THE USE OF RECTAL TEMPERATURE FLUCTUATIONS IN THE STUDY OF CIRCADIAN RHYTHM IN THREE ADULT VERTEBRATE SPECIES IN AWKA, NIGERIA

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ABSTRACT

Biological clocks allow organisms to assess and respond to the oscillating environmental rhythms that result from the earth's movements via the generation of biological rhythms. Circadian clocks are also assumed to enhance survival and reproductive fitness in part by promoting optional timing of behaviour and physiology in relation to regular cycles in the environment. Temperature fluctuations were studied in three different vertebrate species – Sylvilagus floridanus, Rattus norvegicus and Columba livia in relation to the rhythmicity of rectal temperature. Clinical digital thermometer was used to record the core temperature by inserting it 2 – 3 cm deep from the anal sphincter before taking readings 3 minutes after. The highest recorded mean rectal temperature for R. norvegicus and S. floridanus were 38.85 ± 0.40 ^{o}C and 39.83 ± 0.32 ^{o}C respectively with the lowest being 36.58 ± 0.74 ^{o}C and 36.63 ± 0.18 ^{o}C respectively. C. livia failed to exhibit core temperature fluctuation. There were not significant differences in the mean rectal temperature for both sexes for S. floridanus in relation to time of day. Differences in the circadian temperature fluctuations were traced to variation among the animals, of preferred temperature arising from differential behavioural and physiological regulation, in relation to environmental cues. It is also possible that daily changes in illumination were secondary to C. livia. The results also suggest that circadian rhythmicity persists even in artificially imposed selective environment. Rectal temperatures are adequate for monitoring the biorhythms, with each species exhibiting endogenous peculiarities in the various circadian phases.

Keywords: Circadian temperature, Rectal temperature, Vertebrate species, Physiological regulation

INTRODUCTION

Circadian clocks are assumed to enhance survival and reproductive fitness in part by promoting optional timing of behaviour and physiology with respect to regular cycles in the environment. Unlike other sensory modalities, the clock does not respond to any form of energy from the environment. It measures the passage of time internally, and gives the non-directional physical time a direction ("arrow") by virtue of being in a living organism (Frazer, 1996). The clock is, thus, an internal representation of time, a cognitive temporal map analogous to the cognitive spatial maps. This allows the organism to perform internal time measurement, distinction between, before and after, measurement of duration of events, and learning of the local time of day (Shettleworth, 1998). The rising and setting of the sun provides a precise and reliable signal for regulating the phase of

the circadian clock, and it is therefore not surprising that light is a powerful, if not dominant 'zengeber' (entraining stimulus) for most species. However, for many species, daily changes in illumination may be secondary to what is really important, namely, the activity patterns of other denizens of the greater social community, the potential mates, competitors, predators, prey and parasites with which they must share time and space. It may therefore by hypothesize that circadian clock in some, if not all species have evolved a capacity for phase resetting in response to significant interactions with other organism. There is ample evidence from field work to support the general view that animals do coordinate their behaviour in time with that of other animals, of the same or different species, but the mechanisms by which this is achieved are little studied (Frazer, 1996). Studies on circadian rhythmicity and metabolism in mammals and birds include those of Kramm (1975), Daan and Pittendrigh (1976),

Decoursey (1986, 1990), Pohl (1992) and Underwood *et al.* (1997) with specific studies on circadian rhythm of body temperature (CBT) including those of Gordon (1990), Refinetti and Menaker (1992), Krauchi (2002) and Kumar (2004).

In related studies, the data for domestic fowl indicate that the body temperature does fluctuate over a 24-hr period and in the brain the temperature fluctuations are maintained even under constant condition (Winget *et al.,* 1965; Aschoff *et al.,* 1973). Likewise mammals such as humans, hamsters tree shrews, and golden hamsters exhibit robust circadian temperature fluctuations under light and constant light conditions (Colin *et al.* 1968; Refinetti and Menaker, 1992). The present study tackles a similar problem but using rectal temperature of *Sylvilagus floridenus* (cotton tail/rabbits), *Rattus norvegicus* (albino rats) and *Columba livia* (homing pigeons) to study circadian temperature fluctuations over a 24 hours period in other to ascertain the trend in their fluctuation, in the species. The aim was to compare the fluctuation in the anal temperature and to ascertain any differences between species and consistency in relation to time of the day in the Nigerian environment.

