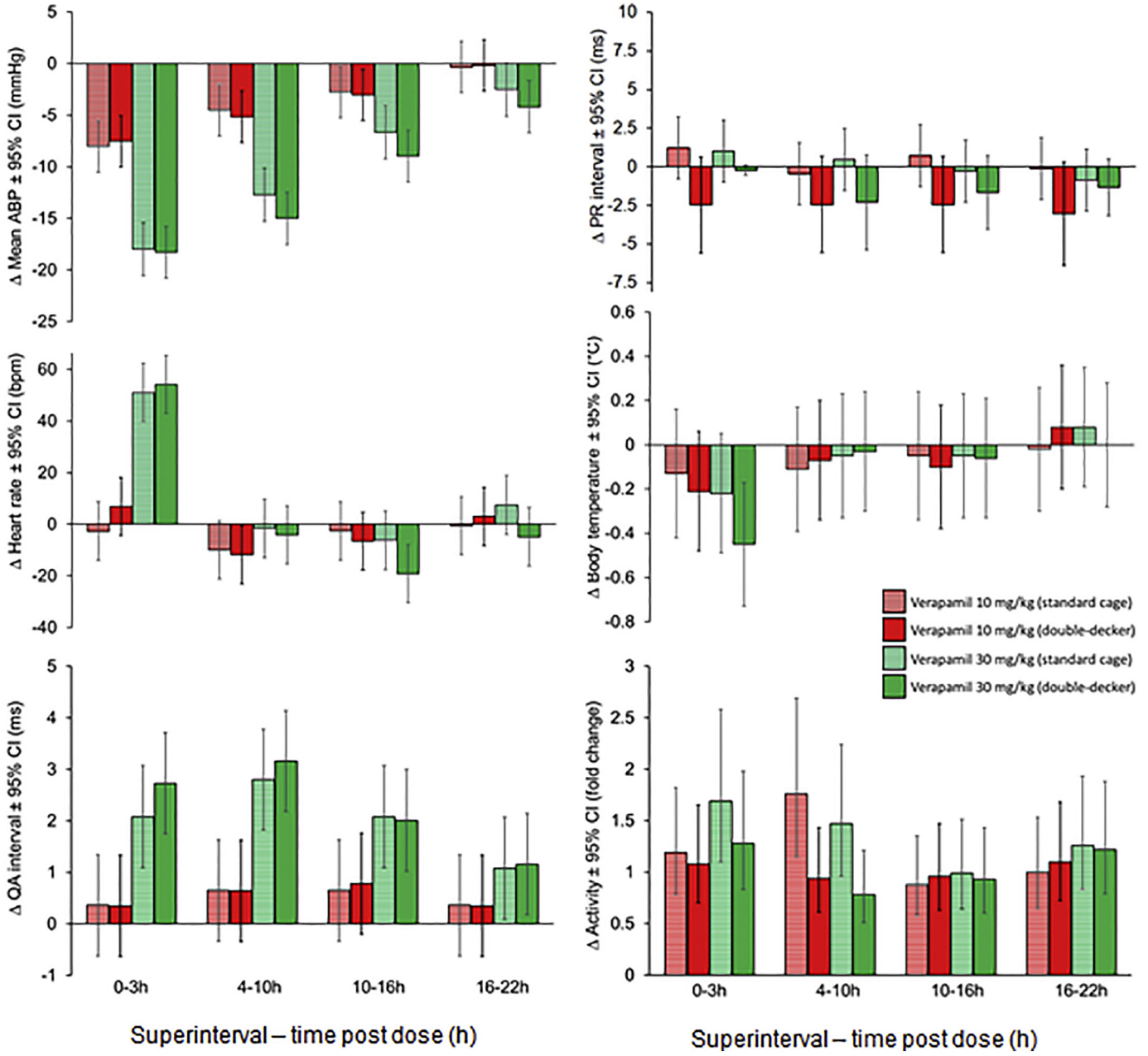


**Fig. 4.** Comparison of effects of verapamil on mean arterial blood pressure, heart rate, QA interval, PR interval, body temperature and activity in standard and double-decker cages. Charts show mean change at each time interval ± 95% confidence interval. Statistically significant changes occur where the 95% CI excludes 0, except for activity where significance occurs where the 95% CI excludes 1.

for assessment of respiratory function (18%), or central nervous system function (e.g. electroencephalograms, 36%).

