

## Introduction

Monkeys, dogs and minipigs are non-rodent species used for cardiovascular safety pharmacology studies. In order to accurately interpret modifications of cardiovascular parameters, it is important to understand the influence on cardiovascular data of the circadian cycle, as well as events such as feeding and drug administration. Changes in hemodynamic parameters must be correctly interpreted and we must be able to distinguish between environmental effects and test item effects. We must also be able to adapt the cardiovascular study design as needed for each species. The present study investigated effects of the circadian cycle, gavage administration and daily feeding in the three non-rodent species usually used in safety pharmacology pre-clinical studies.

## Materials and methods

Cynomolgus monkeys, Beagle dogs and Göttingen minipigs (5 of each species) implanted with telemetry devices (TL11M3-D70-PCT-DSI: Data Sciences International) were used for this study. After implantation the animals were allowed at least 3 weeks to recover from surgery. The animals were introduced to the telemetry laboratory area on several occasions before the experimental recording. On the day of the experiment they were permitted at least one hour habituation prior to recording. Parameters for all five animals (of each species) were recorded on the same day. Cardiovascular parameters (diastolic, systolic and mean arterial pressure, ECG, temperature and activity) were recorded for at least 28 hours, using HEM software v.4.2 (Notocord System). The recording period included 12 hours of light (7:00 a.m. to 19:00 p.m.) and 12 hours of darkness to evaluate the effect of circadian rhythm. Two hours after the beginning of the recording, the administration procedure (gavage with drinking water, 5ml/kg) was performed to evaluate the influence and duration of dosing procedure effects on cardiovascular parameters. The animals were given a daily food allowance (dogs: 400 grams of pelleted diet, minipigs: 350 grams of pelleted diet, monkeys: fruit plus 180 grams of pelleted diet) once during the daylight period (at around 9:00 a.m.) thus allowing us to evaluate the effect of feeding on cardiovascular parameters. All animals had free access to water.

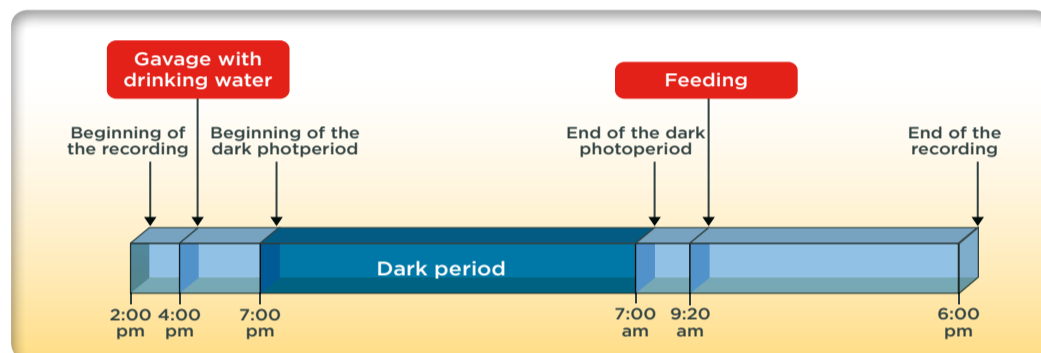


Fig. 1: Design of the recording period

## Results and discussion

### Effect of circadian rhythm

A decrease (-10 to -20%) in heart rate was observed during the dark period in all three species. There were no significant changes in arterial pressure (Fig 2, Fig 3). During the dark period, temperature decreased from 38.3 to 36.8°C (-1.5°C) in minipigs and from 38.3 to 37°C (-1.3°C) in monkeys, but only from 38.1 to 37.6°C (-0.5°C) in dogs (Fig 4). Decreased activity, corresponding to periods of sleep, was noted during the dark period in all three species (Fig 5).

### Effect of administration procedure

Oral gavage with drinking water resulted in significant tachycardia (+ 69 % in primates, + 54% in dogs, and + 33% in minipigs) and increased arterial pressure (ca. 20 to 30 %) when compared to pre-treatment values. Heart rate returned to within 10% of the pre-treatment value more rapidly in minipigs (ca. 20 to 30 minutes) than in dogs or monkeys (ca. 40 to 50 minutes, Fig 6). No changes in temperature were observed. Increased activity peaks were recorded after gavage in dogs and monkeys, but not in minipigs which were found to be less active overall than dogs or monkeys (Fig 5).