MATERIALS AND METHODS

This work was carried out at Awka, Anambra State of Nigeria in month of June 2005. The experimental animals used in this study include, adult *Sylvilagus floridanus* which were purchased from Head bridge market Onitsha while *Rattus norvegicus* (also adults) which were also purchased from College of Medicine, University of Nigeria, Enugu Campus, Enugu, Nigeria. The adult *Columba livia* were purchased from Eke Awka Main Market, Awka, Anambra State. They were appropriately caged, fed and reared. Such pre-rearing was necessary for the acclimatization of the species before the initiation of taking of readings. Each cage was covered with gauze to prevent escape of specimens and to allow adequate ventilation for the animals.

Ten specimens each were used for the study with four replicates made. Equal number of males and females were used for *S. floridanus*, with only males used for *R. norvegicus* and *C. livia* and to allow ventilation for the animals.

Digital thermometer was used take the rectal temperature of the specimens hourly for twenty-four hour period. In taking the measurement the bulb of the clinical digital thermometer was inserted into the rectum to a depth of $2 - 3$ cm from the anal margin or sphincter. The temperature was read from the thermometer 3 minutes after insertion.

The readings were taken for two consecutive days after which further measurements and observations from the replicates were terminated to reduce any stress on the animals. The data obtained from the study were subjected to paired Students t-test, to test whether statistical differences exist between mean rectal temperatures of species; in addition the data was subjected to Levene's test for equality of variances (Levene, 1960).

RESULTS

In the collection of the data for the temperature fluctuation of the three vertebrate species studied which lasted for 24 hours period, on each day, the readings from the replicates were pulled together. The highest recorded mean rectal temperatures in degrees Celsius (^{o}C) for *R. norvegicus* and *S. floridanus* were 38.85 ± 0.40 ^{o}C and $39.9.83 \pm 0.32$ ^{o}C respectively while the lowest temperatures were 36.58 ± 0.74 ^{o}C and 36.63 ± 0.18 ^{o}C respectively. There was no temperature fluctuation for *C. livia* (Table 1).

Furthermore, the mean rectal temperatures for *R. norvegicus* and *S. floridanus* were 37.9 ± 0.06 ^{o}C and 39.11 ± 0.09 ^{o}C respectively (Table 2), were significantly different using t-test ($p \leq 0.05$). Day 1 mean temperature (38.35 ± 0.11 ^{o}C) and those of Day 2 (38.68 ± 0.11 ^{0}C) were significantly different ($p \leq 0.05$). In both cases the F values obtained from Levene's test for equality of variances were greater than 0.5 (Table 2). There were however no significant differences in the rectal temperature variation for both sexes of *S. floridanus* in relation to time of day from the Analysis of Variance (ANOVA) carried out (F ratio = 6.00).

The mean rectal temperature of the male *R. norvegicus* more or less exhibited regularity in fluctuation patterns in relation to time of day but for a slight drop in temperature of 1 ^{o}C at 20.00 hours on Day 2 (Figure 1). The pattern of fluctuation of mean rectal temperature for *S. floridanus* for the 24 hours period was more consistent except for the sharp slight rise for Day 1 and a simultaneous drop (in each case 2 ^{0}C) at 6.00 hours on both days (Figure 2). No temperature fluctuation was however recorded for the mean rectal temperature of male *C. livia* during the 24 hours periods of study, with the mean and temperature maintained at 42 ^{0}C.

DISCUSSION

The results of the study show that biological clocks or circadian clocks are endogenous, inherited timing devices which control rhythms of many physiological

Table 1: Mean rectal temperature fluctuation over a 24 hour period in adult *Sylvilagus floridanus*, *Rattus norvegicus* and *Calumba livia*

