NIGERIAN VETERINARY JOURNAL

CASE REPORT

Effects of Continuous Light Exposure on Testicular Structure and Function of the African Giant Rat (*Cricetomys gambianus*)

Ali, M. N.[1], Onyeanusi, B. I.[1], Ayo, J. O.[2,] Salami, S. A[3], and Hambolu, J. O.[1]

[1]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, ABU Zaria, Nigeria, [2]Department of Veterinary Physiology, Faculty of Veterinary Medicine, ABU, Zaria, [3]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Ilorin, Nigeria. *Corresponding author: Email: ali.magdalene@yahoo.com; Tel No:+234 8039716004

SUMMARY

The aim of this investigation was to study the effects of continuous lighting exposure on the testes of the African giant rats (AGR). Samples of testes from twelve (12) healthy AGR were investigated under two groups. The rats were divided into two groups of 6 rats each as follows: Group I - Control rats (12 h light/12 h darkness); Group II - Rats exposed to continuous light (24 h). The indices of the testes were length, width, and weight of the organ, estimation of serum levels of testosterone, luteinizing hormones, follicle stimulating hormone (FSH) by radioimmunoassay (RIA) technique and histological studies of the testes. The mean body weight of the rats exposed to continuous light was 1.086 ± 0.034 kg which was reduced by 3.9 % from the control (1.131 ± 0.02 kg). The mean weight of the testes in rats, exposed to continuous light was 4.549 ± 0.30 g, which was significantly reduced by 17.8 %, when compared to the control (5.534 ± 0.16 g). The mean length and width of testes of rats exposed to continuous lighting was 0.294 ± 0.71 cm and 0.143 ± 0.48 cm, respectively. The mean serum level of testosterone in control rats was 1.225 ± 0.08 miu/ml and that in rats exposed to lighting, 0.275 ± 0.10 miu/ml. This result showed that the serum level of testosterone in male rats exposed to continuous lighting was reduced by 77.6 % from 1.225 ± 0.08 miu/ml in the control rats to 0.275 ± 0.10 miu/ml. Histological observations showed that the testes of rats exposed to light displayed elongated to round seminiferous tubules each with wide lumen lined by low germinal epithelium, and had wide interstitial spaces. Disruption of spermatogenesis and vacuolization of epithelial cells were evident. This study has shown that exposure of AGR for four (4) weeks of continuous lighting will result in disruption of spermatogenesis and decreased testosterone serum level.

Key words: Light; Testes; African giant rat.

INTRODUCTION

The African giant rats (AGR) (*Cricetomys gambianus*) are found in the rain forest where they are restricted to farmlands, grasslands and human habitations. They are frequently seen at night crossing roads, running along drains and in house compounds. They are social animals and as such several individuals live together in

burrows (Delany and Happold, 1979). In tropical Africa, the AGR is a source of nutritional protein and income for peasant farmers (Ajayi, 1977; Malekani, 2009). The Anti-Persoonsmijnen Ontmijnende Product Ontwikkeling (APOPO) has employed AGR as mine-detection animals for approximately 10 years in sub-Saharan Africa, where the species are indigenous, and now plans to deploy the rats in areas where they are not indigenous (Timothy *et al.,* 2015). Like dogs, AGRs have a highly sensitive sense of smell. The rats, which are relatively large rodents (1-2 kg body weight) that live for 7-8 years in captivity, can be readily trained through operant-conditioning procedures to detect land mines (Poling *et al.*, 2010a, 2010b; Poling *et al.*, 2011) to detect in human sputum the presence of *Mycobacterium tuberculosis*, the bacillus that causes tuberculosis (Weetjets *et al.*, 2009; Poling *et al.*, 2010c; Mahoney *et al.*, 2012); and to detect Salmonella bacteria in horse faeces (Mahoney *et al.*, 2014). The findings of Mahoney *et al.* (2014) suggest that AGRs may be a valuable asset in the global effort to control illicit cigarette trade. Light and dark cycles in circadian rhythm play a significant role in endocrine and reproductive functions (Biswas *et al.,* 1994). It plays a major role in controlling the circadian rhythms (Falcon, 1999) and circadian rhythms might affect cell division in many organisms (Mori and Johnson, 2000). In photoperiodic mammals such as rats, light is an important regulator of secretion of several pituitary hormones (Freeman *et al.,* 2000). In both males and females, short-day exposure reduces hypothalamic synthesis and secretion of gonadotropin-releasing hormone (GnRH) and, subsequently, the pituitary gonadotropins; that is, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Glass, 1986; Buchana and Yellon, 1991). Eventually, these endocrine alterations result in gonadal atrophy or regression, and loss of reproductive function.

