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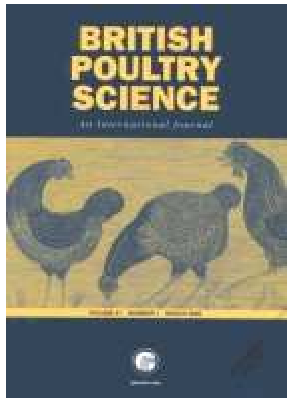
John Savory



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Circadian variation in heart rate, blood pressure, body temperature and EEG of immature broiler breeder chickens in restricted-fed and *ad libitum*-fed states

C. J. SAVORY, L. KOSTAL[1] AND I. M. NEVISON[2]

Avian Science Research Centre, Scottish Agricultural College, Ayr KA6 5HW, Scotland, [1]Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, 900 28 Ivanka pri Dunaji, Slovak Republic and [2]Biomathematics and Statistics Scotland, King's Buildings, Edinburgh EH9 3JZ, Scotland

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Correpondence to: Dr C.J. Savory, Netherham, Station Road, West Linton, Peeblesshire, EH46 7EL, UK

Email: jandesavory@hotmail.com

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Abstract 1. Heart rate, intra-aortic blood pressure, deep body temperature and telencephalic EEG were monitored by radiotelemetry in 6 freely moving immature broiler breeders (3 in each of two years), during routine food restriction and then *ad libitum* feeding, over two 24-h periods in each feeding state.

2. Heart rate, blood pressure and body temperature were all higher during *ad libitum* than restricted feeding, and heart rate and body temperature were higher by day (12 h) than at night (12 h). The decreases in heart rate and body temperature at night were greater during restricted than *ad libitum* feeding. Blood pressure tended to be higher at night, except in year 2 during restricted feeding. Body temperature and ambient temperature were higher in year 2 than year 1.

3. During restricted feeding, marked peaks in heart rate, blood pressure and body temperature in the 15 min after provision of the daily food ration at 09:00 h, when birds were eating, were equivalent to corresponding values seen during *ad libitum* feeding.

4. Relative powers in delta (1-4 Hz) and theta (4-8 Hz) frequency bands of the EEG power spectrum were higher at night in year 2 only, while power in the alpha (8-12 Hz) band was higher at night in both years.

5. It is concluded that most of the variation in heart rate, blood pressure and body temperature between feeding states and times of day/night can be accounted for in terms of variation in food intake and energy expenditure. The greater slow wave (delta, theta) EEG activity seen after lights-off in year 2 may reflect non-paradoxical sleep at that time.

INTRODUCTION

In commercial conditions, all growing broiler breeder chickens are fed on quantitatively restricted rations in order to limit their body weight at sexual maturity and thereby improve health and reproductive performance (Hocking *et al*., 1989). Female birds fed on such recommended rations, which are usually provided once a day and eaten in <10 min, typically eat only a third as much as they would with free access to food, and are highly motivated to feed at all times (Savory *et al*., 1993). They are much more active than unrestricted birds, and (unlike the latter) show increased pacing and oral behaviours that are characteristic of frustration of feeding motivation (Duncan and Wood-Gush, 1972; Kostal *et al*., 1992; Savory and Maros, 1993).

Evidence suggests that such apparently abnormal behaviours may be associated with de-arousal and perhaps serve a coping function (Delius, 1967; Hutt and Hutt, 1970; Dantzer and

Mormede, 1983; Soussignan and Koch, 1985). To address this possibility, which is potentially important in animal welfare terms, a commercially available radiotelemetry system (Data Sciences International, USA) was applied successfully in the (freely moving) chicken for chronic measurement of heart rate, intra-aortic blood pressure, deep body temperature and telencephalic EEG as putative physiological indices of arousal (Savory and Kostal, 1997). By combining this technology with simultaneous videorecording, these parameters were related to ongoing behaviour in 6 restricted-fed immature female broiler breeders. Results obtained were consistent with the idea that apparently abnormal behaviour is related to arousal state in a homeostatic way, being both stimulated by it and reducing it (Savory and Kostal, in press).

