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ARTICLE

Effect of light stress on the adrenal cortex of the Algerian saharan rodent *Gerbillus tarabuli* : Microscopic and morphometric changes

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ABSTRACT In today's world, stress is prevalent, including the widespread exposure to high levels of light at night. This disruption of the natural light-dark cycle significantly affects circadian rhythms, making it a major source of physiological stress. The aim of this study was to analyze the effects of a modified light-dark cycle, as a model of stress, on the adrenal gland morphology in the wild desert rodent *Gerbillus tarabuli*. The animals were divided into two groups of 10 gerbils each. The first group, considered as the control group, was maintained under a standard 12-hour light/12-hour dark cycle. The second group, referred to as the stressed group, was exposed to a disrupted light-dark cycle, switching between a standard 24-hour cycle and a modified cycle with a 20-hour light phase every other day for three months. Following the experimental period, the adrenal glands were removed and processed for histomorphometric analysis. In the stressed group, various histological changes were noticed in the form of loss of normal adrenal architecture. Morphometric results demonstrated a significant increase ($P<0.001$) in the total length and area of the adrenal gland, as well as in its three cortical zones (glomerulosa, fasciculata, and reticularis). We also observed an increase in the diameter of cells and their nuclear area within the adrenal cortex, except for the cells in the zona fasciculata, which demonstrated a decrease in size ($P>0.05$). The findings demonstrate that the histological organisation of the adrenal gland is directly affected by exposure to light stress, highlighting the significant impact of circadian disruption on adrenal structure and function. **Acta Biol Szeged 68(2):151-161 (2024)**

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Introduction

Suprachiasmatic nucleus (SCN), the central clock controlling circadian rhythms in both diurnal and nocturnal species (Challet 2007), plays a pivotal role in maintaining physiological and behavioral synchrony with the light-dark cycle (Xu et al. 2025). However, circadian disruption can occur due to abnormal sleep homeostasis and, when combined with exposure to artificial light, represents one of the most common circadian stressors (Koch et al. 2017). Both factors disrupt the natural light-dark cycle in many individuals, triggering a rapid hypothalamic-pituitary-adrenal (HPA) axis response (Fonken and Nelson 2014; Hirotsu et al. 2015). This response, in turn, affects a wide range of physiological and behavioral functions (Fonken and Nelson 2014).

The adrenal glands, also referred to as the suprarenal glands, are intricate endocrine organs that play a critical role in regulating a wide array of physiological processes, including metabolism, and stress response (Bielohuby et al. 2007; Ashworth et al. 2016; Vinson 2016). The adrenal gland as the main producer of stress hormones plays a major role in responding to physiological challenges and is able to adapt to these physiological needs (Berger et al. 2019).

Structurally, they are composed of two distinct layers: an outer cortex, which secretes corticosteroids, and an inner medulla, responsible for producing epinephrine and norepinephrine. Both the cortex and medulla exhibit rhythmic expression of circadian clock genes over a 24-hour period (Fahrenkrug et al. 2012; Kalsbeek et al. 2012; Kulbitska et al. 2020). This rhythmicity is closely tied to the light-dark cycle; when the organism is placed in constant darkness, the rhythmic secretion is abolished, suggesting that light input is essential for the proper functioning of the adrenal glands (Bedrosian et al. 2016; Koch et al. 2017). Furthermore, constant light exposure has been shown to elevate corticosterone levels and is commonly used as an experimental model of chronic stress (Welberg et al. 2006).

Light serves as a key driver of the suprachiasmatic nucleus, which orchestrates the rhythmic secretion of hormones through the HPA axis. Simultaneously, the SCN projects to the autonomic nervous system, regulating the rhythmic release of norepinephrine and epinephrine (Spiga et al. 2014; Koch et al. 2017; Kulbitska et al. 2020).

The circadian regulation of cortisol production is vital for the body's adaptation to day-night rhythms and daily activities. This intricate interplay between the central clock, peripheral tissues, and the HPA axis highlights the complexity of the body's temporal synchronization mechanisms (Androulakis 2021).

The central clock, located in SCN regulates cortisol production by the adrenal glands through the control of glucocorticoid release. In nocturnal animals, plasma levels of adrenocorticotropic hormone (ACTH) and cortisol gradually increase throughout the inactive phase of the day, reaching a peak just before the onset of the active phase at night. Conversely, these levels decline steadily during the night, reaching their lowest point in the morning (Focke and Iremonger 2020).

Models for studying circadian desynchrony are essential to understand the mechanisms affected by biological rhythms. *Gerbillus tarabuli*, a nocturnal wild desert rodent, has emerged as a species of great interest for research in various fields (Hamidatou Khati et al. 2022), especially for studying the disruption of the light-dark cycle at the metabolic and structural levels (Derbouz Rouibate et al. 2020, 2024).

As a nocturnal species, *G. tarabuli* actively contributes to the conservation of the desert ecosystem. However, urban expansion is occurring in this area, encroaching on its ecological niches, exposing this species to artificial light. Consequently, prolonged exposure to artificial light disrupts the natural circadian cycle, leading to pathophysiological changes, particularly in the endocrine glands. For these reasons, this study is designed to investigate the effects of light stress on the adrenal gland morphology of the Saharan rodent *G. tarabuli*.

