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Full Length Research Paper

Duration-related modulation of body temperature rhythm and reproductive cycle in rats by photoperiodic perturbation

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Studies have shown that light pollution impairs biorhythms through alteration in expressions of circadian clocks. The duration related effect of photoperiodic perturbation on pattern of endogenous rhythms is not clear. 20 adult female rats displaying 4-5 days estrous cycle length and weighing148-154g were randomly divided into four groups of five animals each. While groups A and B were maintained under natural 12 h (light/ dark) cycle (3-20lux) for 1 and 8 weeks respectively, animals in groups C and D were maintained under alternate schedule of 20h light/4h dark cycle (120-150 lux) for 1 and 8 weeks respectively. Body temperature rhythm, estrous cycle pattern and nocturnal plasma melatonin were determined. The results indicated dysrhythmia in body temperature evidenced by the significant change (P<0.05) in nighttime and day time temperatures. There was a significant increase (P<0.05) in estrous cycle length and estrous cycle ratio. There was also a significant decrease (P<0.05) in nocturnal melatonin secretion.. While the effect of photoperiodic perturbation on melatonin secretion and estrous cycle ratio was duration dependent, the effect on nighttime and daytime body temperature, estrous cycle length was not duration dependent. We concluded that photoperiodic perturbation caused no sustainable effect on estrous cycle ratio and nocturnal plasma melatonin secretion.

Keywords: Biorhythm, body temperature, estrous cycle length, melatonin, estrous cycle, estrous cycle ratio

INTRODUCTION

Available evidence indicates that day active animals exhibit temperature rhythm characterized by a bathophase occurring at 4.00am and an acrophase between 4.00pm-6.00pm (Mackowiak *et al*., 1992; Adeniyi *et al.,* 2016). Also, in popular scientific consensus peak melatonin secretion and luteinizing hormone (LH) surge occur in the night (Lowden *et al.,* 2010). The discovery of electric bulbs decades ago and advent of artificial light produced profound changes in biorhythms. For instance, female rats maintained under continuous lighting for 8 months showed prolonged estrus and persistent vaginal cornification due to low serum level of progesterone and high estradiol: progesterone ratio (Lima *et al*., 2004). In shift duty workers, exposure to artificial light resulted in abolishment of melatonin rhythm (Lowden *et al.,* 2010).

Exposure to light at night disrupts nocturnal body temperature and upsets body temperature rhythm (Mahoney, 2010). In humans, melatonin has been shown to interact with the hypothalamic-pituitary- thyroid axis to modulate the circadian rhythm of body temperature (Mazzoccoli *et al.,* 2004; Adeniyi and Agoreyo, 2017).Our previous study demonstrated that photoperiodic perturbation altered estrous cycle, an endogenous rhythm (Adeniyi and Agoreyo, 2018). There is insufficient information about the duration related effect of photoperiodic perturbation on pattern of endogenous rhythms. The study investigated the duration related effect of photoperiodic perturbation on the pattern of endogenous rhythms in rats.

METHODOLOGY

Reagents and supplies

Light Microscope, digital lux meter, glass slides, mercury-in-glass thermometer, weighing balance, dissecting set, needles, syringes and chemicals were obtained commercially.

Animal care and management

Twenty cyclical adult female wistar rats with estrous cycle length of 4-5 days and weighing 148-154 g were used for the research work. The diestrus rats were divided into four groups consisting of five animals each. All rats were housed in plastic cages (0.27m x 0.37m) with stainless steel mesh cover. The rats were kept in four different cages with a wire mesh covering. They were fed pelletized grower's mash adlibitum and provided water through drinking trough. Rats were raised under photoperiod controlled condition and 12 h light/dark cycle respectively.

Experimental procedure

The rats were weighed and randomly grouped into 4 groups as shown in the diagram below (Table 1).

Table 1. Experimental grouping

GROUPS	ADMINISTRATION	LIGHT/DARK CYCLE	LIGHT ON
CONTROL (short term)	distilled water (P.O) (1ml/300 g body weight) (P.O) once per day	Natural 12 h light/12 h dark	7.00am-7.00pm
ALTERED PHOTOPERIOD (short term)	distilled water (P.O) (1ml/300 g body weight) once per day	20 h light/ 4hr dark in alternate daily schedule	7.00am-3.00am
CONTROL (long term)	distilled water (P.O) (1ml/300 g body weight) (P.O) once per day	Natural 12 h light/12 h dark	7.00am-7.00pm
ALTERED PHOTOPERIOD (long term)	distilled water (P.O) (1ml/300 g body weight) once per day	20 h light/ 4 h dark in alternate daily schedule	7.00am-3.00am

Ethical certification

The study was conducted in line with the guidelines of National Institute of Health (NIH) for the use of laboratory rats.