### 3.2. Telemetry recording in standard and double-decker cages

Continuous recordings of physiological parameters were made from both standard and double-decker cages with minimal signal drop-out. The average number of 15 s samples affected by signal drop-out during the 23 h recording was 18 (range 1–51) for standard cages and 3 (range 0–7) for double-decker cages. This was a very small proportion of the total amount of data recorded (0.3% and 0.1% for standard and double-decker cages, respectively).

The effects of verapamil were consistent between the standard and double decker cages. Verapamil caused a dose-related decrease in mean arterial blood pressure after 10 and 30 mg/kg with effects still evident at the last superinterval 16-22 h after the high dose (Figs. 3 & 4). A tachycardia was evident within the first 3 h after 30 mg/kg verapamil and QA interval, known to be affected by cardiac contractility, was also increased after 30 mg/kg with a significant effect still evident at the last superinterval. Verapamil had no notable effect on PR interval. A significant decrease in body temperature was observed after 30 mg/kg verapamil within the first 3 h of dosing in the double-decker phase that was not apparent in the standard caging. When compared statistically using a linear mixed effect model, no significant differences in the verapamil-induced responses were observed between the standard and double-decker cages (Fig. 4). Plasma verapamil concentrations (3 h post-dose) were consistent between both phases: 38 ± 25 and 279 ± 91 ng/mL in the standard caging phase and 43 ± 50 and 224 ± 133 ng/mL in the double-decker phase after 10 and 30 mg/kg, respectively. Concentration-effect data were plotted from each animal

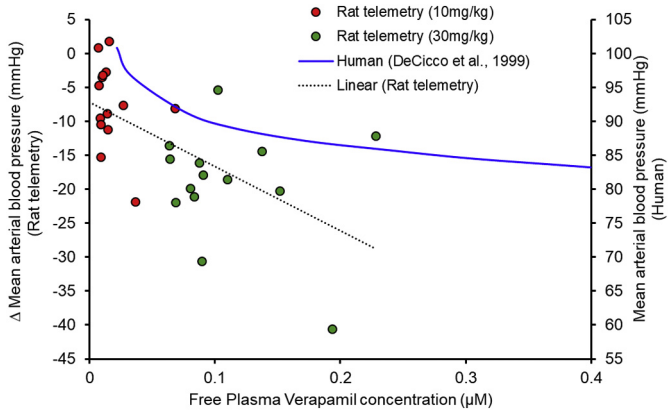


**Fig. 5.** Relationship between plasma verapamil concentrations and mean arterial blood pressure in rats and humans. Rat telemetry data from the current study are shown on the primary y-axis. Change in mean arterial blood pressure from vehicle control are plotted against corresponding free plasma verapamil concentrations for individual animals along with a linear regression ($R^2 = 0.36$). The summarised relationship between actual mean arterial blood pressure and plasma verapamil concentration in humans is plotted on the secondary y-axis (data, taken from De Cicco et al., 1999). Verapamil plasma protein binding data used - $f_u$-0.2 Rat, 0.17 human (Berry, Li, & Zhao, 2011).

using the verapamil plasma concentration data at 3 h post-dose and the mean arterial blood pressure data (5 min average) extracted at 2.9 h post dose (Fig. 5). The magnitude of hypotension was related to the free plasma concentration of verapamil and the effects were consistent with those reported in humans at similar concentrations (De Cicco et al., 1999).

Retrospective statistical power based on the pooled data from the two cage types are presented in Table 1. The effect of variations in group size on the minimal detectable change at 80% power are shown.

## 4. Discussion

This study has shown that social-housing during rat telemetry studies is an achievable refinement, with data recorded from pair-housed animals in standard IVC cages as well as newer double-decker IVCs with expanded vertical space. Although the rats in the current study were telemetered for recording cardiovascular data for safety pharmacology investigations, the principles are equally applicable for telemetry studies measuring other safety pharmacology endpoints, in telemetry studies for academic biomedical research purposes and for telemetry studies with other small animal species.