An additional purpose of this same study was to compare heart rate, blood pressure, body temperature and EEG between daytime and night-time, and between restricted-fed and *ad libitum*-fed states. This was done by switching the 6 birds onto *ad libitum* feeding immediately after they were monitored during food restriction, and then monitoring them again two weeks later. The results of these further comparisons are presented here. A similar approach was taken in another recent study, where heart rate and body temperature were measured over periods of 36 h in restricted-fed and *ad libitum*-fed broiler breeders (de Jong *et al.*, 2002). However, in that work, blood pressure and EEG were not measured, heart rate and body temperature were regarded as stress parameters rather than as indices of arousal, no attention was given to the influence of feeding time during food restriction and there was uncertainty regarding interpretation of results. Here, we conclude that variations in heart rate, blood pressure and body temperature, between feeding states and times of day/night, are associated more closely with variation in food intake and energy expenditure than with either physiological stress or arousal.

MATERIALS AND METHODS

Subjects and husbandry

In each of two successive years, 3 female broiler breeders (Ross 308, Aviagen Limited, UK) were reared in larger groups (of 32 and 13 birds) in floor pens according to a recommended restricted feeding programme (Ross Breeders, 1995). They were fed on a standard layer “starter” diet (201 g/kg crude protein and 11.7 MJ/kg metabolisable energy) for the first 6 weeks of life (in mash form first, then as pellets), and layer “grower” pellets (164 g/kg crude protein and 11.8 MJ/kg metabolisable energy) thereafter. Lights were on from 07:00 to 19:00 h, the daily ration was provided at 09:00 h and water was available *ad libitum*.

At 11 weeks of age, they were housed individually in 3 adjacent cages measuring 36 cm x 46 cm x 52 cm (w x d x h), in a room where lights were on from 07:00 to 19:00 h and ambient temperature was maintained at about 22 ^{o}C. Hardboard panels between the cages prevented birds seeing each other except when they had their heads out of the fronts. Each cage had a food container, water container and radiotelemetry receiver. The 3 receivers were linked to a computer with installed data acquisition card and Dataquest LabPRO software (version 3.0, Data Sciences International, USA) for acquisition and analysis of data (see Savory and Kostal (1997) for details). The daily ration of grower pellets (60 g per bird, all eaten in <10 min) was provided at 09:00 h, when water containers (1 litre) were also filled.

Radiotelemetry implantation

At 13 (year 1) and 12 (year 2) weeks of age, when mean body weights were 1.27 ± SE 0.04 and 1.32 ± 0.03 kg respectively, each bird had a radiotelemetry transmitter (type TL11M2-C50-PXT, Data Sciences International, USA) implanted surgically under halothane anaesthesia (Savory and Kostal, 1997), to allow monitoring of heart rate, intra-aortic blood pressure, deep body temperature and telencephalic EEG. The tip of the device's pressure sensing catheter was introduced into the descending aorta *via* a leg (ischiadic) artery and, although the catheter was tied in place, circulation in the leg was maintained through collateral arteries and leg function was not impaired. EEG was recorded from the device's paired sensing electrodes positioned on the surface of the telencephalon (using stereotaxic apparatus), the electrode leads being passed under the skin and held in place on the posterior skull with dental acrylic.

Data recording

All birds recovered well and ate all their food ration rapidly on the day after surgery. At 15 (year 1) and 13 (year 2) weeks of age, when they weighed 1.71 ± 0.02 and 1.45 ± 0.04 kg, the physiological parameters and behaviour of the 3 birds were monitored over two 24-h periods (midnight to midnight), separated by two days. Immediately after the second period, food restriction ceased and birds were fed *ad libitum* thereafter. At 17 (year 1) and 15 (year 2) weeks, when they weighed 2.29 ± 0.04 and 2.22 ± 0.12 kg, they were monitored again, in the *ad libitum*-fed state, over two 24-h periods separated by two days. In all 4 monitoring periods, each physiological parameter was recorded from a 5-s sample in every minute.