Determination of estrous cycle

Phases of estrous cycle were assessed using the method of Marcondes *et al.* (2002). 10microliter of distilled water was introduced into the vagina to produce vaginal lavage. With the aid of light microscope (x40 objective lens), epithelial cells, cornified cells and leucocytes were identified.

Diestrus phase: was predominantly characterized by leucocytes with little or no cornified cells.

Proestrus phase: was predominantly characterized by epithelial cells with little or no leucocytes.

Estrus phase: was characterized by cornified cells.

Metestrus phase: was characterized by leucocytes, cornified cells and few epithelial cells.

Rats cycle from diestrus (D) to proestrus (P) to estrus (E) to metestrus (M). The cycle length was then determined. The total number of each phase in two weeks was determined and the percentage of each phase was calculated.

Estrous Cycle Ratio (ECR) using the method of Agoreyo and Adeniyi, (2018) as:

$$\frac{\text{Percentage of proestrus} + \text{Percentage of estrus}}{\text{Percentage of metestrus} + \text{Percentage of diestrus}}$$

RESULTS

Body temperature rhythm

There was a significant ($P<0.05$) decrease in nighttime

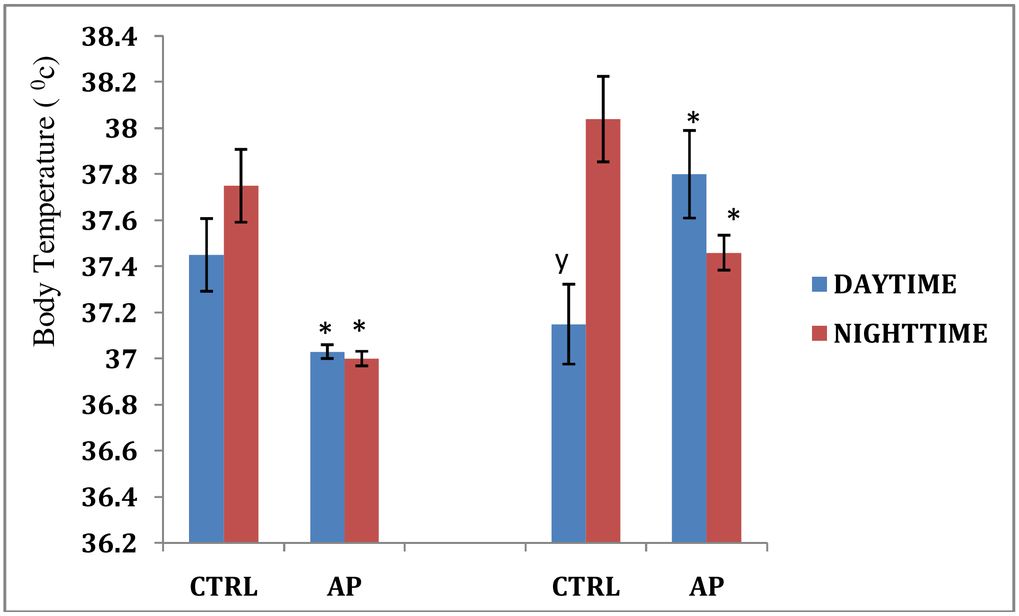

Figure 1. Duration-related effect of altered photoperiod on body temperature rhythm. CTRL- control, AP- Altered photoperiod.*represents significant difference from ctrl at P<0.05. [y] represents significant difference (P<0.05) from 1wk.

body temperature in rats exposed to altered photoperiod for one week and eight weeks respectively. Daytime temperature significantly (P<0.05) decreased and increased after one week and eight weeks respectively. In rats administered distilled water (control group), there was a duration-dependent decrease in daytime temperature (Figure 1).

Nocturnal plasma melatonin

There was a significant decrease in nocturnal plasma melatonin in rats exposed to altered photoperiod for one week when compared with control. There was a significant increase in nocturnal plasma melatonin in rats exposed to altered photoperiod for eight weeks when compared with control. Altered photoperiod exposure caused a duration dependent decrease in nocturnal plasma melatonin (Figure 2).

Estrous cycle length

There was a significant increase in estrous cycle length in rats exposed to altered photoperiod for one week when compared with control. There was a significant increase in estrous cycle length in rats exposed to altered photoperiod for eight weeks when compared with control. Altered photoperiod exposure caused no duration dependent effect in estrous cycle length (Figure 3).