### Effect of feeding

Feeding increased heart rate when compared to pre-feeding values. The increases were higher in dogs (+ 139%) than in minipigs (+55%) or monkeys (+38%). Increased heart rate lasted for up to 2 hours after feeding in minipigs, but for only 30 to 60 minutes in dogs and monkeys (Fig 7). In the absence of an unfed control group we cannot exclude that the duration of the increase in HR after feeding may in part be due to the underlying effects of circadian cycle in HR. Feeding also increased mean arterial pressure (MAP). This increase in MAP (Fig 3) was higher on dogs (+50%) than in minipigs (+28%) or monkeys (+16%). Increased activity peaks were recorded after feeding in dogs and monkeys, but not in minipigs which were found to be less active overall than dogs or monkeys (Fig 5).

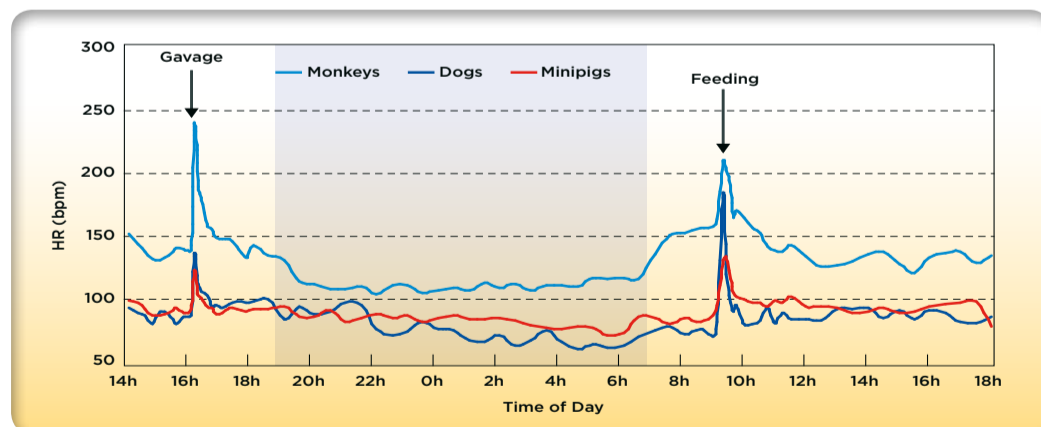


Fig. 2: Variation of HR in monkeys, dogs and minipigs during circadian cycle

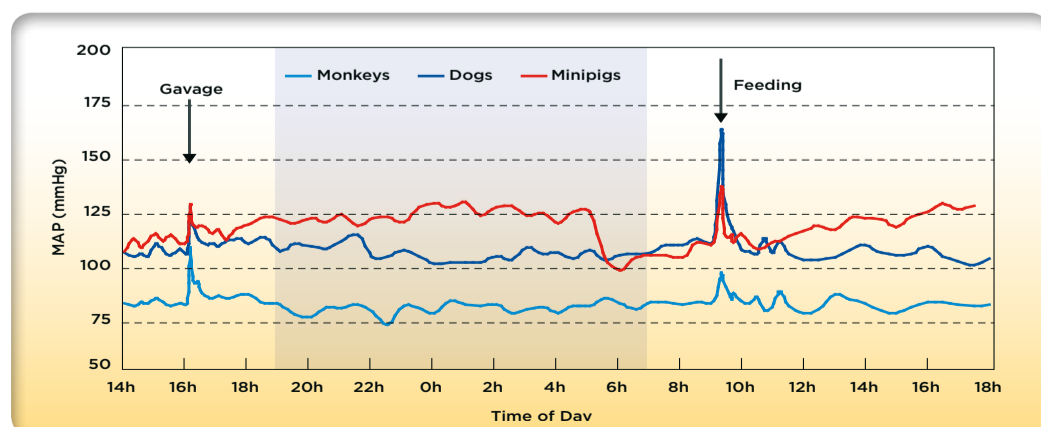


Fig. 3: Variation of Mean Arterial Pressure in monkeys, dogs and minipigs during circadian cycle