Materials and methods

Animals

The animals were collected in a desert region in the Northwestern area of the Algerian Sahara, wilaya of Béni-Abbès (30° 4´ 48˝ N, 2° 6´ 0˝ W). All procedures were conducted in strict accordance with the recommendations of the EU Directive 2010/63/EU on the protection of animals used for scientific purposes. The animal experimentation protocol was conducted in accordance with the recommendations of the "Association Algérienne des Sciences en Expérimentation Animale (AASEA) (http://www.aasea.asso.dz/).

Experimental work

A total of 20 gerbils were acclimated for 4 weeks before the start of the experiments in two air-conditioned rooms. The rooms were specially designed for chronobiological investigations. Throughout the trial, they were housed in individual cages maintained at 22 ± 2 °C and the relative humidity was about 50-60%, with free access to food (barley grains) without water.

The animals were randomly divided into two groups of 10 gerbils each, as follows: **Group I** (control group) was housed under a 24-hour light-dark cycle (12 h light - 12 h dark). **Group II** (stressed group) was exposed to a disrupted light-dark cycle, switching between a standard 24-hour cycle and a modified cycle with a 20-hour diurnal phase every other day for 3 months. At the end of the experiment, the animals were sacrificed at the same circadian time, between 9:00 and 11:00 AM, to minimize variations in physiological parameters due to daily rhythms. The adrenal glands were rapidly removed, dissected, and prepared for microscopic examination.

Histology

Adrenal specimens were collected immediately after euthanizing the animals and immersed in the Bouin's fluid. Fixed samples were then dehydrated through a graded series of ethanol, cleared in xylene, and embedded in paraffin blocks. Serial sections of 5 µm thickness were cut using a rotary microtome and mounted on clean and dry glass slides. The prepared sections were stained using Mallory's trichrome and Haematoxylin and Eosin (H&E) (Martoja and Martoja-Pierson 1967). All paraffin sections were examined using a Zeiss Axoplan standard light microscope and photographed with a High-Resolution Optics Microscope Camera (MA88-500/ Premiere®, 5.0 Megapixels with a 1280 x 1024 resolution) using TSView 6.2.4.5 software. None of the collected images were manipulated.

Measurement of adrenal cortex parameters

Morphometric analysis was conducted using AxioVision 4.8 (Carl Zeiss MicroImaging, Germany), and the capsule thickness, adrenal gland length and area, and cortex (including its constituent zones) were quantified from 30 measurements of each parameter. All measurements were calibrated using a micrometer slide to convert pixel values into micrometers (µm).

Results were expressed as means ± standard error of the mean (SEM). The Student t-test was used to compare the two groups. A P-value less than 0.05 was considered statistically significant.

Results

Histomorphometric evaluation

Adrenal gland histology

Analysis of adrenal gland sections from gerbils revealed that both studied groups exhibit a typical structure consisting of a cortical zone (cortex) and a medullary zone (medulla). These glands were encased in a fibrous connective tissue capsule (Fig.1, A1 and B1).

The adrenal cortex was divided into three zones: zona glomerulosa (ZG), located in a small area just below the fibrous capsule, followed by zona fasciculata (ZF), formed the great part of adrenal cortex thickness; and the deepest zona reticularis (ZR) (Fig.1, A2, B2).

The morphometric results demonstrated that the capsule thickness increased in Group II (8.343 ± 0.583 µm) compared to Group I (6.706 ± 0.943 µm), although this difference was not statistically significant ($P>0.05$, Fig. 3A, Table 1). Additionally, we observed a significant increase in adrenal gland length (4111 ± 75.83 µm vs. 3059.31 ± 56.39 µm, Fig. 3B) and area ($9.39 \times 10^6 \pm 7.41 \times 10^5$ µm² vs. $4.99 \times 10^6 \pm 4.28 \times 10^5$ µm², Fig. 3C) in Group II compared to the control group, representing gains of 34% and 88%, respectively ($P<0.001$, Table 1).

Adrenal cortex

All three cortical zones of the adrenal gland in both groups of gerbils were clearly visible: ZG, ZF and ZR. In Group II (stressed group), various histological changes were observed, including the loss of normal adrenal architecture. Capsular alterations were noted, such as hyperplasia and lamellar separation of the capsule (Fig. 1, B1). Additionally, there was an enlargement of the glomerulosa, fasciculata, and reticularis zones, along with a shortening of the medulla compared to the control group (Fig. 1, B1 and B2).

Comparative morphometric analysis demonstrated an increased cortex length in Group II, with an average of 1092 ± 40.02 µm vs. 717.70 ± 30.37 µm. This increase is highly significant ($P<0.001$, Fig. 3D) and represents a gain of 52%. We also noticed an increase in all three cortical zones (glomerulosa, fasciculata, and reticularis), as seen in table 1 and Fig. 3E.

Zona glomerulosa

The ZG of Group I is organized into closely packed ovoid clusters, separated by thin connective trabeculae enclosing narrow sinusoidal capillaries. The ZG cells are relatively small and round, exhibiting pink-stained vesicular cytoplasm with lipid inclusions. The nuclei are round and feature a prominent nucleolus (Fig. 2, A1).