The use of rat telemetry is a common practice within the pharmaceutical industry. This is apparent from the survey data and from published literature from the safety pharmacology community (Bhatt et al., 2016; Collins et al., 2018; Fermini et al., 2017; Segreti et al., 2016; Tang et al., 2016). Given the main purpose of the studies is usually early screening, to de-risk cardiovascular liabilities and aid candidate selection prior to regulatory studies, it is likely that multiple compounds per project are investigated. The group size for rat telemetry studies varies between organisations, using between 4 and 9 rats per compound tested (as per the previously referenced papers). Cross-over study designs are most usual where the same animals receive multiple doses, but parallel group designs are also often required to achieve the study objectives. Most organisations maintain colonies of telemetered rats and re-use animals for testing of multiple compounds, thereby reducing the number of animals used; however, it is likely that within the pharmaceutical industry many thousands of rats undergo these procedures annually worldwide.

**Table 1**
The table shows the change that can be detected in each variable with a group size of n = 4, 6 or 8 at 80% power. Note that data was pooled from the studies performed in socially housed rats housed in both standard and double-decker cages.

| Parameters | N = 4 | N = 6 | *N* = 8 |
|---|---|---|---|
| HR (bpm) | 32 | 24 | 20 |
| Diastolic ABP (mmHg) | 6 | 4 | 3 |
| Systolic ABP (mmHg) | 7 | 5 | 4 |
| Mean ABP (mmHg) | 7 | 5 | 4 |

All European respondents to the survey house their rats socially on non-recording days, with the majority (79%) maintaining social housing during telemetry data recordings. This greatly contrasts with respondents from outside Europe, where 50% house their rats individually at all times and only 14% socially-house during telemetry data recordings. Similar trends are reflected in the literature, with publications from the USA often reporting the use of individual housing (Accardi et al., 2016; Segreti et al., 2016; Tang et al., 2016) and those from Europe reporting the use of social housing (Collins et al., 2018). The most common method employed for social housing during telemetry recordings is to house with an unrecorded companion animal(s), although the survey did not determine whether they were uninstrumented or instrumented and recorded on a separate day. This is likely to be due to the use of legacy telemetry hardware which is unable to record from group-housed animals simultaneously due to signal transmission on one frequency and the potential for cross-talk. Improved hardware and alternative telemetry systems are now available that enable simultaneous recordings from socially-housed telemetered animals; however, the current survey suggests that they are not yet widely used (only three European respondents reported recording from multiple animals in a cage). This could reflect the relatively small number of survey respondents and/or the relatively recent introduction of some of these new systems and hardware. A reason for using unrecorded companion animals rather than recording from multiple telemetered animals in each cage may also be due to the preferred latin-square study design commonly used. This design would result in cagemates receiving different dose levels and a perceived risk of cross-contamination. This is also an issue for non-rodent telemetry studies (Prior et al., 2016); however, the risk is deemed low in rats due to the lack of vomiting reflex although grooming and copraphagia are potential sources of cross-contamination in rodents.

The ability to socially house rats during telemetry recordings satisfies the legal requirements (2010/63/EU, 2010) or best-practice guidelines (ILAR, 2011) for social housing of laboratory animals, but data indicates that social housing also confers an improvement to the animals' welfare. Undisturbed heart rate, measured as an index of stress, and stress responses to common laboratory procedures have been shown to be reduced by social housing compared with individual housing in Sprague Dawley rats (Azar, Sharp, & Lawson, 2011; Sharp, Zammit, Azar, & Lawson, 2002). In the present study we socially housed rats with uninstrumented companions in standard and double-decker cages. Double-decker cages offer additional benefits to animal welfare by allowing more floor space than standard IVC cages and, importantly, enabling true upright postures to be adopted. Upright posture has been shown to be an integral and important part of a rat's welfare (Buttner, 1993; Makowska & Weary, 2017) and, whilst the time spent in this position was not quantified in the current study, rats were often seen in full upright position with a straight back when housed in the double-decker cage (Fig. 1b). Housing rats in multilevel caging systems has also been shown to induce a positive affective state and to improve overall wellbeing (Wheeler et al., 2015).

Our data shows that the use of double-decker IVCs had no adverse effect on the quality of the telemetry data recorded with less periods of signal drop-out noted compared to the standard caging when using a configuration of 4 multiplexed receivers. The hardware arrangement used in this study with double-decker cages does require the user to have a surfeit of receivers which may not always be available. In this

situation, an alternative would be to remove the upper shelf of the double-decker cage on recording days and use one receiver positioned underneath the cage bottom (as with the standard cages). Removal of the upper floor reduces the total available floor space for the rats yet still allows full upright posture to be adopted. The use of other telemetry systems or hardware would also alleviate this problem since, with some new systems, a single receiver is enough for recording from animals in multiple cages housed in a cage rack.