Time of the Day	*Rattus* Day I	*norvegicus* Day II	*Sylvilagus* Day I	*floridamus* Day II	*Columba* Day I	*livia* Day II
01.00	38.38 ± 0.20	38.3 ± 0.20	33.63 ± 0.18	39.45 ± 0.07	42.00	42.00
02.00	37.6 ± 0.46	38.58 ± 0.28	38.03 ± 0.75	39.48 ± 0.13	42.00	42.00
03.00	37.38 ± 0.30	38.60 ± 0.48	37.08 ± 0.18	39.35 ± 0.14	42.00	42.00
04.00	37.3 ± 0.56	37.65 ± 0.40	38.65 ± 0.48	39.98 ± 0.31	42.00	42.00
05.00	37.60 ± 0.12	38.63 ± 0.20	38.70 ± 0.29	39.03 ± 0.40	42.00	42.00
06.00	36.58 ± 0.74	38.60 ± 0.35	38.30 ± 0.46	39.03 ± 0.38	42.00	42.00
07.00	37.08 ± 0.57	38.0 ± 0.28	38.10 ± 0.69	38.93 ± 0.28	42.00	42.00
08.00	37.63 ± 0.26	37.85 ± 0.14	38.15 ± 0.56	39.00 ± 0.37	42.00	42.00
09.00	37.63 ± 0.25	37.80 ± 0.26	38.68 ± 0.42	39.53 ± 0.36	42.00	42.00
10.00	37.63 ± 0.14	37.55 ± 0.05	38.95 ± 0.16	39.53 ± 0.03	42.00	42.00
11.00	38.00 ± 0.15	37.88 ± 0.36	39.05 ± 0.16	39.53 ± 0.18	42.00	42.00
12.00	37.73 ± 0.10	37.90 ± 0.22	39.05 ± 0.26	39.83 ± 0.32	42.00	42.00
13.00	37.68 ± 0.24	37.70 ± 0.04	38.93 ± 0.31	39.70 ± 0.09	42.00	42.00
14.00	37.73 ± 0.14	37.55 ± 0.20	39.02 ± 0.08	39.73 ± 0.31	42.00	42.00
15.00	38.20 ± 0.23	37.93 ± 0.83	38.25 ± 0.20	39.65 ± 0.17	42.00	42.00
16.00	38.23 ± 0.43	38.08 ± 0.41	39.39 ± 0.18	39.63 ± 0.18	42.00	42.00
17.00	38.48 ± 0.44	38.00 ± 0.38	38.38 ± 0.23	39.53 ± 0.13	42.00	42.00
18.00	37.80 ± 0.44	37.73 ± 0.34	39.40 ± 0.10	39.50 ± 0.17	42.00	42.00
19.00	38.30 ± 0.12	37.83 ± 0.16	39.40 ± 0.16	39.30 ± 0.10	42.00	42.00
20.00	38.10 ± 0.15	38.20 ± 0.05	39.48 ± 0.23	38.50 ± 0.43	42.00	42.00
21.00	38.05 ± 0.28	38.05 ± 0.31	39.48 ± 0.20	39.53 ± 0.37	42.00	42.00
22.00	38.35 ± 0.19	38.15 ± 0.28	39.58 ± 0.20	39.40 ± 0.15	42.00	42.00
23.00	38.53 ± 0.06	38.85 ± 0.40	39.45 ± 0.16	39.33 ± 0.13	42.00	42.00
24.00	38.45 ± 0.10	38.48 ± 0.08	39.40 ± 0.11	39.38 ± 0.22	42.00	42.00

Mean temperatures (^{0}C) obtained in Day I and II

Table 2: Variation in mean rectal temperatures of adult *Rattus norvegicus* and *Sylvilagus floridanus* for each for Day 1 and Day 2, over 24 hour period

Parameter	Mean	Probability values for associated t-test Unequal S^2	Equal S^2	F values for Levene's test for equality of Variances
Rattus norvegicus	37.93 ± 0.06	11.019* (p = 0.000)	11.019* (p=0.000)	2.482 [n.s] (p=0.119)
Sylvilagus floridanus	39.11 ± 0.09			
Day one mean	38.35 ± 0.11			
Day two mean	38.68 ± 0.11	2.09* (p=0.039)	2.094* (p=0.039)	0.516[n.s] (p=0.474)

Note: S = variance, S.E. standard error of mean; ns = not significant, * = significant at 0.05 probability level

and behavioural functions (Decoursey *et al.,* 2000). These results are in line with the observations of Bligh and Johnson, 1973 who recorded difference among animals of preferred body temperature arising from variability in their behavioural and physiological regulation, and in relation to environmental cues. This no doubt is reflected in the differences in the temperature regimes obtained for the three vertebrate species with at those of *C. livia* being highest followed by *S. floridanus* and then *R. norvegius* (Table 1).

The statistical differences obtained in the comparison of the mean rectal temperatures of *R norvegicus* and those of *S floridanus*, (Table 2) is also a reflection of the differences in the preferred temperature of these species (Bligh and Johnson 1973), and supported by the existence of

nonhomogeneity of variance implicit in Levene's (1960) test carried out (Table 1).