In Group II, exposed to stress light, ZG cells remained intact, displaying uniform cytoplasm with visible lipid inclusions, pale rounded nuclei, and barely discernible nucleoli. The sinusoidal capillaries appeared more distended compared to the control group (Fig. 2, B1).

The length of ZG increased significantly by 88%, ($P<0.01$) in Group II compared to Group I (109.22 ± 4.06 µm *vs.* 59.47 ± 2.60 µm, respectively) (Table 1, Fig. 3E).

Additionally, the diameter (21.74 ± 1.18 µm *vs.* 17.30 ± 0.54 µm, $P<0.01$, Fig. 4A), area (367.88 ± 25.09 µm² *vs.* 243 ± 16.03 µm², $P<0.01$, Fig. 4B), and nuclei area (54.46 ± 2.70 µm² *vs.* 46.62 ± 2.40 µm², $P<0.05$, Fig. 4C) of ZG cells were significantly increased by 27%, 51%, and 17%, respectively, compared to the control group (Table 1).

Zona fasciculata

The ZF of Group I consists of large polyhedral cells with rounded nuclei and slightly basophilic cytoplasm containing numerous lipid inclusions. The cells are organized in long, straight cords, separated by tiny sinusoidal capillaries (Fig. 2, A2). In Group II, exposed to stress light, ZF exhibited narrower cells that were inclined towards each other, resulting in a more compact architecture. The cells displayed lighter cytoplasm, eccentric nuclei, and numerous lipid inclusions. Additionally, the sinusoidal spaces appeared more dilated (Fig. 2, B2).

The length of ZF increased significantly by 60%, ($P<0.01$) in Group II compared to Group I (523.46 ± 18.54 µm *vs.* 328.16 ± 23.69 µm, respectively) (Table 1, Fig. 3E).

Furthermore, the diameter (18.22 ± 1.054 µm *vs.* 19.05 ± 0.94 µm, Fig. 4A), area (320.35 ± 21.90 µm² *vs.* 335.50 ± 22.82 µm², Fig. 4B), and nuclei area (56.80 ± 3.24 µm² *vs.* 60.26 ± 3.76 µm², Fig. 4C) of ZF cells were decreased by -4%, -5%, and -6%, respectively, compared to the control group, although this difference was not statistically significant ($P>0.05$, Table 1).

Zona reticularis

The ZR of Group I consisted of small, densely packed, profoundly eosinophilic polyhedral cells. These cells merged with the chromaffin cells of the adrenal medulla and were arranged in a short, irregular network of amalgamated cords, separated by blood sinusoids (Fig. 2, A3). In Group II, exposed to light stress, the ZR cells appeared condensed, displaying a partially compact structure with clearly visible, slightly enlarged nuclei and coarse chromatin. Confluent sinusoids were scattered throughout, and collagen fibers extended around the vessels in this region (Fig. 2, B3).

