



EFFECTS OF VARYING PHOTOPERIODS ON THE MORPHOMETRY AND HORMONAL ACTIVITY
OF THE THYROID GLAND OF THE AFRICAN GIANT RATS (*CRICETOMYS GAMBIANUS*,
WATERHOUSE)

ALI, M.N.^{1*} MAIDAWA, S.M.¹ IMAM, J.¹ AND IBE, C.S.²

¹Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

²Department of Veterinary Anatomy, Michael Okpara University of Agriculture, Umudike, Nigeria

ABSTRACT

This study aimed to investigate the effects of continuous exposure to light and darkness on the thyroid gland of the captive African giant rats (*Cricetomys gambianus*). A total of thirty-six (36) apparently healthy African giant rats (AGR) of both sexes were randomly divided into three different photoperiodic groups (n = 12/group); viz; Group I: rats were exposed to continuous darkness. Group II: were exposed to continuous (24h) artificial light and group III: (Control) rats were exposed to a 12h light:12h dark cycles for a period of four (4) weeks of experimentation. The effects of continuous light and darkness exposures on the thyroid activity were studied. Serum concentration of free triiodothyronine (FT₃), free tetrathyronine (FT₄), and thyroid - stimulating hormones (TSH) were determined by radioimmunoassay (RIA) technique. In the rats exposed to continuous darkness the weight of the thyroid gland increased significantly ($P < 0.001$) and the serum level of T₃, T₄ and TSH was higher than that of the control. The weight and width of the thyroid gland also increased significantly ($P < 0.001$) and ($P < 0.05$) respectively in rats exposed to light when compared with the control. However, the serum level of T₃, T₄ and TSH was higher than the control with no significant difference ($P > 0.05$). The organo-somatic thyroid index for the control and rats exposed to darkness and lighting were 3.772 ± 0.03 , 4.398 ± 0.04 and 4.085 ± 0.04 respectively, with no significant ($P > 0.05$) difference. In conclusion, this study has shown that exposure of AGR for four (4) weeks of continuous lighting resulted in significant increase in thyroid activity.

Keywords: African giant rat, Continuous artificial light, Continuous darkness, Thyroid gland hormones, Thyroid gland parameters

***Correspondence:** ali.nkweshi33@gmail.com, 08039716004

INTRODUCTION

The thyroid is the largest and the first recognizable endocrine gland during development in vertebrates. Marked variation in location, gross and histological features of the gland have been observed in different vertebrate [1]. The thyroid gland secretes hormones that regulate growth and metabolism and plays a critical role in tissue development and differentiation [2]. Weighing and histological evaluations of the thyroid are classically required for the evaluation of thyroid function. Additional evaluations include biomarkers that demonstrate the effects of thyroid hormones on metabolism, thyroid function, and blood levels [3]. Thyroid hormones are produced by the follicular cells that compose the bulk of the parenchyma. They are stored in the follicular fluid and later broken down to yield the final products, which are then released into the blood stream [1]. Thyroid hormones production is control by the pituitary gland. If there is an inadequate amount of thyroid hormone circulating in the blood, the

pituitary gland amplified the release of TSH to stimulate more thyroid hormones production. In contrast, when there is too much of circulating thyroid hormone, TSH level reduced as the pituitary attempts to diminish the production of the thyroid hormone [4 - 7]. Photoperiodism is the physiological reaction of organisms to the length of night or a dark period [8], and the ability of plants and animals to measure environmental day length to ascertain time of year [9]. Photoperiodism is classified into three groups according to the photoperiods; total light, total darkness and normal day/night regime [10]. A number of biological and behavioural changes are photoperiodism dependent. Together with temperature changes, photoperiod provokes changes in the color of fur and feathers, migration, entry into hibernation, sexual behaviour, and even the resizing of sexual organs [11]. In photoperiodic mammals such as rats, light is an important regulator of secretion of several pituitary hormones [12]. The African giant rat (AGR) also known as giant pouched rat, is by size one of Africa's largest rodents and is