Exposure of male albino rats to continuous lighting for 70 days increase the weight of testis and serum testosterone level (Biswas *et al.*, 2013). In Djungarian hamsters, long photoperiods markedly stimulate testicular development, increase body weight and initiate summer molting. In another small rodent, *Microtus agrestis*, a similar cycle of body weight with maxima at the time of maximal gonadal activity and minima at the time of gonadal quiescence is observed. Hamsters kept in continuous darkness develop pronounced atrophy of the gonads and delayed growth of accessory sex organs (Wade and Bartness, 1984). Those hamsters maintained on long photoperiod of 16 hours of light and 8 hours of darkness have larger gonads than those maintained at normal lighting rhythm of 12 hours of light and 12 hours of darkness (Hasting *et al.,* 1989). Photostimulation in the immature golden hamster increases testicular activity and testosterone secretion (Hance *et al.*, 2009). However, in the adult marmoset (*Callithrix jacehus*), continuous light exposure for 60 days exerts no effect on the rhythmicity of plasma testosterone and spermatogenesis (Kholkute and Javaramans, 1987). The present investigation has been undertaken to determine the effects of continuous lighting exposures on the structure and function of the testes of the AGR.

MATERIALS AND METHODS
Experimental animals
Mature AGRs were purchased from surrounding villages around Zaria, Nigeria. They were transported to the experimental animal unit of the Department of Anatomy, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. They were housed in standard laboratory cages, measuring 50 cm (H) by 40 cm (W) by 40 cm (L). The handling method in this study was in accordance with the guidelines of the American Society of Mammalogists (Gannon and Sikes, 2007). The animals were fed with feed supplement, maize

grains, ground-nut, dried cassava tubers and sweet potatoes. Water was given *ad libitum.*

Protocol of study
 A total of 12 male adult AGRs were used for this study. The rats were divided into two groups of 6 rats each as follows: Group I - Control rats (12 h light/12 h darkness); Group II - Rats exposed to continuous lighting (24 h). The control rats were kept in the Departmental animal room that had normal lighting rhythm of 12 hours of light and 12 hours of darkness. The rats exposed to continuous lighting were housed in a small room in the Department. The room was illuminated with 122 cm fluorescent lamp that gave a mimicked natural daylight. The lamp was switched on permanently for four weeks, covering the duration of the experiment.

Sample collection
After the period of exposure, the rats were lightly anaesthetized using chloroform in a closed container. They were then weighed using a Mettler balance (Model P1421) with a sensitivity of 0.1gm. Using a 21-gauge needle attached to a 5 ml syringe, intracardiac blood was collected into sample bottles and allowed to clot. The blood samples were later centrifuged at 3,000 rpm for 30 minutes. After this, the rats were euthanized by cervical dislocation, they were them placed on supine position and the abdomen and thorax were cut open. The scrotal sacs of the rats were deserted open and the pair testes exposed. The testes were examined *in situ* and then exteriorised. The testes, trimmed of fat and extraneous tissue, were measured using a micrometer screw gauge and then weighed using a Mettler balance P1210 (Mettler Instrument AG, Switzerland), with a sensitivity of 0.001 g.