The length of the ZR increased significantly by 48%,

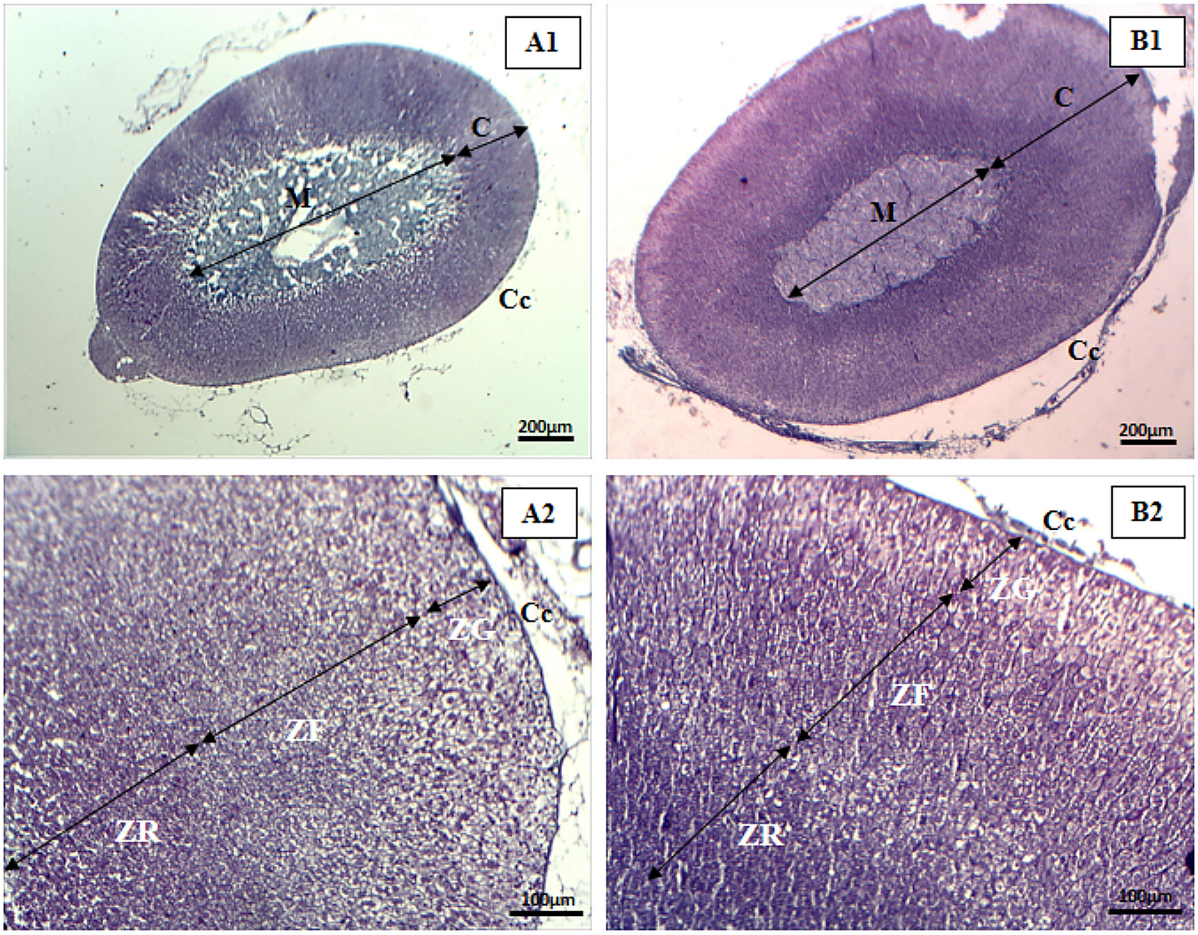


Figure 1. Histopathological evaluation of adrenal glands in both control and stressed groups. Group I (control group) is represented with A1 and A2. Group II (stressed group) is represented with B1 and B2. (A1, B1; objective magnification ×2,5 and A2, B2; objective magnification x10, Mallory's trichrome). A1: Adrenal cortex (C) and medulla (M) enclosed within a capsule (Cc). A2: Three zones of adrenal cortex showing clusters of cells within zona glomerulosa (ZG) just beneath the capsule (Cc), cords of cells within zona fasciculata (ZF) and anastomosing cords of cells in zona reticularis (ZR). B1: An increase in length of the adrenal cortex and a reduced adrenal medulla (M) along with detachment of the fibrous capsule (Cc). B2: An expansion of the three cortical zones: ZG, ZF and ZR.

($P<0.01$) in Group II compared to Group I (413.06 ± 30.14 µm *vs.* 279.13 ± 16.21 µm, respectively) (Table 1, Fig. 3E).

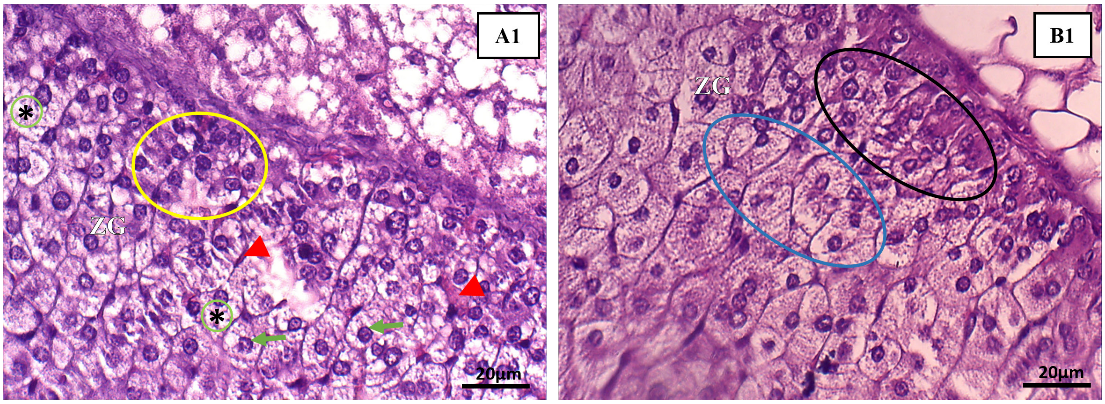
In addition, the diameter of ZR cells (19.75 ± 0.58 µm *vs.* 18.89 ± 0.82 µm, $P>0.05$, Fig. 4A), and nuclei area (76.69 ± 5.98 µm^2 *vs.* 63.78 ± 4.55 µm^2, $P<0.05$, Fig. 4C) increased by 5% and 20%, respectively. However, the area of ZR cells (225.29 ± 11.069 µm^2 *vs.* 245.67 ± 13.49 µm^2, $P>0.05$, Fig. 4B) decreased by -8% compared to the control group (Table 1).

Discussion

The negative effects of artificial light at night (LAN) have been well documented, showing disruption of biological clocks. The ecological consequences of LAN are widely recognised and considered a major threat due to its impact on global biological rhythms, with potential implications for the health and well-being of many organisms, including humans (Helm et al. 2024).