Data analysis

The data obtained were analysed in two ways. Firstly, overall mean values for heart rate, blood pressure, body temperature, and relative powers in delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz) and beta (12-30 Hz) frequency bands of the EEG power spectrum were calculated for every 12 h of light (day) and darkness (night), and every bird and monitoring period. These were analysed by a nested analysis of variance which tested the significance of effects of feeding state (restricted, *ad libitum*), time (day, night), year (1, 2), and their interactions. Secondly, overall mean values from all birds and both monitoring periods were calculated for every 15 min over 24 h, to show circadian variation in restricted-fed and *ad libitum*-fed states.

When the 5-s EEG signal waveforms were viewed (on computer screen print-outs), there were sometimes obvious artefacts or other anomalies, due to the presence of one or more very large peaks, major deviations in the baseline, or either partial or total absence of a signal. Many of these would presumably have been movement artefacts, sometimes apparent during feeding, and in at least one bird there may occasionally have been tension on the sensing electrode contacts when its neck was extended due to insufficient slack in the electrode leads. (All birds were examined after being killed by lethal injection at the end of experimentation, and the dental acrylic holding electrodes in place was still firmly attached to the skulls of all of them.) The problem of artefact rejection is a common feature of EEG research (*e.g.* Grasing and Szeto, 1992; Berger and Phillips, 1994), and here all waveforms with one or more obviously anomalous peak, or major deviation in baseline, or less than 3 s of signal were excluded from the analyses. Out of 34560 (6 birds x 4 d x 24 h x 60 min) EEG waveforms that were inspected visually, 2006 (5.8%) were rejected (subjectively) on the basis of any of these criteria. Proportions rejected in individual birds were 14.1, 6.0, 5.0, 4.7, 2.7 and 2.4%. Apart from 3 very low heart rate values (<60 bpm), which were rejected, there were no such anomalies in the heart rate, blood pressure and body temperature data.

RESULTS

Significant (P<0.05) effects on the physiological variables of feeding state, time of day/night, year, and their interactions are shown in the Table. Thus, heart rate, blood pressure and body temperature were all higher during *ad libitum* feeding than restricted feeding (F). Heart rate and body temperature were also higher by day than at night (T). The differences in heart rate and body temperature between day and night were greater during restricted than *ad libitum* feeding (FxT). The decrease in overall mean heart rate at night was greater in year 2 than year 1 (TxY). Blood pressure tended to increase from day to night, except in year 2 during restricted feeding (FxTxY). Overall mean body temperature was higher in year 2 (41.11 ^{o}C) than year 1 (40.69 ^{o}C) (Year), possibly because ambient temperature during monitoring periods was also higher (P=0.007, by t-test) in year 2 (23.4±0.2 ^{o}C) than year 1 (21.5±0.4 ^{o}C). The decrease in body temperature at night during restricted feeding was greater in year 1 than year 2 (TxY, FxTxY).

Table near here

Relative powers in delta (1-4 Hz) and theta (4-8 Hz) frequency bands of the EEG power spectrum were higher at night than by day, but only in year 2, when they were also higher (by day and at night) than in year 1 (T, Y, TxY). Power in the alpha (8-12 Hz) band was higher at night than by day in both years (T). There were no other significant effects.

During restricted feeding, there were marked peaks in heart rate (Figure 1a), blood pressure (Figure 1b) and body temperature (Figure 1c) in the 15 min after provision of the daily ration at 09:00 h, when birds were feeding (all food was eaten in <10 min). After feeding, there was a gradual decline in heart rate that continued until after lights-off (19:00 h). There was no such consistent change in either blood pressure or body temperature; indeed the latter took 2-3 h to fall to the night-time level after lights-off, and started rising toward the day-time level about 1 h before lights-on (07:00 h). Circadian variations in heart rate, blood pressure and body temperature during *ad libitum* feeding were associated only with lights-on and lights-off.