Hormonal assay
The serum levels of LH and FSH were measured by radioimmunoassay according to the method of Moudgal and Madhwa Raj. (1974). Serum samples were assayed in duplicate and the amount of gonadotropins were expressed as miu/ml serum. The radioimmunoassay of testosterone was carried out as described by Auletta *et al.* (1974). The measurements were all done using ELISA kits (AccuBind Microwell, Lake Forest, California, USA). The instructions in the booklet that accompanied the kit were followed carefully for the preparation of standard curve and determination of hormone levels using the gamma-counter. The standard curves were plotted and the values of the serum level for hormones in the samples were determined by extrapolating the counts of each sample to the standard curve. All samples were run in duplicate in a single assay to avoid interassay variation.

Light microscopy
Tissues for light microscopy were preserved in labelled bottles, containing 10 % neutral-buffered formalin. After fixation, the tissue was dehydrated through a series of graded ethanol solutions (50 – 100 %), and embedded in paraffin (Bancroft and Sevens, 1990). Paraffin sections (5 µm) were cut and stained with haematoxylin-eosin and trichrome stains. The sections were observed and subjected to photomicrography using Olympus light microscope (BX63+ DP72)

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

RESULTS
The mean body weight of the rats exposed to continuous light was 1.086 ± 0.034 kg which was reduced by 3.9 % from the control. The mean weight of the testes in rats, exposed to continuous light was 4.549

Table I: Mean values of body weight and testes parameters in male African Giant rats exposed to continuous lighting (Mean ± SEM)

parameters	Control (n = 6)	Lighting (n = 6)
Body weight, (kg)	1.131 ± 0.02^{ab}	1.086 ± 0.034^{a}
Testis Weight, (g)	5.534 ± 0.16^{b}	4.549 ± 0.30^{a}
Length, (cm)	0.329 ± 0.31^{b}	0.294 ± 0.71^{a}
Width, (cm)	0.157 ± 0.19^{b}	0.143 ± 0.48^{a}

a, b = Means along the same column with different superscript letters differ significantly (P < 0.05)

Table II: Mean values of serum levels of testosterone, luteinizing hormone and follicle-stimulating hormone in male African Giant rats exposed to continuous lighting (Mean ± SEM)

Hormones, (miu/ml)	Control (n = 6)	Lighting (n = 6)
Testosterone	1.225 ± 0.08^{c}	0.275 ± 0.10^{a}
Luteinizing hormone	0.175 ± 0.48	0.550 ± 0.26
Follicle stimulating hormone	0.350 ± 0.03^{a}	0.525 ± 0.05^{b}

a, b = Means along the same column with different superscript letters differ significantly (P < 0.05)

± 0.30 g, which was reduced by 17.8 %, when compared to the control (5.534 ± 0.16 g). The mean length and width of testes of rats exposed to continuous lighting were 0.294 ± 0.71 cm and 0.143 ± 0.48 cm, respectively. The mean length and width of rats exposed to lighting were lower from the control (Table I). The mean serum level of testosterone in control rats was 1.225 ± 0.08 miu/ml and in rats exposed to lighting was 0.275 ± 0.10 miu/ml. This result showed that the mean serum level of testosterone in male rats exposed to continuous lighting reduced by 77.6 % from 1.225 ± 0.08 miu/ml in the control rats to 0.275 ± 0.10 miu/ml. The mean serum level of LH in rats exposed to continuous lighting increased by 214.3 % from 0.175 ± 0.48 miu/ml in the control to 0.550 ± 0.26 miu/ml. The mean serum level of FSH in rats, exposed to continuous lighting was higher by 50 % from 0.525 ± 0.05 miu/ml to 0.350 ± 0.03miu/ml in the control (Table II).