arguably becoming Africa's most intriguing rodent because of its scientific attributes such as the detection of landmines [13] and also in the medical diagnosis of pulmonary tuberculosis [14], disease vectors [15], potential pest species status [16] among many other. Research interest in the AGR has increased in order to understand its biology, potential for domestication and adaptation for laboratory use with these research covering photoperiods on reproduction and endocrine organs [17 - 20], anatomy of the spinal nerves [21], spinal cord [22], feed and water consumption [23] and Skeleton [24] of the AGR. However, there is paucity of information on the effect of varying photoperiods on the thyroid gland and thyroid hormones of the African giant rat. The aim of the present study was to evaluate the effects of continuous lighting and darkness exposure on the thyroid gland of the African giant rat.

MATERIALS AND METHODS

Study site

The experiment was conducted at the Department of Veterinary Anatomy, Ahmadu Bello University Zaria, (11° N, 7 42 E), located in the Northern Guinea Savannah zone of Nigeria, at an altitude of 686 m above maximum sea level, and has nearly equatorial monthly photoperiod of 12.13 ± 0.13 h [25]. The zone is characterized by three major seasons: harmattan (November - February), hot-dry (March - May) and rainy (June - October) seasons [25, 26].

Animal husbandry and management.

Mature AGRs (*Cricetomys gambianus*) obtained from rural farmers in the surrounding villages around Zaria, Nigeria were used for the study. They were transported by vehicle to the experimental animal unit of the Department of Anatomy, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria. They animals were housed in metal standard laboratory cages, measuring 50 x 40 x 40 cm Throughout the experiment, the rats were fed with feed supplement, mixtures comprising of maize grains, ground-nuts, dried cassava tubers and sweet potatoes and drinking water was given provided ad libitum. All animal procedures and protocols were carried out according to the strict guidelines prescribed by the Animal Care and Use Committee of the American Society of Mammalogists for the use of wild mammals in research [27].

Experimental design

A total of thirty (36) adult AGRs of both sexes were used for this study. After a 2-week acclimatization period, the rats were randomly divided into three photoperiodic groups of twelve (12 rats) each; as follows: Group I - Control rats (12 h light/12 h darkness; lights on 7:00h, lights off 19:00). Group II - Rats exposed to continuous

lighting (24 h, lights on day/night) and Group III- Rats exposed to continuous darkness: (with lights switched off permanently, except 5-10 min duration of candle light per day, for food access and cleaning purposes). All the experimental rats exposed were housed in separate small room illuminated with two white bulb (12W) that provided an approximate light intensity of 350 Lux, while, the rats exposed to constant darkness had their room windows covered with a thick black cotton material) throughout the 4 week of experimentation period.

Tissue and blood sampling

After the exposure, the rats were sacrificed by cervical dislocation, the rats were then placed on a dorsal recumbency, and a mid-line incision was made, to exposed the thoraco-lumbar cavity and a careful dissection was made through the structures of the neck to expose the trachea to locate the thyroid gland. The thyroid gland was trimmed of fat and connective tissues and measured using a Mettler balance P1261 (Mettler Instrument Ag, Greifensee, Switzerland). Based on the values obtained, the organo-somatic index (OSI) of thyroid was calculated by dividing organ weight with body weight and quotient being multiple by 100 (weight of organ (g) / weight of body (kg) X 100) [28]. Using a 21- gauge needle attached to a 5ml syringe, cardiac blood was collected and allowed to clot. The blood samples were later centrifuged at 3,000 rpm for 30 minutes. The sera were then pipetted into 1.5 mL labelled Eppendorf tubes and stored at 20° C until subsequently analyzed for the hormonal assay. The serum levels of T₃, T₄ and TSH were measured by radioimmunoassay according to the method of Moudgal and Madhwa Raj. [29]. Serum samples were assayed in duplicate and the amount of gonadotropins were expressed as mIU/mL. The measurements were all done using RIA kits (Kodak Clinical Diagnostics Ltd., Amersham, Buckinghamshire, England). The instructions in the booklet that accompanied the kits were followed carefully for the preparation of standard curve and determination of hormone levels using the gamma-counter.