Temperature rhythm was exhibited by the three vertebrates species studied with each rhythm exhibiting peculiarities, with the circadian temperature fluctuation of the *S. floridanus* being sharper than that of *R. norvegicus* (Figures 1 and 2). These observations are in line with those of Refinetti and Menaker who reported robust circadian temperature fluctuations, even under light and light conditions. For the studied vertebrate species. Nuesslein-Hildeshelm *et al.* (1995) also observed even among juveniles a high level of core temperature for rabbits and pronounced core temperature rhythm in several strains of rats. There is also evidence from the study of temperature rhythm of *S. floridanus* that when behaviours are
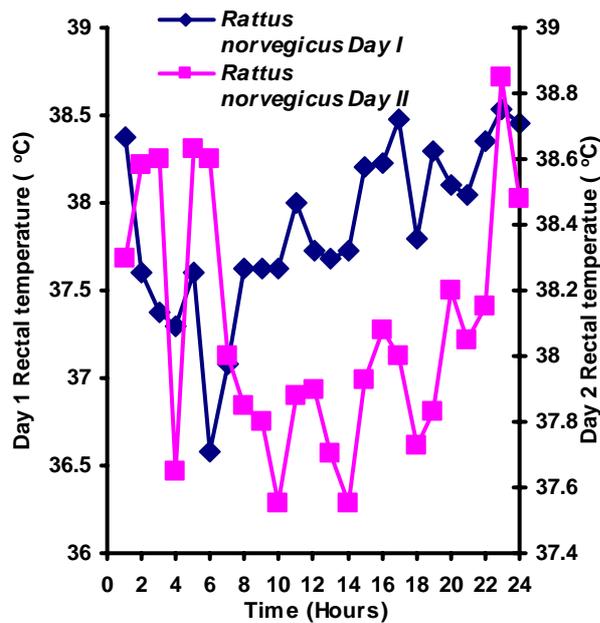
Figure 1: Rectal temperature fluctuation for days 1 and 2 over a 24 hour period in adult *Rattus norvegicus*
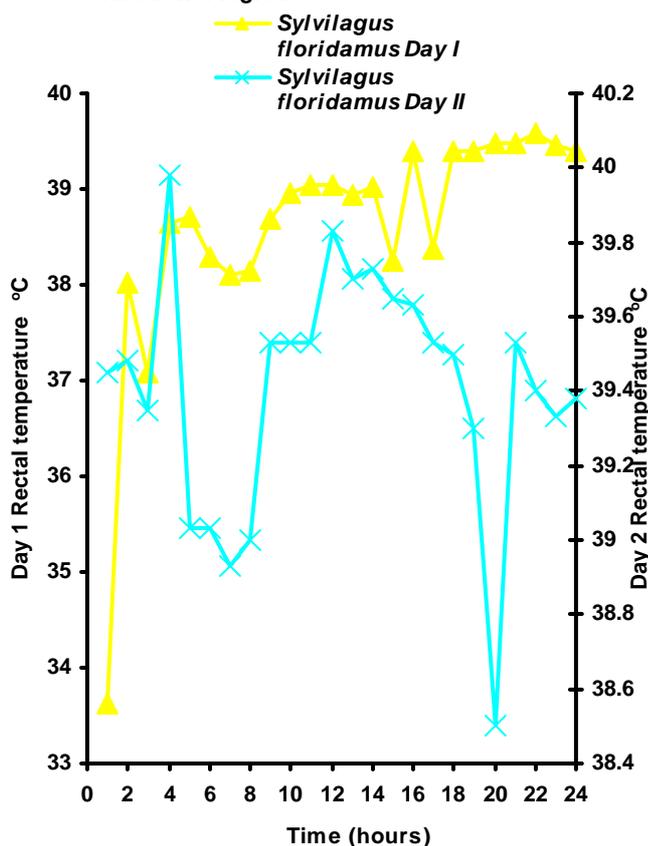

Figure 2: Rectal temperature fluctuation for days 1 and 2 over a 24 hour period in adult *Sylvilagus floridanus*

elicited outside the usual circadian phase of activity, the stimulus is considered to mask true circadian phase (Figure 1) (Aschoff, 1998). The slight drops in the core body temperature of the rats might be attributed to physiological thermoregulatory systems which have been implicated in infant rats (Sullivan *et al.,* 1988) as well as in these adult rats. It is also possible that the constancy of rectal temperature fluctuation observed for *C. livia* is peculiar to the species. Evidently it is possible that daily changes in illumination were secondary to these bird species (Decoursey, 1990) with these birds moderating to unique response to dusk and day in contrast to the other animal species. Earlier results on the domestic fowl by Aschoff *et al.* (1973) are also in line with these observations, since they reported that body temperature failed to fluctuate over a 24 hour period with the result that brain and body temperature fluctuations were maintained under constant conditions.

In the long run evidence abound from the study that circadian rhythmicity does not disappear even in artificially imposed selective environment as obtained from similar studies on animal groups other than vertebrates (Sheeba *et al.*, 1999). It can also be concluded that rectal temperatures are adequate for monitoring biorhythms, with each species showing uniformity in their response to environmental cues. There is also evidence that even though these clocks are organic structures, and not absolutely precise, they have also evolved as means by which external timing cues like dawn and dusk can entrain the phase of the clock to the local time (Decoursey, 1990).

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