In the current study, oral administration of the calcium channel antagonist verapamil to socially housed rats caused the expected haemodynamic effects of hypotension, tachycardia and an increase in QA interval (reflecting a reduction in cardiac contractility), in accordance with data from previous studies in rats (Adeyemi et al., 2009; Fermini et al., 2017; Tang et al., 2016) and humans (De Cicco et al., 1999). Verapamil had no effect on PR interval despite being a known negative dromotrope. The reason for the lack of effect is unknown but previous studies in humans have shown the dromotropic effects of verapamil to be reduced following oral administration (Echizen, Vogelgesang, & Eichelbaum, 1985). There were no notable differences between the effects of verapamil when assessed in rats housed in double-decker versus standard IVCs. This suggests that the use of double-decker caging is unlikely to affect the sensitivity of detections of drug-induced effects, important when used in early drug safety screening.

An assessment of the statistical power was made from the current data pooled across both the cage types. Whilst this data is based on only a small number of studies and should be considered preliminary, it demonstrates that social housing of rats during telemetry studies is compatible with the detection of small drug-induced changes in cardiovascular parameters. The minimal detectable change in arterial blood pressure and heart rate was 4-5 mmHg and 24 bpm, respectively, in our socially housed rats with a group size of $n = 6$ at 80% power. This compares with a minimal detectable change of 5-6 mmHg and 24 bpm, respectively, in a previously published study in telemetered rats (Bhatt et al., 2016) in which the animals were individually housed (T Wisialowski, personal communication). Other authors have shown that cardiovascular studies performed in socially housed dogs and cynomolgus monkeys can have similar or even greater statistical power than those performed in individually housed animals (Prior et al., 2015, Xing et al., 2015).

## 5. Conclusion

Whilst the majority of the industry survey respondents do not socially house telemetered rats on recording days, there are opportunities for adoption of this refinement within industry and academia worldwide. Companion animals can be used in rat telemetry studies improving animals' welfare without adversely affecting the detection of drug-induced cardiovascular changes. The use of double-decker cages is also technically possible in rat telemetry studies allowing socially housed rats to exhibit periods of full upright posture known to be an integral and important part of a rat's welfare.

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## References

2010/63/EU (2010). *Directive 2010/63/EU of the European Parliament and of the council of 22 September on the Protection of Animals used for Scientific Purposes, OJ L276/33.* Brussels: European Commission.

Accardi, M., Troncy, E., Abtout, S., Ascah, A., Maghezzi, S., & Authier, S. (2016). Rat cardiovascular telemetry: Marginal distribution applied to positive control drugs. *Journal of Pharmacological and Toxicological Methods, 81*, 120–127. https://doi.org/10.1016/j.vascn.2016.03.005.

Adeyemi, O., Roberts, S., Harris, J., West, H., Shome, S., & Dewhurst, M. (2009). QA interval as an indirect measure of cardiac contractility in the conscious telemeterised rat: Model optimisation and evaluation. *Journal of Pharmacological and Toxicological Methods, 60*, 159–166. https://doi.org/10.1016/j.vascn.2009.03.006.

Azar, T., Sharp, J., & Lawson, D. (2011). Heart rates of male and female Sprague-Dawley and spontaneously hypertensive rats housed singly or in groups. *Journal of the American Association for Laboratory Animal Science, 50*, 175–184.

Berry, L. M., Li, C., & Zhao, Z. (2011). Species differences in distribution and prediction of human Vss from preclinical data. *Drug Metabolism and Disposition, 39*, 2103–2116.

Bhatt, S., Li, D., Flynn, D., Wisialowski, T., Hemkens, M., & Steidl-Nichols, J. (2016). Statistical power analysis of cardiovascular safety pharmacology studies in conscious rats. *Journal of Pharmacological and Toxicological Methods, 81*, 128–135.

Buttner, D. (1993). Upright standing in the laboratory rat–time expenditure and its relation to locomotor activity. *Journal of Experimental Animal Science, 36*, 19–26.

