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RESEARCH ARTICLE

Alterations of the Oestrous Cycle and Increase in the Blood Pressure in Normotensive Female Rats by Continuous Exposure to Light

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ABSTRACT

The study aims to investigate changes on some cardiac parameters of normotensive female rats due to continual exposure to light. Three months old female Wistar rats were subjected to varying light cycles and intensities, following which cardiovascular parameters were recorded continuously at days 0, 10, 15, 20, 25 and 30. We calculated and recorded the mean arterial pressure, pulse pressure, peripheral resistance and cardiac output. It was observed that continuous exposure to light for 10 days significantly increased the mean arterial pressure (MAP; 103±1 mmHg control vs. 117±6 mmHg at 20 min; $P<0.05$) and the systolic blood pressure (SBP; 111±1 mmHg control vs. 130±5 mmHg at 20 min; $P<0.05$). Heart rate (HR) did not significantly change during the exposure to light. However, the pulse pressure (PP) increased at day 20 on exposure to light (18±1 mmHg control vs. 32±4 mmHg at 20 min; $P<0.05$). It also reduced the peripheral resistance, while increasing the cardiac output in the female rats. It also elevated oestrogen plasma levels and LF/HF ratio, which is a spectral component of ECG denoting heart rate variability (HRV). Continuous exposure to light may induce stress in female rats, leading to hormonal blood pressure, and sympathovagal imbalances.

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INTRODUCTION

Blood pressure like many physiological measurements goes through several cycles and variations in the course of the day. These variations could be attributed to fluctuations in endocrine hormonal releases, which could also be controlled by circadian and diurnal rhythms (Goldberg *et al*., 2018; Castiglioni *et al*., 2020). These rhythms are also under the influence of sleep and wakeful cycles, with reported morning rise and a nocturnal dip in majority of individuals (Chae *et al*., 2019), and can regulate autonomic, hemodynamic, and hemostatic functions (Scheer *et al*., 2010). These fluctuations have been linked with metabolism and nutritional intakes (Young and Latimer, 2021), cardiocirculatory adaptations (Castiglioni *et al*., 2020), age of the individual which correlates positively with arterial stiffness index (Isobe *et al*., 2017; Kikuya *et al*., 2019), genetic presentations like dysautonomia, which involves fluctuations in blood pressures secondary to afferent baroreflex failure and could be correlated with renal dysfunction (Goldberg *et al*., 2018).

Blood pressure viabilities are increasingly linked with renal dysfunctions and hormonal imbalance, necessitating hemodialysis and cardiovascular mortality (Karpetas *et al*., 2017), with plasma catecholamines and cortisol levels which are hormones of stress, peaking during the circadian

/ light rhythms, while vagal parasympathetic markers are highest during the biological night (Scheer *et al*., 2010). Renal dysfunctions are also possible, with variations in eGFR and proteinuria levels (Goldberg *et al*., 2018), arteriolar hyalinosis and arteriosclerosis were also observed (Isobe *et al*., 2017). These renal and cardiovascular pathologies are often due to the renin angiotensin aldosterone system (RAAS), often linked to a circadian rhythm, and positively correlated with the levels of albumin excretion, urinary protein, and BP (Isobe *et al*., 2017). These hormones can influence the brain's response to stress, and as such are often associated with circadian dependent variations (Braga *et al*., 2002).

These fluctuations and their effects on the determination and diagnosis of hypertension in individuals have necessitated the documentation of guidelines, which takes cognisance of daytime and night-time readings. This highlights the usefulness of 24 h pattern of blood pressure fluctuations, and other biological parameters in the management of patients (Omboni *et al*., 2015). The timing of onset of adverse cardiovascular events, cardiac morbidities and mortalities based on the circadian and diurnal rhythms could go a long way in correct biomarker testing, proteomic and metabolic diagnosis, which will maximize therapy (Tsimakouridze *et al*., 2015). Previous studies on the psychosocial stress observed in hospital nurses undergoing night shifts suggest that there is a modulation of the autonomic nervous adrenal activity, which causes an alteration in heart rates and urinary adrenal excretion (Fujiwara, 1992).