Light microscopic findings
Testicular capsules of all groups of rats were very prominent with numerous blood vessels. In the control rats, the testes consisted of a number of seminiferous tubules, lined by the germinal epithelium and separated by interstitial tissue which was observed to contain more spermatozoa and distinct Leydig cells. The seminiferous tubules possessed epithelia, containing Sertoli cells and germ cells at various stages, covering the complete cycle of spermatogenesis (Plates 1 and 3). The testes of rats exposed to light displayed elongated to round seminiferous tubules, with wide lumen lined by low germinal epithelium, and had wide interstitial spaces. Disorientation of epithelial cells was common in majority of the seminiferous tubules. Disruption of spermatogenesis and vacuolization of epithelial cells were evident (Plates 2 and 4).

Plate 1: Testis of control a rat showing capsule (C) and seminiferous tubules (double arrow heads) lined by germinal epithelium (GE). Hematoxylin and Eosin, Scale bar = 200 µm
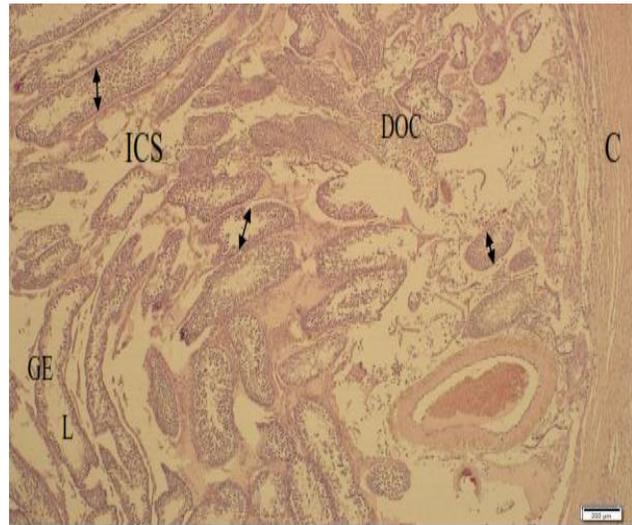

Plate 2: Testis of a rat exposed to light showing the seminiferous tubules (arrows) with wide lumen (L) lined by low germinal epithelium (GE), disorientation of epithelial cells (DOC) and having capsule (C). Masson Trichrome, Scale bar = 200 µm
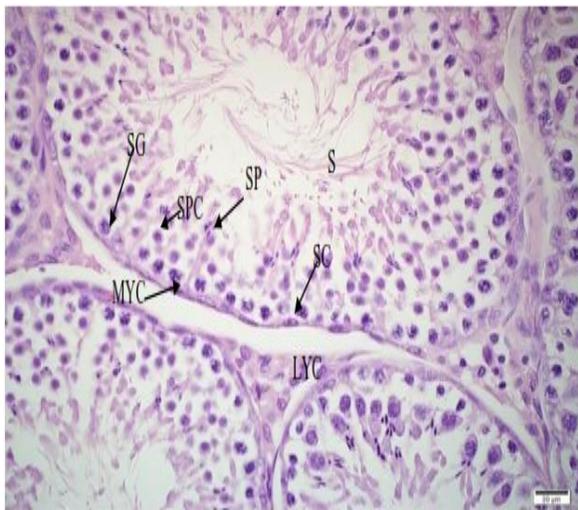

Plate 3: Seminiferous tubules of control rat containing, myloid cells (MYC), Sertoli cells (SC), Leydig cells (LYC) and germ cells at various stages of spermatogenesis such as spermatogonia (SG), spermatocyte (SPC), spermatid (SP) and spermatozoa (S). Hematoxylin and Eosin, Scale bar = 20 µm
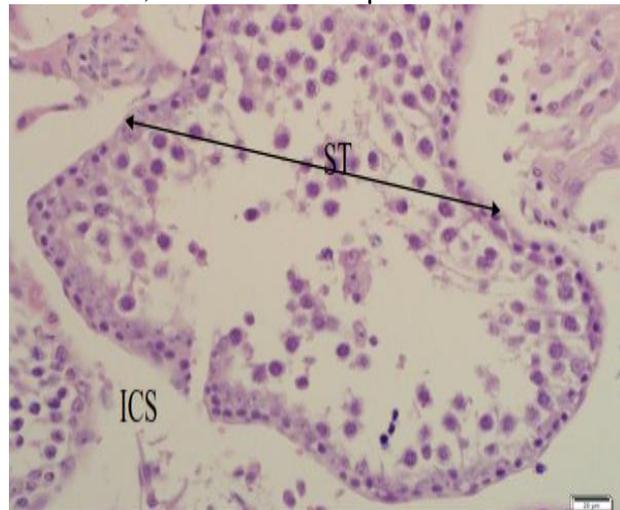