Estrous cycle ratio

There was a significant increase in estrous cycle ratio in rats exposed to altered photoperiod for eight weeks when compared with control. Altered photoperiod exposure also caused a duration dependent increase in estrous cycle ratio (Figure 4).

DISCUSSION

Photoperiodic perturbation has widely been reported to disrupt biorhythms. In the present study, we observed that exposure of female rats to light pollution in form of altered photoperiod impaired rhythm of body temperature in a duration-independent pattern. Previous works by Mahoney, (2010) and Lowden *et al.* (2010) have documented derangement in temperature rhythm in night shift workers. Although, rats are known to be nocturnal, the increase in daytime body temperature observed in rats exposed to altered photoperiod for 8 week and the duration-dependent decrease in daytime temperature seen in animals administered distilled water plausibly indicated adaptation of internal clocks to other external cues such as ambient temperature and anthropogenic factors (Fonken and Nelson, 2014). Previous studies have attributed light pollution induced disruption in feeding/fasting cycle to impaired suprachiasmatic expression of circadian genes such as period, circadian locomotor oscillation cycles of caput (CLOCK), brain and

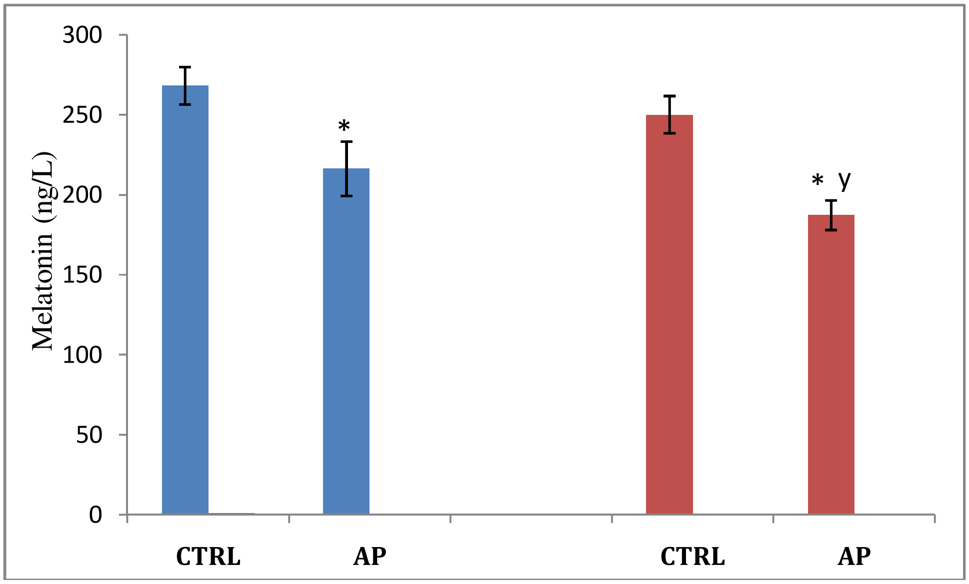

Figure 2. Duration-related effect of altered photoperiod on nocturnal plasma melatonin. CTRL- control, AP- Altered photoperiod.*represents significant difference from ctrl at P<0.05. [y] represents significant difference (P<0.05) from 1wk.