There were no obvious changes in relative powers in delta (Figure 2a), theta (Figure 2b), alpha (Figure 2c) or beta (Figure 2d) frequency bands of the EEG power spectrum around feeding time during restricted feeding. In that feeding state, however, powers in theta and alpha bands did appear to be reduced between lights-on and shortly after the daily meal. Powers in delta and theta bands peaked about an hour after lights-off and then declined gradually, in year 2 only (significant TxY interactions, Table). These peaks, and also one in alpha power then, were more marked during restricted feeding, but there was no significant FxT interaction with any EEG band in the Table.

Figures 1 and 2 near here

DISCUSSION

The increase in energy expenditure above resting metabolic rate that occurs after eating a meal has been referred to as "diet-induced thermogenesis" (Rothwell and Stock, 1979) or "the thermic effect of food" (Hill *et al*., 1985). Presumably it explains why heart rate, blood pressure and body temperature of the broiler breeders here were all increased significantly during *ad libitum* feeding monitoring periods, when their mean daily food intake (208 ± 7 g in year 1 and 178 ± 11 g in year 2) was about 3 times higher than the 60 g daily ration provided during restricted feeding monitoring periods. It should also explain why heart rate, blood pressure and body temperature were all as high in the 15 min after provision of the daily meal during restricted feeding as they were during *ad libitum* feeding (Figures 1a, 1b, 1c).

Total removal of food has been found to cause progressive reduction in heart rate in young broiler and layer fowls (Yamamoto and Mimura, 1976; Shimada and Koide, 1978). Chronic food restriction caused reductions in fasting and resting rates of heat production in adult laying hens (Macleod and Shannon, 1978), broilers and broiler breeders (Macleod *et al*., 1993; Koh and Macleod, 1999) and pigeons (Phillips *et al*., 1991), and it also caused reductions in rectal and foot-surface temperatures in broiler breeders (Macleod *et al*., 1993). The peaks in heart rate and blood pressure found in the present study in association with the single daily meal during food restriction are like those found to occur during a 1-h feeding period in restricted-fed rats (van den Buuse, 1999). It appears that no previous study has examined blood pressure or EEG in relation to either food restriction or time of day/night in domestic fowls, and here there was no effect of feeding state on EEG (Table).

Reductions in heart rate, heat production and body temperature at night have been reported previously for fowls (Yamamoto and Mimura, 1976; Shimada and Koide, 1978; Macleod *et al*., 1980; Macleod and Jewitt, 1984; de Jong *et al*., 2002), Japanese quail (Hohtola *et al*., 1991; Underwood *et al*., 1999) and pigeons (Graf *et al*., 1989; Ostheim, 1992; Phillips *et al*., 1991, 1993). Just as in the present study, these reported nocturnal declines were greater during food restriction or total food withdrawal than during *ad libitum* feeding. It has been proposed that the more marked nocturnal hypothermia observed during restricted feeding enhances energy savings already being achieved through lowered thermal conductance (Phillips *et al*., 1991). From an earlier comparison of heat production of restricted- and *ad libitum*-fed broiler breeders (Macleod *et al*., 1993), using the same restricted feeding programme as in the present study, it can be calculated that mean thermal conductances across 24 h (*i.e.* day and night) were 38 and 71 kJ/^{o}C, respectively.

In a pilot radiotelemetry trial with one restricted-fed chicken (Savory and Kostal, 1997), heart rate and blood pressure were correlated negatively, the former being higher and the latter lower by day than at night. Although similar opposite trends were observed in the present study, the difference in blood pressure between day and night was not consistent in year 2 during food restriction. Such a tendency for heart rate and blood pressure to be correlated negatively in the fowl appears to differ from the rat, in which heart rate and blood pressure changed in parallel between day and night (van den Buuse, 1999). It may also differ from the turkey, in which mean arterial blood pressure has been found to be slightly lower at night than by day (Krista *et al.*, 1981). With humans, it was concluded that the 24-h profile of blood pressure, as observed under normal circumstances, is the sole result of environmental and behavioural factors such as the occurrence of sleep, and has no endogenous circadian component (van Dongen *et al.*, 2001).