Plate 4: Seminiferous tubules of a rat exposed to light with disrupted seminiferous tubule (ST) wider interstitial spaces (ICS). Haematoxylin and Eosin, Scale bar = 200 µm

DISCUSSION

Light and dark cycles in circadian rhythm play a significant role in endocrine and reproductive functions (Biswas *et al.*, 1994; Aleandri *et al*., 1996). Continuous light exposure in the male rats resulted in a significant decrease in body weight, when compared to the control. This result contradicts that of Steven (1994) who reported that the body weight of male Djungarian hamster reared in long days or after 30 long days were significantly greater than those of male exposed to short days. This may probably be due to the fact that the

AGR are nocturnal and can see well in the dark and even feed better. Continuous light exposure resulted in a significant decrease in testes weight, length and width, significant testosterone decrease and elevated FSH serum level when compared with the control. The control rats had a significant increase in testes weight, significant increase in testosterone level and a decrease in FSH. This result agrees with Rodriquez *et al*. (2005) who reported that long term exposure to continuous light inhibits gonadal growth in European male seabass but contradicts that of Olatunji-Bello and Sofola (2001) who reported an increase in size of testes and elevation of hormones, LH and FSH in rats exposed to continuous light when compared with the control. Biwas *et al*. (2013) also reported an increased testes weight and serum levels of testosterone, LH and FSH in male albino rats exposed to continuous light for 70 days. Photostimulation in the immature golden hamster increases testicular activity and testosterone secretion (Hance *et al*., 2009). But in adult marmoset (*Callithrix jacehus*), continuous light exposure for 60 days had no effect on the rhythmicity of plasma testosterone and spermatogenesis (Kholkute and Javaramans, 1987). The seminiferous tubules of the testes of the control rats possessed epithelia containing Sertoli cells and germ cells of various stages, covering the complete cycle of spermatogenesis. There were more spermatozoa in the central lumen of the tubules.This observation did not agree with that of Olatunji-Bello and Sofola (2001), who reported that the seminiferous tubules of the rats exposed to light contained more spermatozoa than the controls. This study has shown that continuous light for four weeks has a detrimental effect on the structure and function of the testes of AGR.

REFERENCES

Ajayi, S. S. (1977). Live carcass weights of Giant rats (*Cricetomys gambianus,* Waterhouse) and domestic rabbits (*Oryclolagus cuniculus*). *Africa Journal of Ecology*, 15: 223-227.

ALEANDRI, V.; SPINA, V. and MORINI, A. (1996). The pineal gland and reproduction. *Humn Report Update*, 2: 225–235

BANCROFT, J.D. and STEVENS, A. (1990). Theory and Practices of Histological Techniques. 3rd Ed., Churchill Livingston, London, UK.

BISWAS, R.; SARKAR, M. and BISWAS, N.M. (1994). Protection of testicular activity by continuous light in rats treated with lithium. *Med. Sci. Res*. 24: 297-298

BRONSON, F.H. (1989). *In Mammalian Reproductive Biology*, Chicago: University of Chicago Press.

BUCHANA, K.L. and YELLON, S.M. (1991). Delayed puberty in the male Djungarian hamster: effect of short photoperiod or melatonin treatment on the GnRH neuronal system. *Neuroendocrinology*, 54: 96–102

DELANY, M. J. and HAPPOLD, D. C. W (19 79). Taxonomy of African mammals. In : Ecology of Africa Mammamls, p.4.