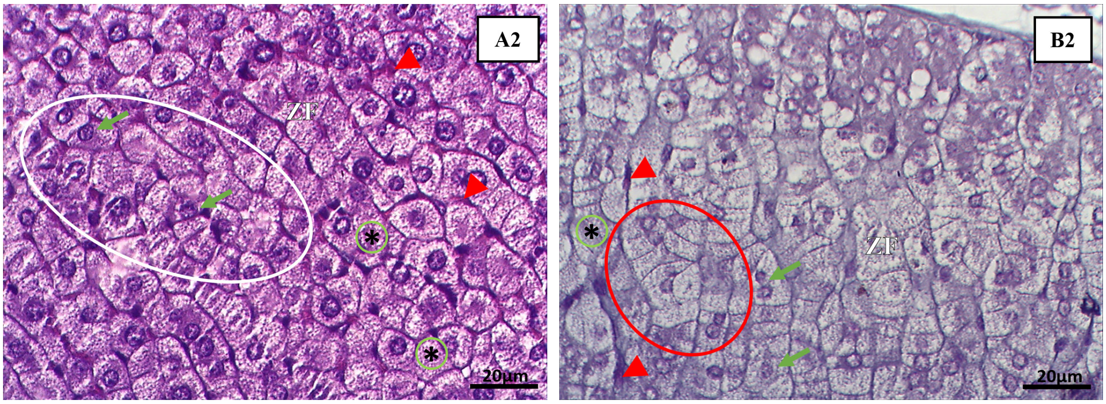
The aim of this work is to examine the structure of the adrenal gland in a nocturnal desert rodent; *G. tarabuli*, subjected to chronic light stress, compared to animals subjected to a standard circadian rhythm, and this is to evaluate the stressful effects of circadian desynchrony on adrenal gland morphology.

Histological examination and morphometric analysis of the adrenal glands of gerbils subjected to lengthening of the diurnal phase as light stress revealed numerous structural and cellular changes in the cortical region, including an increase in total adrenal size. This enlargement seemed to be caused mainly by cell hypertrophy in



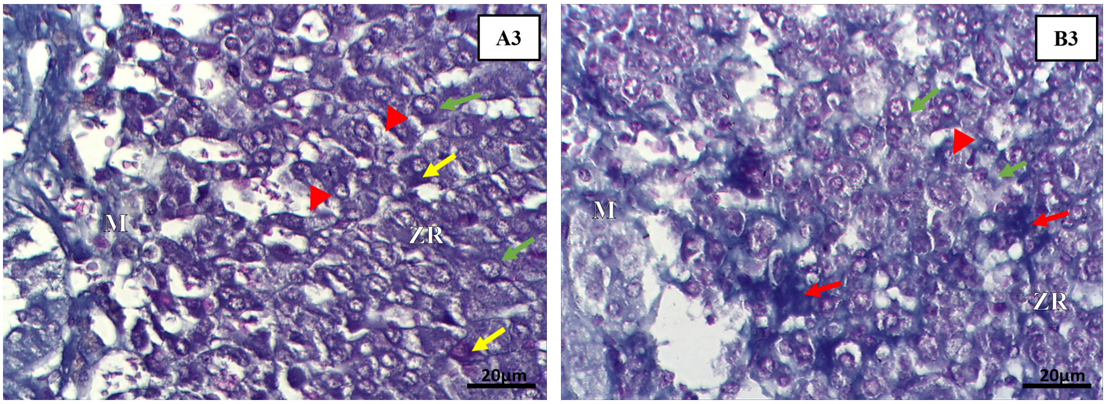
A1: The ZG is arranged in closely packed ovoid clusters (circle) separated by sinusoidal capillaries (arrowheads). The cells of the ZG are relatively small and round with an eosinophilic appearance and clear lipid microvacuoles (asterisks). The nuclei are round with central position (green arrows) (H&E X40).

B1: The ZG cells are compact, forming a bulging architecture over the capsule (black circle). They are small, with moderately abundant dense eosinophilic cytoplasm and a regularly enlarged nucleus. In the inner region of the zone (blue circle), the architecture is hepatoid, with larger, polygonal cells, abundant microvacuolar cytoplasm, with small and regular nuclei (H&E X40).



A2: The ZF is composed of large, polyhedral spongiocytes (white circle), rich in lipid vacuoles (Asterixs) pushing nuclei peripherally (green arrows), arranged in parallel cords separated by sinusoidal capillaries (arrowheads) (H&E X40).

B2: The spongiocytes are less wide and inclined towards each other, resulting in a compact architecture (circle) rich in small lipid vacuoles (Asterix) centred on small nuclei (green arrows). These cells compress the sinusoids (arrowheads) (Mallory's trichrome X40).



A3: The ZR in line with chromaffin cells of the adrenal medulla (M), the cells are arranged in anastomosing cords interspersed with sinusoids (arrowheads). The ZR contains ovoid reticulocytes with large central nuclei and marginal chromatin (green arrows), granular basophilic cytoplasm, and lipofuscin pigments (yellow arrows) (Mallory's trichrome X40).

B3: The ZR infiltrates the adrenal medulla and the reticulocytes are condensed cells with a partially compact structure, featuring clearly visible, slightly enlarged nuclei and coarse chromatin (green arrows). They are interspersed with confluent sinusoids (arrowheads), and collagen fibers extend around the vessels in this zone (red arrow) (Mallory's trichrome X40).

Figure 2. Microphotographs of the adrenal gland showing cellular morphology of the three cortical zones in both control and stressed groups. Group I (control group) is represented with A1, A2 and A3. Group II (stressed group) is represented with B1, B2 and B3. ZG; Zona Glomerulosa A1 and B1, ZF; Zona Fasciculata A2 and B2, ZR; Zona Reticularis A3 and B3.