Regardless of feeding state, relative powers in the slower wave (delta (1-4 Hz), theta (4-8 Hz)) frequency bands of the EEG power spectrum were higher at night than by day in the present study in year 2 only, while power in the alpha (8-12 Hz) band was higher at night in both years. There was no such difference between day and night in fast wave (beta (12-30 Hz)) power. Slow wave (delta band) activity has also been shown to reach high levels at night in the pigeon (Berger and Phillips, 1994). With the rat, the trend in relative delta power over the daytime, inactive period (when nocturnal rodents sleep) resembles that seen in human subjects during sleep, with peak levels occurring at the onset, followed by a steady decline during remaining hours of the daytime rest period (Grasing and Szeto, 1992). This is like the marked peaks in delta, theta and alpha powers shown by broiler breeders after lights-off during restricted feeding in the present study. There was a smaller peak in delta power after lights-off during ad libitum feeding. In a previous study with domestic hens, it was reported that delta activity decreases across the night, reflecting a transition from (mainly slow wave) non-paradoxical to (less slow wave) paradoxical sleep (van Luijtelaar *et al.*, 1987).

Overall mean body temperature was significantly higher in year 2 than year 1, possibly because ambient temperature during monitoring periods was also higher in year 2 than year 1 (see Results). This may explain why the decline in body temperature at night during restricted feeding was greater in year 1 (4.5%) than year 2 (3.1%) (significant FxTxY interaction, which also accounts for the significant TxY interaction). It might also explain why the increase in (overall mean) heart rate by day was greater in year 2 (22%) than year 1 (14%), why delta and theta (slower wave) EEG powers were (36% and 39%) greater in year 2 than year 1, and why the increases in delta and theta powers at night were much greater in year 2 (19% and 12%) than year 1 (0% and 2%). The significant interactions may also be due partly to the small sample size used (3 birds in each of two years), which was a consequence of the high cost and labour intensity of this type of work.

Another purpose of this study, reported elsewhere (Savory and Kostal, in press), was to test a hypothesis that some behaviours observed during food restriction are associated with de-arousal (see Introduction). For this, it was assumed that arousal is associated with increased heart rate, blood pressure, body temperature and beta (fast wave) EEG power, and reduced delta and theta (slow wave) EEG powers, and that de-arousal is associated with opposite effects. Based on powers in the different EEG frequency bands, there was no evidence here of any consistent difference in arousal or de-arousal between restricted-fed and *ad libitum*-fed states. Also, it can be concluded from the present results that variations in heart rate, blood pressure and body temperature, between feeding states and times of day/night, are associated more closely with variation in food intake and energy expenditure (see above) than with either arousal state or physiological stress (cf. de Jong *et al.*, 2002).

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Table. *Overall mean heart rate (HR), blood pressure (BP), body temperature (BT), and relative powers in different frequency bands (delta, theta, alpha, beta) of the EEG power spectrum, during the day (12 h) and night (12 h), in restricted-fed (R) and ad libitum-fed (AL) states; and significance of effects of feeding state (F), time of day/night (T), year (Y), and their interactions (P values, by ANOVA)*