Collins, T., Gray, K., Bista, M., Skinner, M., Hardy, C., Wang, H., ... Harmer, A. R. (2018). Quantifying the relationship between inhibition of VEGF receptor 2, drug-induced blood pressure elevation and hypertension. *British Journal of Pharmacology, 175*, 618–630. https://doi.org/10.1111/bph.14103.

*Data Sciences International. Rodent social housing solutions. (2018).* https://www.datasci.com/docs/default-source/default-document-library/rodent-social-housing-solutions.pdf?sfvrsn=3afde265_2 Website accessed 27 July 2018.

De Cicco, M., Macor, F., Robieux, I., Zanette, G., Fantin, D., Fabiani, F., ... Boiocchi, M. (1999). Pharmacokinetic and pharmacodynamic effects of high-dose continuous intravenous verapamil infusion: Clinical experience in the intensive care unit. *Critical Care Medicine, 27*, 332–339.

Echizen, H., Vogelgesang, B., & Eichelbaum, M. (1985). Effects of d,l-verapamil on atrioventricular conduction in relation to its stereoselective first-pass metabolism. *Clinical Pharmacology & Therapeutics, 38*, 71–76.

*EMKA technologies.* http://www.emka.fr/product/implants-for-rats/ Website accessed 27 July 2018.

Ericson, A.-C., Kågström, J., Laumola, E.-L., Martinsson, F., & Eriksson, A. B. (2012). Effects of hNaV1.5 blockers on the rat QRS interval. *Journal of Pharmacological and Toxicological Methods, 66*, 160.

Farraj, A. K., Hazari, M. S., & Cascio, W. E. (2011). The utility of the small rodent electrocardiogram in toxicology. *Toxicological Sciences, 121*, 11–30. https://doi.org/10.1093/toxsci/kfr021.

Fermini, B., Ramirez, D. S., Sun, S., Bassyouni, A., Hemkens, M., Wisialowski, T., & Jenkinson, S. (2017). L-type calcium channel antagonism - translation from in vitro to in vivo. *Journal of Pharmacological and Toxicological Methods, 84*, 86–92. https://doi.org/10.1016/j.vascn.2016.11.002 (Epub 2016 Nov 15).

Fryer, R. M., Harrison, P. C., Muthukumarana, A., Nodop Mazurek, S. G., Ng, K. J., Chen, R. R., ... Reinhart, G. A. (2012). Strategic integration of in vivo cardiovascular models during lead optimization: Predictive value of 4 models independent of species, route of administration, and influence of anesthesia. *Journal of Cardiovascular Pharmacology, 59*, 369–376. https://doi.org/10.1097/FJC.0b013e31824485dd.

Hawkins, P. (2014). Refining housing, husbandry and care for animals used in studies involving biotelemetry. *Animals, 4*, 361–373. https://doi.org/10.3390/ani4020361.

Hawkins, P., Morton, D., Bevan, R., Heath, K., Kirkwood, J., Pearce, P., ... Webb, A. (2004). Husbandry refinements for rats, mice, dogs and non-human primates used in telemetry procedures. *Laboratory Animals, 38*, 1–10.

Institute for Laboratory Animal Research (2011). *Guide for the care and use of laboratory animals* (8th ed.). Washington, DC: The National Academies Press.

*KAHA Sciences. Rat telemetry system. (2018).* http://www.kahasciences.com/rat-telemetry-systems Website accessed 27 July 2018.

Kilkenny, C., Browne, W., Cuthill, I. C., Emerson, M., & Altman, D. G. (2010). Animal research: Reporting *in vivo* experiments: The ARRIVE guidelines. *British Journal of Pharmacology, 160*, 1577–1579.

Kotańska, M., Śniecikowska, J., Jastrzębska-Więsek, M., Kolaczkowski, M., & Pytka, K. (2017). Metabolic and cardiovascular benefits and risks of EMD386088 – A 5-$HT_6$ receptor partial agonist and dopamine transporter inhibitor. *Frontiers in Neuroscience, 11*, 1–10.

Krohn, T. C., Hansen, A. K., & Dragsted, N. (2003). The impact of cage ventilation on rats housed in IVC systems. *Laboratory Animals, 37*, 85–93.