Table 1. Selected morphometric parameters (mean±SEM) of adrenal gland in both groups of gerbils

	Parameter	Group I (Control group)	Group II (Stressed group)	Percentage Difference	Probability
Capsule	Thickness (µm)	6.706 ± 0.943	8.343 ± 0.583	24%	P>0.05
Adrenal gland	Length (µm)	3059.31 ± 56.39	4111 ± 75.83	34%	***P<0.001
	Area (µm^2)	$4.99x10^6 \pm 4.28x10^5$	$9.39x10^6 \pm 7.41x10^5$	88%	
Adrenal cortex	Length cortex (µm)	717.70 ± 30.37	1092 ± 40.02	52%	***P<0.001
			Zona glomerulosa (ZG)		
	Length (µm)	59.47 ± 2.60	109.22 ± 4.06	88%	***P<0.001
	Cell diameter (µm)	17.30 ± 0.54	21.74 ± 1.18	27%	
	Cell area (µm^2)	243 ± 16.03	367.88 ±25.09	51%	
	Nuclear area (µm^2)	46.62 ± 2.40	54.46 ± 2.70	17%	*P<0.05
			Zona fasciculata (ZF)		
	Length (µm)	328.16 ± 23.69	523.46 ± 18.59	60%	***P<0.001
	Cell diameter(µm)	19.05 ± 0.94	18.22 ± 1.054	-4%	P>0.05
	Cell area (µm^2)	335.50 ± 22.82	320.35 ± 21.90	-5%	
	Nuclear area(µm^2)	60.62 ± 3.76	56.80 ± 3.24	-6%	
			Zona reticularis (ZR)		
	Length (µm)	279.13 ± 16.21	413.06 ± 30.14	48%	***P<0.001
	Cell diameter (µm)	18.89 ± 0.82	19.75 ± 0.58	5%	P>0.05
	Cell area (µm^2)	245.67 ± 13.49	225.29 ± 11.069	-8%	
	Nuclear area(µm^2)	63.78 ± 4.55	76.69 ± 5.98	20%	*P<0.05

Significant (*P*<0.05) difference from control group according to a Student's *t* test.

certain cortical zones, suggesting adaptive-compensatory changes in the organ.

In a previous work, it was reported that a forced desynchronization of the locomotor activity rhythm, a so-called internal forced desynchronisation protocol (T22), in which rats are subjected to a symmetric 22-h light-dark cycle (11:11LD), revealed a greater increase in adrenal gland mass, a significant increase in total adrenal gland area and a significant hypertrophy of the cortical area (de Oliveira et al. 2019). In addition, another study reported that rats subjected to intrauterine growth restriction have hypercorticosteronemia and adrenal gland hypertrophy (de Oliveira et al. 2016). It has also been reported that the effects of continuous exposure of female rats to constant light demonstrated an increase in both the absolute and relative volumes of ZG and ZF compared to controls, leading to an enlargement of the cortical region area (Milosević et al. 2005).

Studies have shown cellular hypertrophy in the adrenal glands of rats exposed to chronic stress (Kulbitska et al. 2020). The ability of the adrenal gland to adapt during stressful challenges is of paramount importance in the body's stress response (Berger et al. 2019). Thus, photic desynchronization in animals appears to be a stressful condition for our species as well. In line with this, our team has shown through body temperature monitoring that *G. tarabuli* is primarily active at night (Issad et al. 2021). It appears that disrupting its circadian cycle, particularly by prolonging its resting phase, could contribute to adrenal dysfunction.

In the current study, we observed a significant increase in the size of the zona glomerulosa in the group subjected to the modified cycle, accompanied by an increase in cell density. In parallel, the size of the cells and their nuclei has increased in favour of the cytoplasm.

Microscopic and morphometric changes of the adrenal glands revealed that the application of a thermal lesion to adult male rats showed a gradual increase in the width of all cortical areas. particularly in the zona glomerulosa and zona fasciculata, in which the area of corticocytes and their nuclei increased significantly (Kulbitska and Nebesna 2022).

Other studies have shown the effect of chronic exposure to light (600 lux) in adult Wistar rats on the growth and function of adrenal zona glomerulosa, The results obtained in animals exposed to chronic lighting indicated that the absolute and relative volume of the ZG increased insignificantly by 5% (p>0.05), and the volume of ZG cells and their nuclei demonstrated an insignificant change of 1% (p>0.05) compared to controls (Milosević et al. 2005).

According to Doi, the disruption of the two cryptochrome genes Cry1 and Cry2, which are core components