Statistical analysis

All recorded weights and dimensions were expressed as mean \pm standard error of the mean (mean \pm SEM), and subjected to statistical analysis using Statistical Package for Social Science (SPSS), version 17.0. Variation in parameters in groups was analyzed using one-way analysis of variance (ANOVA) and further subjected to a post-hoc test, Duncan multiple range tests. Values of $P < 0.05$ were considered significant.

RESULTS

The mean body weight of the rat exposed to continuous darkness was 1.159 ± 0.03 kg, which was 7.02% greater than the control (1.083 ± 0.02 kg). The mean body weight of the rat exposed to continuous light was 1.106 ± 0.03 kg, which was less by 2.12% from control. The mean weight of the thyroid gland in rats exposed to continuous darkness was significantly increased ($P < 0.001$) by 23.53% from 0.034 ± 0.002 g in the control to 0.042 ± 0.024 g in the rat exposed to darkness (Table 1). On the other hand, the mean weight of the thyroid gland exposed to continuous light was significantly increased by 35.29% ($P < 0.001$) from 0.034 ± 0.002 g in the control to 0.046 ± 0.002 g in the exposed rats. The values of, the length and width of the thyroid gland were 0.729 ± 0.134 cm, 0.729 ± 0.013 cm, 0.728 ± 0.08 cm and 0.405 ± 0.106 cm. 0.414 ± 0.101 cm and 0.437 ± 0.098 cm for the control, continuous dark and continuous artificial light photoperiodic Groups respectively, with the width been significantly higher in the rat exposed to light ($P < 0.05$) (Table 1).

Hormonal assay

The mean serum level of T3 for the control rats and those exposed to continuous darkness and lighting were 0.83 ± 0.27 ng/mL, 1.00 ± 0.10 ng/mL and 1.18 ± 0.15 ng/mL, respectively. The percentage serum level increase was 20.48% and 42.2% for rats exposed to continuous darkness and lighting, respectively. The mean serum level of T4 for the control rats was 8.88 ± 4.90 ng/mL, rats exposed to darkness, 17.00 ± 2.94 ng/mL and the rats exposed to lighting, 21.50 ± 8.97 ng/mL. The percentage serum level increase was 91.4% and 142.1% for rats exposed to darkness and lighting respectively. The mean serum level of TSH in the control rats, those exposed to darkness and lighting were 0.40 ± 0.06 ng/mL, 1.02 ± 0.67 ng/mL and 0.89 ± 0.14 ng/mL, respectively. The percentage serum increase for TSH was 155% and 122.5% for rats exposed to darkness and lighting, respectively. There was no significance difference across the groups ($P > 0.05$) (Table 2).

Table 1: Mean values of bodyweight, organo-somatic thyroid index (OSTI), thyroid weights, length and width of the thyroid glands of different groups of African Giant rats (mean \pm SEM)

Parameter	Control (n = 12)	Darkness (n = 12)	Lighting (n = 12)
Body weight (kg)	1.083 ± 0.018	1.159 ± 0.026	1.106 ± 0.234
OSI	(3.772 ± 0.03)	(4.398 ± 0.04)	(4.085 ± 0.04)
Thyroid weight (g)	0.034 ± 0.002^a	0.042 ± 0.024^b	0.046 ± 0.002^b
Thyroid length (cm)	0.729 ± 0.134	0.720 ± 0.0134	0.728 ± 0.08
Thyroid width (cm)	0.405 ± 0.106^a	0.414 ± 0.101^{ab}	0.437 ± 0.098^b

a, b Values with different letter superscript in same row are significantly different ($P < 0.05$).

Figures in parentheses indicate organ-somatic indices.