Feeding state	R	R	AL	AL	Significance of effects						
Time	Day	Night	Day	Night	F	T	Y	FxT	FxY	TxY	FxTxY
HR (bpm)	226.0	176.7	356.9	317.5	<0.001	<0.001	0.376	0.007	0.939	<0.001	0.722
BP (mmHg)	109.3	111.6	117.8	122.2	0.007	0.104	0.774	0.603	0.393	0.090	0.046
BT (^{o}C)	41.20	39.62	41.61	41.16	<0.001	<0.001	0.012	<0.001	0.393	<0.001	<0.001
EEG (mV^2/Hz)											
delta (1-4 Hz)	0.111	0.130	0.112	0.117	0.341	0.029	0.060	0.165	0.475	0.033	0.523
theta (4-8 Hz)	0.066	0.072	0.063	0.066	0.222	0.010	0.016	0.390	0.723	0.043	0.484
alpha (8-12 Hz)	0.037	0.040	0.036	0.038	0.304	0.001	0.290	0.291	0.501	0.492	0.109
beta (12-30 Hz)	0.019	0.020	0.019	0.020	0.724	0.297	0.417	0.602	0.398	0.768	0.497

Figure 1. *Circadian variation in overall mean heart rate (a), blood pressure (b) and body temperature (c), in restricted-fed (bold line) and ad libitum-fed (fainter line) states. Vertical lines indicate times of lights on (0700 h) and lights off (1900 h), and the vertical dashed line indicates feeding time (0900 h).*

Figure 2. *Circadian variation in overall mean relative powers (mV^2/Hz) in the delta (a, 1-4 Hz), theta (b, 4-8 Hz), alpha (c, 8-12 Hz) and beta (d, 12-30 Hz) frequency bands of the EEG power spectrum, in restricted-fed (bold line) and ad libitum-fed (fainter line) states. Vertical lines indicate times of lights on (0700 h) and lights off (1900 h)), and the vertical dashed line indicates feeding time (0900 h).*

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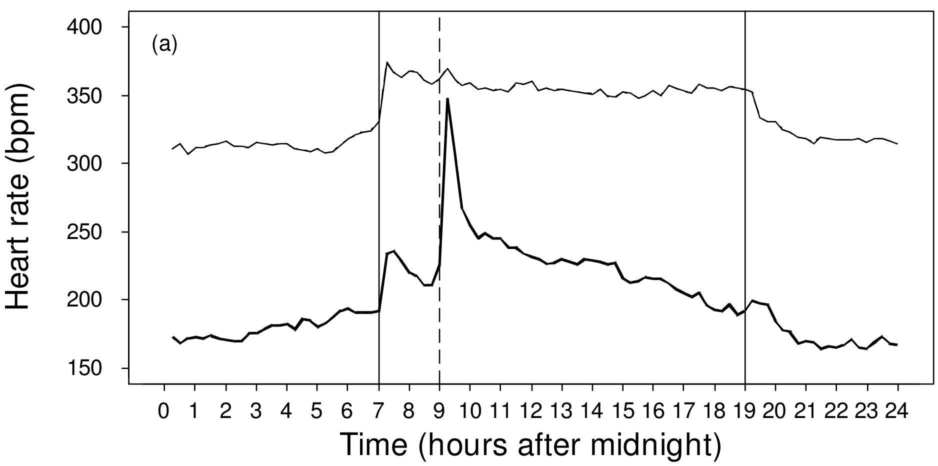
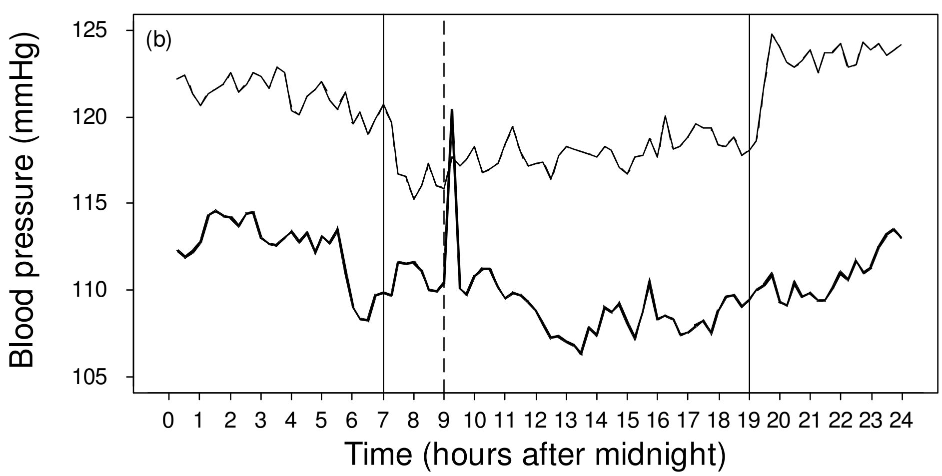
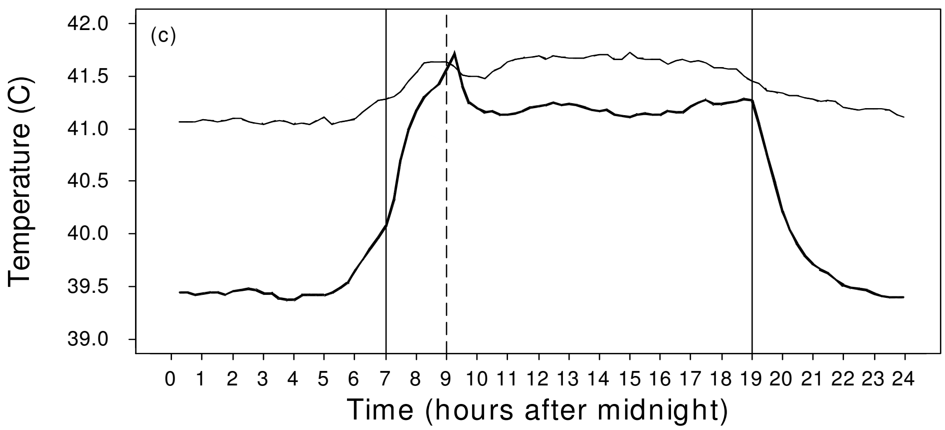
1a