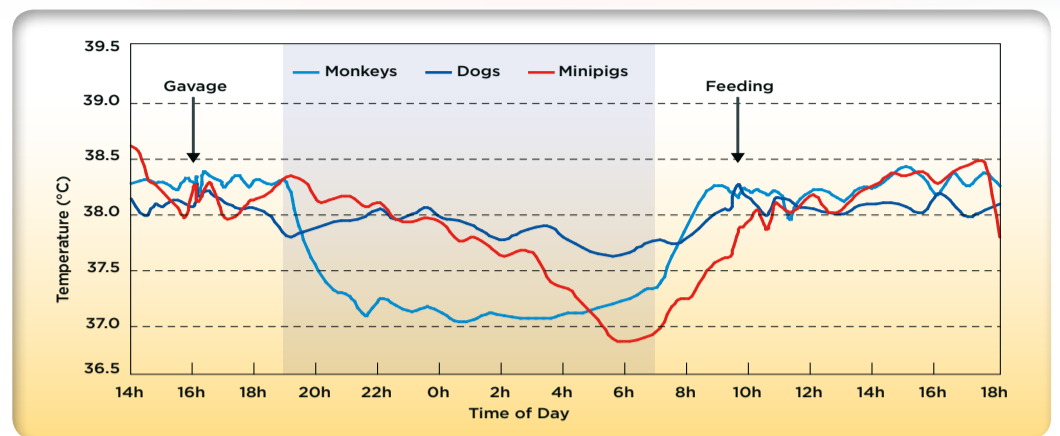


Fig. 4: Variation of Temperature in monkeys, dogs and minipigs during circadian cycle

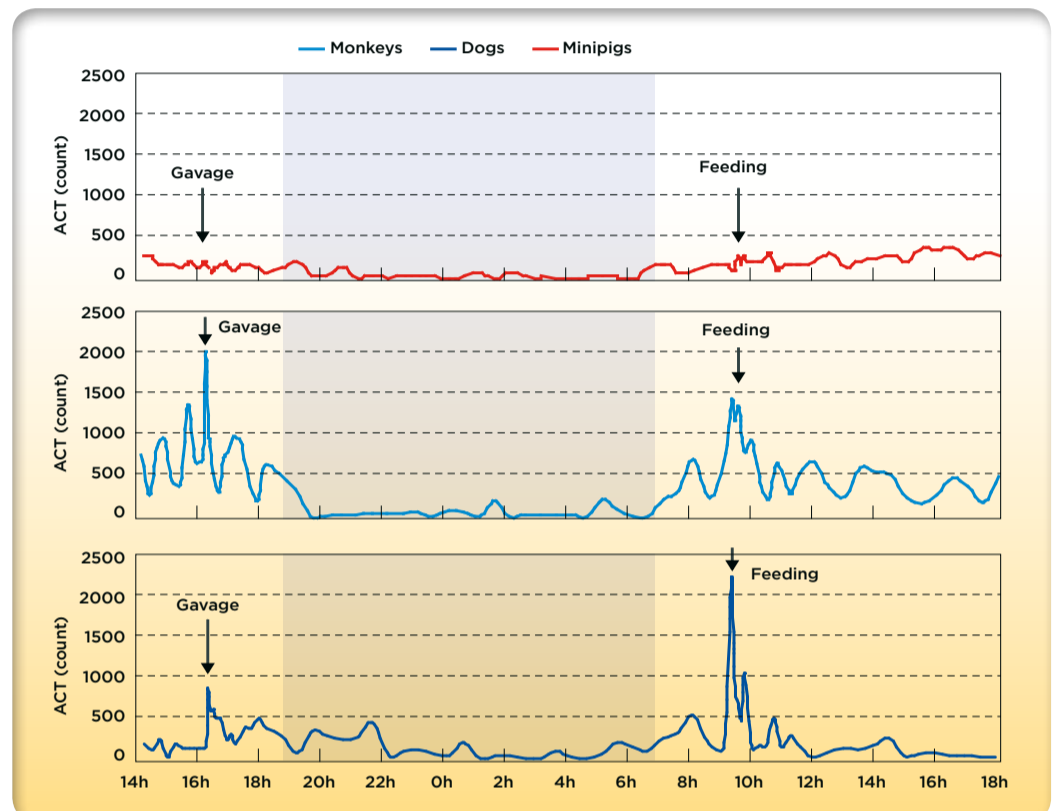


Fig. 5: Variation of activity in monkeys, dogs and minipigs during circadian cycle