Table 2: Mean values of serum levels of triiodothyronin (T3), thyroxin (T4) and follicle stimulating hormone (TSH) of different groups of African Giant rats (Mean \pm SEM)

Parameter	Control (n = 12)	Continuous Darkness (n = 12)	Continuous Artificial Light (n = 12)
T3 (ng/mL)	0.83 ± 0.27^a	1.00 ± 0.10^a	1.18 ± 0.15^a
T4 (ng/mL)	8.88 ± 4.90^a	17.00 ± 2.94^a	21.50 ± 8.97^a
TSH (ng/mL)	0.40 ± 0.06^a	1.02 ± 0.67^a	0.89 ± 0.14^a

a, b Values with same letter superscript on same row does not differ significantly ($P > 0.05$)

DISCUSSION

There was a slight increase in body weight of rats exposed to constant darkness but the increase was not statistically significant, this might be enlightened by the

facts that darkness and hormone termed melatonin [30, 31] may decrease the body metabolism and/or increase the food intake in rats, hence, affects the entire body mass in rat [32, 33]. Continuous lighting exposure resulted in an increase in weight of thyroid gland and elevation of the thyroid hormones (T3 and T4), when compared to the control. This result corroborates with the findings Olatunji-Bello and Sofola [34], who reported an increase in thyroid weight and thyroid hormone (T4) in male rats exposed to continuous light. Halder *et al.* [35] also reported that continuous lighting had no effect on thyroid function as shown by the non-change in weight and plasma T4 parameters in the female Indian palm squirrel (*Funambulus pennanti*). Rats subjected to continuous darkness exposure in this study also showed an increase in thyroid weight, elevated concentration of thyroid hormones when compared with the control. This result was in accordance with Eleiwe *et al.* [36], who reported that the period of continuous darkness for no more than one month had more or less no apparent risky effect on thyroid tissues while longer dark periods brought detrimental outcomes on the thyroid gland architectures of adult Wistar albino rats. The result was contrary to the findings of Olatunji-Bello and Sofola [34], who reported a significant reduction in thyroid weight and unchanged serum level of T4 in rats exposed to continuous darkness for four weeks when compared to the control. Halder *et al.* [35] demonstrated that continuous darkness significantly reduced thyroid weight and plasma level of thyroxine in Indian female palm squirrel (*Funambulus pennanti*). Ghadiri *et al.* [37] reported that prolonged periods of darkness in adult Wistar rats reduce thyroid activity and serum level of thyroid hormones, hence they suggested that darkness can be considered as a probable factor in hypothyroidism. The exclusive production of melatonin at night has proven that; melatonin is a chemical expression of darkness [38]. Besides, melatonin has a direct inhibitory effect on T4 and, also, depresses the response of the thyroid to TSH (39). Thus, the anti-thyroid effect of melatonin, as observed earlier by Olatunji-Bello and Sofola (40) was not observed in the present study. Continuous exposure to darkness ensured that melatonin production proceeded unabated and exerted the anti-thyroidal effect of melatonin on thyroid tissue (39). The increase in thyroid weight and elevation of concentration of thyroid hormones in rats exposed to continuous darkness observed in the present study may be due to the hibernation nature of the AGR. It is known that melatonin is continuously synthesized and secreted during a period of continuous darkness, and that its synthesis and secretion are inhibited during a period of continuous light, provided that the light is a bright artificial light [41, 42] there is a high quality correlation between usual level of endogenous melatonin and

thyroid hormones role in various bodily vital naturally processes such as reproduction, so the endocrine means of animal seasonal reproduction and the confirmation that long day light and the thyroid hormones, are mandatory to the appropriate timing of reproduction [43]. The changes in thyroid structure observed in the present work may thus be related, directly or indirectly, to changes in melatonin levels during continuous darkness and continuous light.

CONCLUSION

Continuous lighting exposure significantly ($P < 0.05$) increased thyroid activity, while continuous darkness did not exert adverse effects on the thyroid gland, suggesting the absence of anti-thyroid activity of melatonin on AGR, this may probably be due to the hibernation nature of the AGR.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to the Ahmadu Bello University, Zaria for funding part of the research work.

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