(a)
Heart rate (bpm)
400
350
300
250
200
150
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Time (hours after midnight)

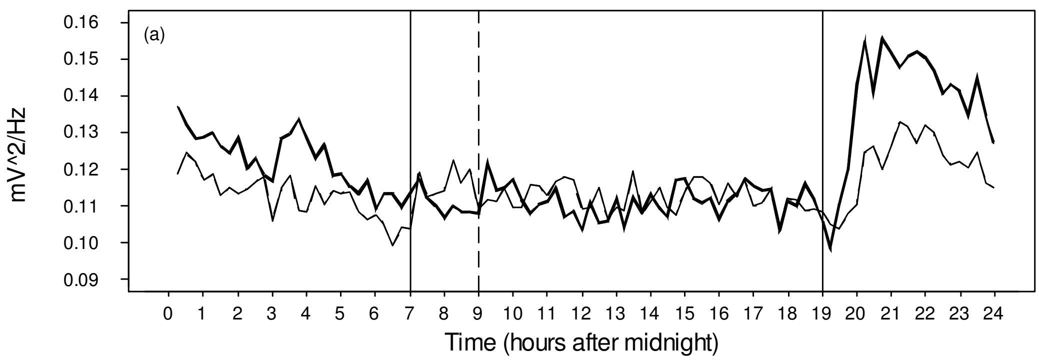
1b


(b)
Blood pressure (mmHg)
125
120
115
110
105
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Time (hours after midnight)