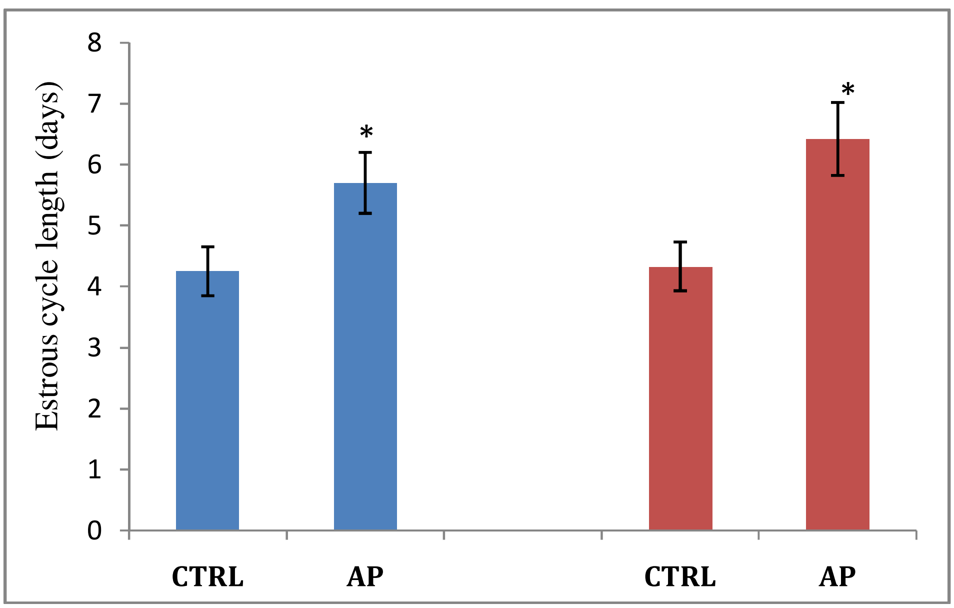

Figure 3. Duration-related effect of altered photoperiod on estrous cycle length. CTRL- control, AP- Altered photoperiod.*represents significant difference from ctrl at P<0.05. [y] represents significant difference (P<0.05) from 1wk.

muscle arnt like protein (BMAL-1) resulted in impaired feeding rhythm, glucose dishomeostasis, hypoinsulinemia and diabetes (Marcheva *et al.,* 2010; Grimaldi *et al.,* 2010; Cavas *et al.,* 2012).

The contribution of these clock genes to temperature rhythm is unclear.

Our study also demonstrated that exposure to altered photoperiod suppressed melatonin secretion with the lowest secretion occurring after 8 weeks period. The tendency of light to depress melatonin secretion is known as the negative masking effect of light. Prolongation of estrous cycle noticed in female rats exposed to altered

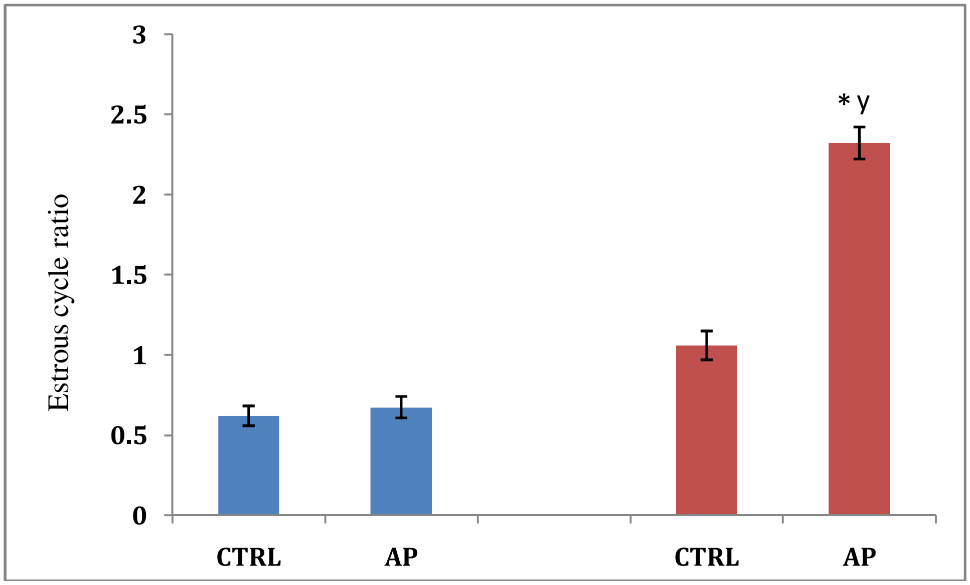

Figure 4. Duration-related effect of altered photoperiod on estrous cycle ratio. CTRL-control, AP- Altered photoperiod. *represents significant difference from ctrl at P<0.05. [y] represents significant difference (P<0.05) from 1wk.

photoperiod for one week may be attributed to poor gonadotropin output and disruption of ovary-gonadotropin communication (Barrett *et al.,* 2010) probably due to decreased expression of ovarian PER1. Unlike estrous cycle length, estrous cycle ratio provides information about the relative durations of luteogenesis and luteolysis (Agoreyo and Adeniyi, 2018; Adeniyi and Agoreyo, 2019). Therefore the significant increase in estrous cycle ratio in rats maintained under altered photoperiod for 8 weeks indicated prolongation of follicular phase and delay in ovulation due to low progesterone and high estradiol: progesterone ratio (Lima *et al*., 2004; Mahoney*,* 2010). We observed that exposure to altered photoperiod led to duration-dependent increase in estrous cycle ratio. This implies that as the frequency of exposure to altered photoperiod increased, there was an increase in likelihood of an ovulation.

The study concluded that photoperiodic perturbation caused no sustainable effect on estrous cycle ratio and nocturnal plasma melatonin secretion

Authors' declaration

We declared that this study is an original research by our research team and we agree to publish it in the journal.

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