FREEMAN, M.E.; KANYICSKA, B.; LERANT, A. and NAGY, G. (2000). Prolactin structure, function and regulation of secretion. *Physiology Review*, 80: 1523–1631

GANNON, W.L. and SIKES, R.S. (2007).The Animal care and use Committee of the American Society of Mammalogists. Guidelines of the American society of mammalogists for the use of wide mammals in research. *Journal of Mammalogy,* 88: 809–823

GLASS, J.D. (1986). Short photoperiod-induced gonadal regression: effect on the gonadotropin-releasing hormone (GnRH) neuronal system of the white-footed mouse, *Peromyscus leucopus. Biology of Reproduction,* 35: 733–743

HANCE, M. W., MANSON, J. I. and MENDIS-HANDAGAMA, S. M. (2009). Effect of photostimulation and

non-stimulation of golden hamster from birth to early puberty on testis structure and function. *Histology and Histopathology*, 24: 1417 – 1424.

HASTINGS, M. H., WALKER, A. P., POWER, J. B., HUTCHISON, J., STEEL, E. A. and HERBERT, J. (1989). Differential effects of photoperiodic history on the responses of gonadotrophins and prolactin to intermediate day-lengths in male Syrian hamster. *Journal of Biological Rhythms,* 4: 334 – 35.

HOFFMANN, K. and LLLNEROVA, H. (1986). Photoperiodic effects in the Djungarian hamster. Rate of testicular regression and extension of pineal melatonin pattern depend on the way of change from long to short photoperiods. *Neuroendocrinology*, 43: 317–321

IVANIŠEVI C-MILOVANVIC O.; DEMAJO, M.; KARAKAŠVIC A. and PANTI´C, V. (1995). The effect of constant light on the concentration of catecholamines of the hypothalamus and adrenal glands, circulatory adrenocorticotropin hormone and progesterone. *Journal of Endocrinology*, 18:378–383

KHOLKUTE, S.D. and JAVARAMANS, R.A. (1987). Continuous light environment has no effect on the circadian testosterone rhythm, spermatogenesis or fertility of the marmoset (*Callithrix jacchus).* *International Journal of Andrology,* 10: 635–642

KNOPPER, L. D. and BOILY, P. (2000). The energy budget of captive Siberian hamsters, Phodopus sungorus, exposed to photoperiod changes: mass loss is caused by a voluntary decrease in food intake. *Physiology and Biochemical Zoology,* 73: 517–522 Mahoney, A., Edward, T. L., La Londe, K., Beneye, N., Cox, C., Weetjens, B. J. and Poling, A. (2014). Pouched rats (*Cricetomys gambianus)* detection of

Salmonella in horse faeces. *Journal of Veterinary Behaviour,* 9: 124 – 126.

MAHONEY, A., LA LONDE, K., EDWARD, T. L., COX, C., WEETJENS, B. J. and POLING, A. (2014). Detection of cigarettes and other tobacco products by giant African pouched rats (*Cricetomys gambianus*). *Journal of Veterinary Behaviour*, 9: 262 – 268.

MAHONEY, A., WEETJENS, B. J. COX, C., BEYENE, N., REITHER, K., MAKINGI, G., JIBITANA, M., KAZWALA, R., MFINANGA, G. S., KAHWA, A., DURGIN, A. and POLING, A. (2012). Detection of tuberculosis in human sputum: comparison to culturing and polymerase chain reaction. *Tuberculosis Research and Treatment*, http://dx.doi.org/10.1155/2012/716989 .

MAHONEY, A., WEETJENS, B. J. COX, C., BEYENE, N., REITHER, K., MAKINGI, G., JIBITANA, M., KAZWALA, R., MFINANGA, G. S., KAHWA, A., DURGIN, A. AND POLING, A. (2012). Detection of tuberculosis in human sputum: comparison to culturing and polymerase chain reaction. *Tuberculosis Research and Treatment*, http://dx.doi.org/10.1155/2012/716989 .