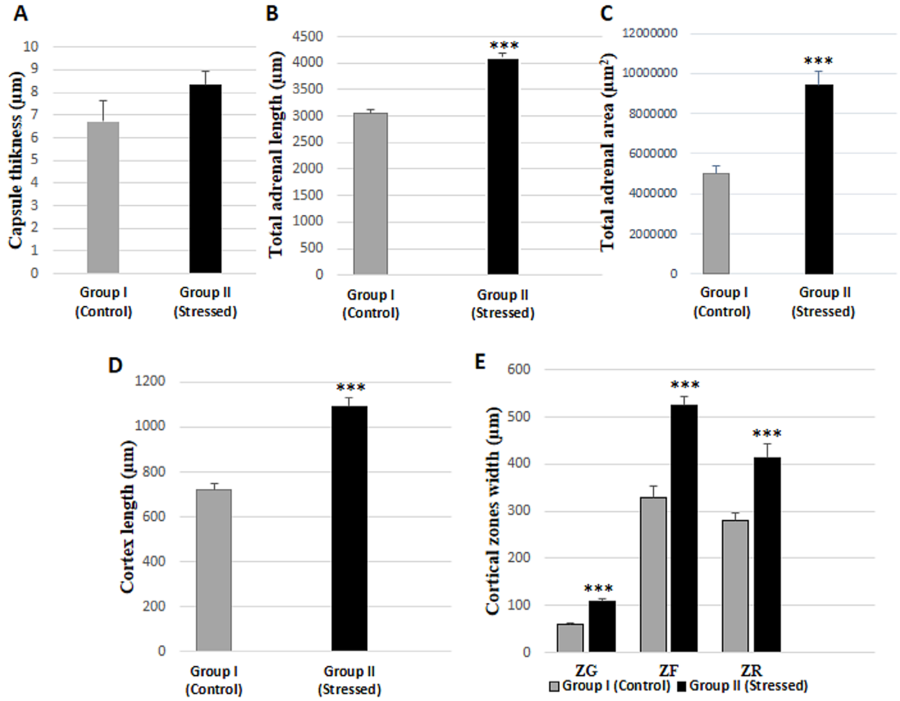


Figure 3. Capsule thickness, adrenal gland length and area, cortex length, and cortical zones width of group II (stressed group) compared to group I (control group) (A, B, C, D and F, respectively). ZG; Zona Glomerulosa, ZF; Zona Fasciculata, ZR; Zona Reticularis. Values are means ± SEM. (n = 10); *** p<0.01.

of the circadian clock, induces changes in the adrenal gland. Histologically, Cry-null mice exhibit bilateral hyperplasia of ZG cells. In addition, aldosterone secretion from ex vivo slice cultures of Cry-null adrenal glands is autonomously increased compared to wild-type adrenal glands. These results suggest that the abnormality in Cry-null mice is localized in the adrenal gland (Doi 2012).

Furthermore, angiotensin II is known to exert trophic effects on the cells of the zona glomerulosa in response to stress (Bollag 2014). On the other hand, other studies have shown that exposure to chronic and variable stress in adult male rats has a negative impact on the zona glomerulosa, leading to a reduction in both its size and that of its cells (Ulrich-Lai et al. 2006).

Our results demonstrated that a prolonged light phase significantly increased the thickness of the zona fasciculata, while a reduction in cell size and lipid reserves was observed. Similar results were reported in female rats subjected to a disrupted light cycle (20L/4D) for 47 days to study the effect of photic disruption on fertility in this species. Histological examination of the adrenal gland revealed a significant increase in the thickness of the zona fasciculata (Mardanpour-Fariman et al. 2021).

Comparable results were also reported by Milosevic and colleagues, who demonstrated that prolonged exposure to continuous light in adult Wistar rats activated stress system, leading to significant hypertrophy of the zona fasciculata cells. There was also a marked increase in serum corticosterone levels. These results suggest that continuous exposure to constant light stimulates the growth and secretory activity of ZF cells (Milosević et al. 2005) and may be explained as a consequence of increased ACTH cell activity (Milošević et al. 2003). Several researchers have suggested that stressful stimuli, which activate the HPA axis, subsequently trigger the zona fasciculata (ZF) to produce glucocorticoids from intracellular (Hayashi et al. 2014; Abdel Malak and Amin 2018).

In addition, studies have shown that a subchronic inverted light–dark cycle pattern caused higher corticosterone production ; possibly due to the sustained stress caused by the inverted sleep pattern (Walker et al. 2020; Shawky et al. 2023). Light exposure, as a key zeitgeber for the circadian rhythm of cortisol, modulates its production, with light levels influencing the cortisol stress response (Petrowski et al. 2021). Exposure to light at night could dysregulate the HPA axis, potentially increasing the risk of cortisol-related disorders (Dijk et al. 2012; Nelson et al. 2022). It could be suggested that cortical cells were maximally stimulated by the oxidative stress resulting from inverted sleep rhythm, leading to its release of all its corticosterone contents. This functional impairment suggests that the adrenal gland is very sensitive to this