Kurtz, T. W., Griffin, K. A., Bidani, A. K., Davisson, R. L., & Hall, J. E. (2005). Recommendations for blood pressure measurement in humans and experimental animals: Part 2: Blood pressure measurement in experimental animals: A statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. *Hypertension, 45*, 299–310. https://doi.org/10.1161/01.HYP.0000150857.39919.cb.

Makowska, I. J., & Weary, D. M. (2017). The importance of burrowing, climbing and standing upright for laboratory rats. *Royal Society Open Science, 3*, 160136. https://doi.org/10.1098/rsos.160136.

Prior, H., Billing, R., Wallace, I., South, M., Oldman, K., Moors, J., & Milne, A. (2015). Pair housed dog telemetry: Animal welfare refinement with early indications of similar study sensitivity. *Journal of Pharmacological and Toxicological Methods, 75*, 182.

Prior, H., Bottomley, A., Champéroux, P., Cordes, J., Delpy, E., Dybdal, N., ... Chapman, K. (2016). Social housing of non-rodents during cardiovascular recordings in safety pharmacology and toxicology studies. *Journal of Pharmacological and Toxicological Methods, 81*, 75–87.

Prior, H., Gellatly, N., & Jackson, S. (2018). Why are non-rodents not socially housed

during cardiovascular telemetry recordings on safety pharmacology studies? *Journal of Pharmacological and Toxicological Methods, 93*, 158 (poster at SPS meeting, Berlin 2017).

Prior, H., Marks, L., Grant, C., & South, M. (2015). Incorporation of capillary micro-sampling into whole body plethysmography and modified Irwin safety pharmacology studies in rats. *Regulatory Toxicology and Pharmacology, 73*, 19–26.

Segreti, J., Polakowski, J., Blomme, E., & King, A. (2016). Simultaneous measurement of arterial and left ventricular pressure in conscious freely moving rats by telemetry. *Journal of Pharmacological and Toxicological Methods, 79*, 23–33. https://doi.org/10.1016/j.vascn.2016.01.003.

Sgoifo, A., Stilli, D., Medic, D., Gallo, P., Aimi, B., & Musso, E. (1996). Electrode positioning for reliable telemetry ECG recordings during social stress in unrestrained rats. *Physiology & Behavior, 60*, 1397–1401. https://doi.org/10.1016/S0031-9384(96)00228-4.

Sharp, J. L., Zammit, T. G., Azar, T. A., & Lawson, D. M. (2002). Stress-like responses to common procedures in male rats housed alone or with other rats. *Contemporary Topics in Laboratory Animal Science, 41*, 8–14.

Sivarajah, A., Collins, S., Sutton, M. R., Regan, N., West, H., Holbrook, M., & Edmunds, N. (2010). Cardiovascular safety assessments in the conscious telemetered dog: Utilisation of super-intervals to enhance statistical power. *Journal of Pharmacological and Toxicological Methods, 62*, 12–19. https://doi.org/10.1016/j.vascn.2010.05.011.

Skinner, M., Xing, G., Lu, J., Ren, J., & Oldman, K. (2017). Detecting drug-induced changes in ECG parameters using jacketed telemetry: Effect of different data reduction techniques. *Journal of Pharmacological and Toxicological Methods, 85*, 38–48.

Tang, H.-M., Ju, H., Zhao, S., LaDuke, C., Hahn, S., Glick, J., ... Friedrichs, G. (2016). Translational assessment of cardiac contractility by echocardiography in the telemetered rat. *Journal of Pharmacological and Toxicological Methods, 77*, 24–32. https://doi.org/10.1016/j.vascn.2015.09.005.

*TSE website.* https://www.tse-systems.com/product-details/stellar-telemetry Website accessed 27 July 2018.

Wheeler, R. R., Swan, M. P., & Hickman, D. L. (2015). Effect of multilevel laboratory rat caging system on the well-being of the singly-housed Sprague Dawley rat. *Laboratory Animals, 49*, 10–19. https://doi.org/10.1177/0023677214547404.

Xing, G., Lu, J., Hu, M., Wang, S., Zhao, L., Zheng, W., Schofield, J., Oldman, K., Adkins, D., Yu, H., Platz, S., Ren, J., & Skinner, M. (2015). Effects of group housing on ECG assessment in conscious cynomolgus monkeys. *Journal of Pharmacological and Toxicological Methods, 75*, 44–51. https://doi.org/10.1016/j.vascn.2015.05.004.