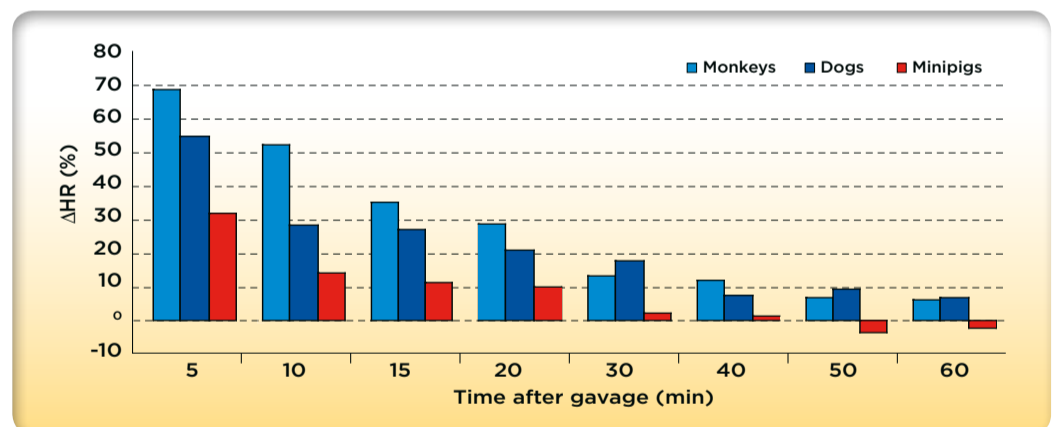


Fig. 6: Variation of HR (%) after gavage in monkeys, dogs, and minipigs

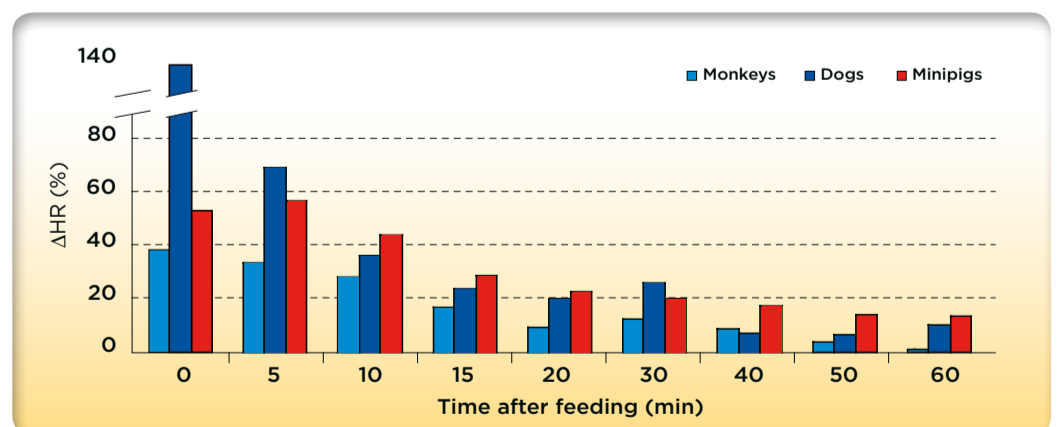


Fig. 7: Variation of HR (%) after feeding in monkeys, dogs, and minipigs

## Conclusion and discussion

The results of this study demonstrated that the circadian cycle, as well as gavage and feeding have a considerable influence on blood pressure and heart rate. This should be taken into consideration when adapting the design of cardiovascular studies to experimental animal models and interpreting results. The timing of oral test item administration should take into account the T<sub>max</sub> and half-life of the compound. If possible, the study should be designed to enable the expected effects of the test item to be recorded during the same photoperiod as pre-treatment data. If drug-induced effects are expected rapidly after oral test item administration, minipigs seem to be a good choice of animal model, since cardiovascular parameters are less disturbed in minipigs after the gavage procedure than in the other models tested. The results of this study also indicate that animals should be fasted for several hours before the beginning of the recording period.