MALEKANI, J. M. (2009). *Cricetomys* farming for improving animal production in development countries. Available at: http://www.orskovfoundation.org/communityprojects_congo002.pd Accessed on January 31, 2009.

OLATUNJI-BELLO, I. and SOFOLA, O.A. (2001). Effect of Continuous Light and Darkness Exposure on the Pituitary-Gonadal Axis and thyroid Activity in Male rats. *African Journal of Biomedical Research*, 4: 119 – 122

ORTEGA, H.H.; LORENTE, J.A.; MIRA, J.A.; BARAVALLE, C. and

SALVETTI, N.R. (2004). Continuous light exposure causes dissociation in gonadotrophins secretion and inhibits partially neuroendocrine differentiation of leydig cells in adult rats. *Reproduction in Domestic Anim*als, 39: 417–423

POLING, A., WEETJENS, B. J., COX, C., BEYENE, N., BACH, H. AND SULLY, A. (2010a). Teaching giant African pouched rats to find landmines: operant conditioning with real consequences. *Behaviour Analysis in Practice,* 3: 19 – 23.

POLING, A., WEETJENS, B., COX, C., BEYENE, N. AND SULLY, A. (2010b). Using Giant African pouched rats (*Cricetomys gambianus*) to detect landmines. *Psychological Record*, 60: 715 – 727.

POLING, A., WEETJENS, B., COX, C., BEYENE, N. AND SULLY, A. (2011). Using trained pouched rats (*Cricetomys gambianus*) to detect land mines: another victory for operant conditioning. *Journal of Applied Behaviour Analysis*, 44: 351 – 355.

POLING, A., WEETJENS, B. J., COX, C., MGODE, G., JUBITANA, M., KAZWALA, R., MFINANGA G. AND VELD, D. (2010c). Using giant African rats to detect tuberculosis in human sputum samples: 2009 findings. *American Journal of Tropical Medicine and Hygiene,* 83: 1308 – 1310.

PRENDERGAST, B.J. and NELSON, R.J. (2001). Spontenous regression of enhanced immune function in a photoperiodic rodent, *Peromyscus maniculatus. Proceedings in Biological Science*, 268: 2221–2228

REITER, R. J. (1991b). Melatonin: the chemical expression of darkness. *Molecular Cell Endocrinology*, 79: C153-C159

REITER, R.J. (1991c). Pineal Gland: interface between the photoperiodic environment and the endocrine system. *Trends in Endocrinology and Metabolism*, 2: 13–19

RODRIGUEZ, L;, BEGTASH, I.; ZANUY, S. and CARRILLO, M. (2005). Long term exposure to continuous light inhibits precocity in European male seabass (Dicentrarchus labrax): hormonal aspects. *General and Comparative Endocrinology,* 140: 116–125

STEVEN M. YELLON (1994). Effects of photoperiod on reproduction and the GnRH immunoreactive neurone system in the postpubertal male Djungarian hamster. *Biology of Reproduction*, 50: 368–372

TIMOTHY, L. E., CHRISTOPHE, C. BART, W. AND ALAN P. (2015). Influences of castration on the performace of landmine-detection rats (*Cricetomys gambianus*). *Journal of Veterinary Behaviour,* 10: 369 – 372.

WADE, E. P. AND BARTNESS, J. T. (1984). Seasonal obesity in Syrian hamsters: effect of age, diet, photoperiod and melatonin. *American Journal of Physiology*. 247: R328 – R334.

WEETJENS, B. J., MGODE, G. F., MACHANG'U R. S., KAZWALA, R., MFINANGA, G., LWILLA, F., COX, C., JUBITANA, M., KANYAGHA, H., MTANDU, R., KAHWAS, A., WESSONGO, J., MARKINGI, G., MFAUME, S., STEENBERGE, V., BEYENE, N. W., BILLET, M. AND VERHAGEN, R. (2009). African pouched rats for detection of pulmonary tuberculosis in sputum samples. *International Journal of Tuberculosis and Lung Disease,* 13: 737 – 743.