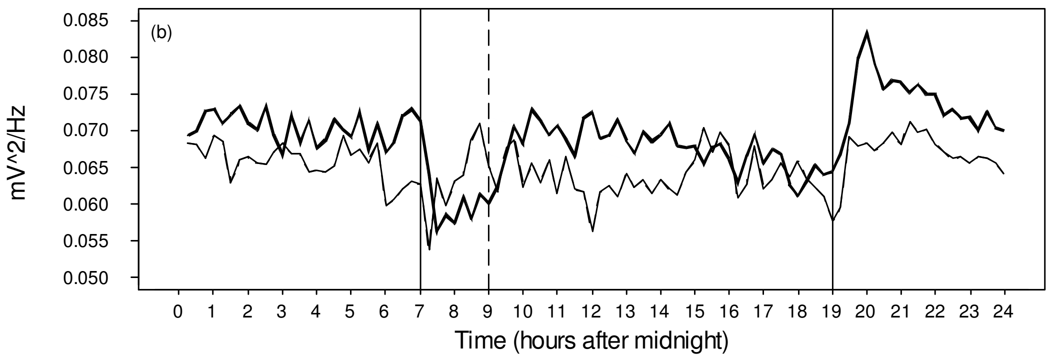
1c


(c)
42.0
41.5
41.0
40.5
40.0
39.5
39.0
Temperature (C)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Time (hours after midnight)

2a

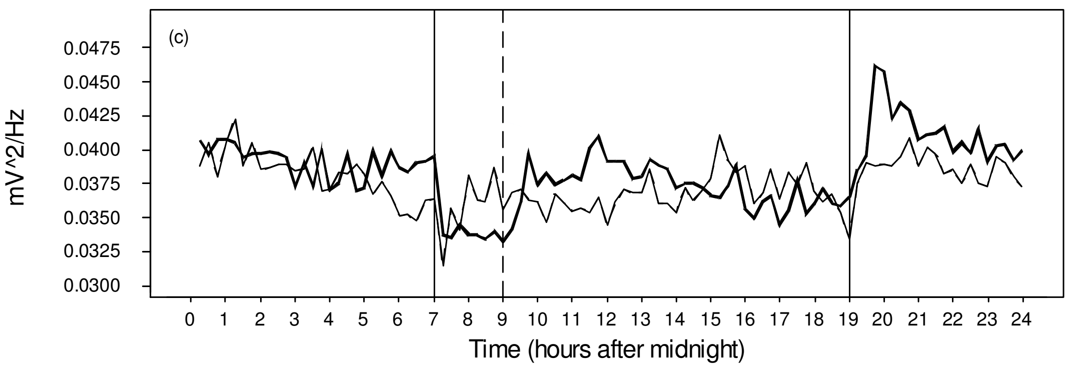

(a)
mV^2/Hz
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Time (hours after midnight)

2b

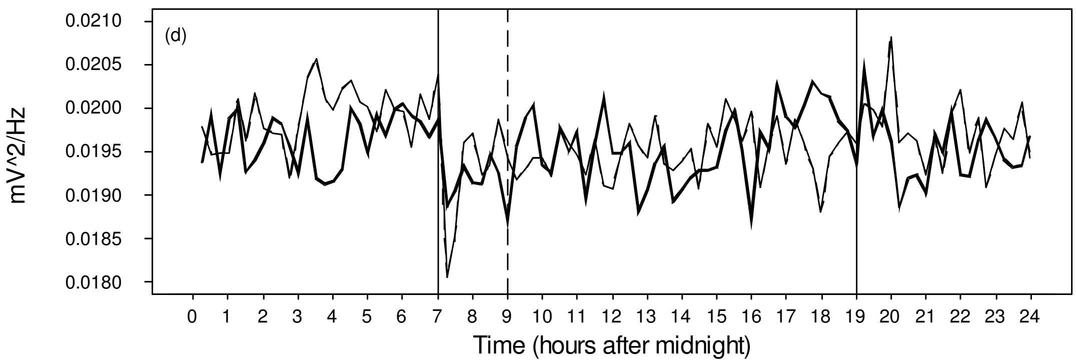


(b)
0.085
0.080
0.075
0.070
0.065
0.060
0.055
0.050
mV^2/Hz
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Time (hours after midnight)

2c


(c)
mV^2/Hz
0.0475
0.0450
0.0425
0.0400
0.0375
0.0350
0.0325
0.0300
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Time (hours after midnight)

2d


(d)
mV^2/Hz
0.0210
0.0205
0.0200
0.0195
0.0190
0.0185
0.0180
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Time (hours after midnight)