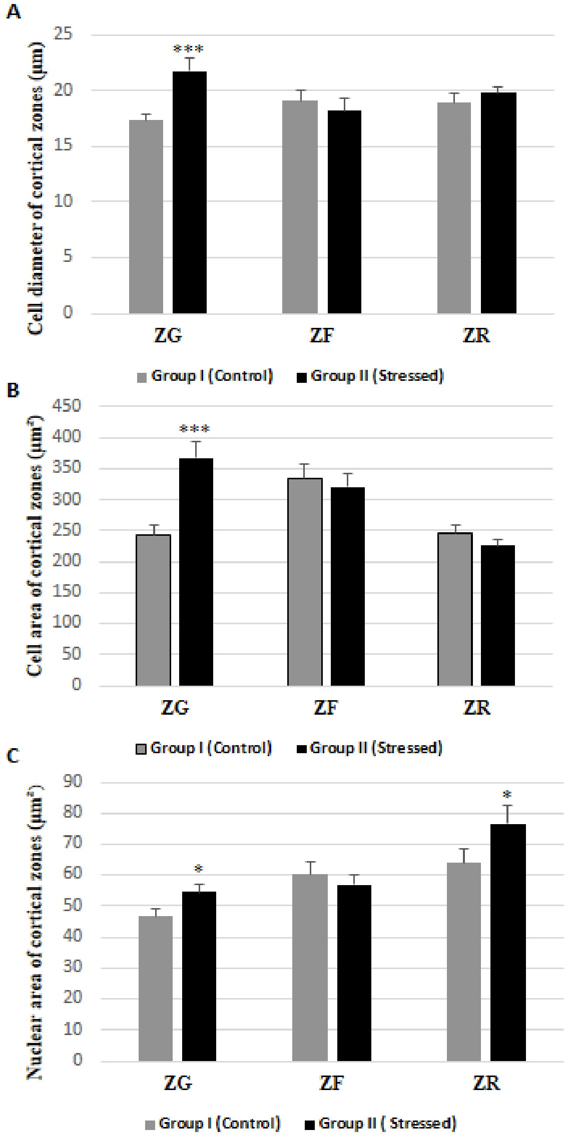


Figure 4. Cell diameter, cell area, and nuclear area of cortical zones group II (stressed group) compared to group I (control group) (A, B, and C, respectively). ZG; Zona Glomerulosa, ZF; Zona Fasciculata, ZR; Zona Reticularis. Values are means ± SEM. ($n = 10$); * $p<0.05$, *** $p<0.01$.

stress (Shawky et al. 2023).

Other authors report that changes in the length of daytime affect the morphofunctional state of the adrenal glands of rats. Specifically, the corticocytes of the zona fasciculata demonstrated a decrease in both the number and size of secretory inclusions, and their borders were poorly defined (Kulbitska et al. 2020). Furthermore, histological and morphometric studies have shown that the presence of animals under altered light conditions stimulates the morphofunctional activity of the zona fasciculata of the adrenal cortex, indicating hyperproduction of corticosteroids (Mamotenko 2014).

It has also been reported that chronic variable stress in adult male Sprague-Dawley rats selectively induces cellular hyperplasia in the outer zona fasciculata (Ulrich-Lai et al. 2006). In addition, repeated stress (immobilization stress, 1 h/day for 7 consecutive days) in male rats increases the thickness of the adrenal zona fasciculata and depletes lipid stores in ZF cells. Moreover, it increases plasma corticosterone and progesterone concentrations, which could indicate activation of this zone in response to an increase in the release of ACTH (Pellegrini et al. 1998). On the other hand, the study used the forced swimming protocol to create an animal model of depression. In depressed rats, there was an increase in cortical thickness, hypertrophy of zona fasciculata cells, and statistically significant increase in corticosterone levels compared to control rat (Eldomiaty et al. 2023).

In the current study, the zona reticularis of the group subjected to the disrupted cycle showed thickening, with cells merging with the chromaffin cells of the adrenal medulla, associated with cellular dystrophy resulting from cell hyperplasia, which may indicate cellular dysfunction in response to the disruption of the rhythm.

In accordance with our results, AboBakr and colleagues (2022) demonstrated that the adrenal glands of rats subjected to an inverted sleep pattern exhibited a deformed organisation of the cells that included areas of separation, the ZR and adrenal medulla were overlapping, and the tissue of the adrenal medulla included reticularis cells.

Previous studies postulating the influence of chronic unpredictable mild stress on the structure of the rat adrenal cortex for 4 weeks showed that an apparent increase in the thickness of the ZF was associated with an apparent reduction of thickness of ZG and ZR which revealed some vacuolated cells and some karyolitic nuclei together with some mononuclear cells (Sadek et al. 2020).

Furthermore, numerous experts have pointed out that stress leads to an increase in the production of free radicals, contributing to the development of various pathologies (Ganesan et al. 2011; Altayeb and Salem 2017). On measuring some oxidative stress markers, it was observed that the inverted light–dark cycle group exhibited a significant increase in free radicals and a notable decrease in antioxidant enzyme activities compared to the control group (Shawky et al. 2023). Many studies have linked psychosocial stress to oxidative stress and the development of various diseases (Moshfegh et al. 2019; Janšáková et al. 2021; Kim et al. 2021). Additionally, it has been demonstrated that chronic stress induces dynamic structural changes in the adrenal gland (Bozzo et al. 2011).

Thus, our results highlight the effect of the disrupted cycle on the adrenal gland, which is characterised by structural changes. In line with our observations, these results are consistent with those previously reported in the liver of *G. tarabuli* (Derbouz Rouibate et al. 2020, 2024), where both structural and ultrastructural changes were observed. These findings illustrate the close relationship between biological rhythms and peripheral clocks, emphasizing how circadian misalignment can affect multiple organs involved in physiological regulation.

Conclusion

In conclusion, this study demonstrates that chronic circadian disruption, induced by exposure to an altered light-dark cycle as a model of stress, leads to microscopic alterations in the adrenal cortex of the nocturnal rodent *G. tarabuli*. These results support the hypothesis that circadian desynchrony, commonly experienced during night shift work, may serve as a significant risk factor for adrenal dysfunction and could contribute to the development of various stress-related pathological conditions.

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