We investigate the role of continuous light exposure on cardiac parameters in normotensive female rats, so as to understand the role of the heart rate viability on the ECG, the sympathovagal response and the plasma estrogen levels.

MATERIALS AND METHODS

Animals: Animals used in this study were from the Height Institute at the Universidad Arturo Prat, and weighed about 180-200g. Institutional study approval was received before the commencement of study (#275/2020). All rats were housed in temperature-controlled room, with free access to rat chow and water. Vaginal smear was used to determine estrus cycle changes, as described by our group in a previous study (Palacios *et al*., 2016). The normal estrous cycle in rats has 4 phases, proestrus (14 h), estrus (24-48 h), metestrus (6-8 h), and diestrus (48-72 h) (Martinez *et al*., 2019).

In this study, a cohort of 4 rats were used under different protocols (Fig. 1):

- Protocol 1 (Baseline): light-cycled (8:00 to 20:00 hours) for 10 days.
- Protocol 2 (light 24 h): stimulation for 10 days constant 24 hours per day with fluorescent light with 1,000 lumens of intensity at 1 m on the floor.
- Protocol 3 (normal): light-cycled (8:00 to 20:00 hours) for 10 days.

Cardiovascular parameters were determined continuously, at days 0, 10, 15, 20, 25 and 30.

Non-invasive determination of blood pressure and heart rate: The cardiac parameters (Diastolic Blood Pressure (DBP), Systolic Blood Pressure (SBP) and Heart rates were measured through the non-invasive tail cuff method (BIOPAC Systems) and Acknowledge 3.9.1 computer software program, following a 20min animal acclimatization. This has been previously described in our earlier publications (Palacios *et al*., 2018). Cardiac parameters were calculated using the following formulas (Cifuentes *et al*., 2009; Nwokocha *et al*., 2017):

Mean Arterial Pressure: MAP = P diastole + 1/3 (P systole – P diastole)
Pulse Pressure: PP = (SBP – DBP)
Cardiac Output: CO = stroke volume x HR; PP ≈ stroke volume.
Peripheral Resistance: PR = MAP/CO

Heart rate variability (HRV) analysis: Rhythmic analysis was performed by determining heart rate variability (HRV). The frequency bands were; P: 0 to 3 Hz (total power), LF: 0.20 to 0.75 Hz (power in the low-frequency), and HF: 0.75 to 3.0 Hz (high-frequency). The LF to HF ratio (LF/HF) represents the sympathovagal balance (Cifuentes *et al*., 2016).

Statistical analysis: To compare the significant differences between the mean of each group, a one-way analysis of variance (ANOVA) was used, followed by a *post hoc* test, Bonferroni (GraphPad Prism v 5.01, CA, USA). Value $P<0.05$ means statistically significant. Standard error of the mean (SEM) was used.

RESULTS

Effect of continuous exposure to light on the number of cornified endometrial cells: We found that animals exposed to continuous light for 10 days showed a persistent estrus, and once the stimulus ceased, the estrous cycle returned to normal.

Effect of continuous exposure to light on blood pressure in female rats: To determine whether light stress alters the cardiovascular parameters, non-invasive determination of blood pressure, and heart rate recordings were performed. We found that the continuous exposure to light for 10 days significantly increased the mean arterial pressure (MAP; 103±1 mmHg control vs. 117±6 mmHg at 20 min; $P<0.05$, n = 4; Fig. 2A), the systolic blood pressure (SBP; 111±1 mmHg control vs. 130±5

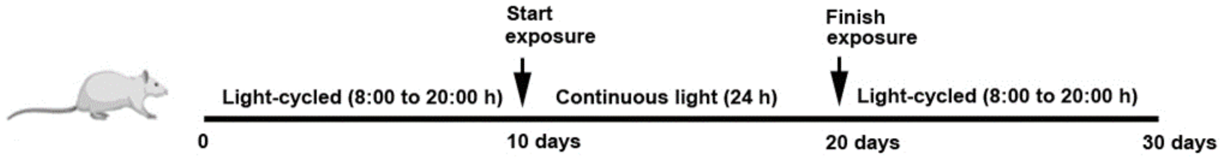

Fig. 1: Scheme of exposure to light in the stress model.

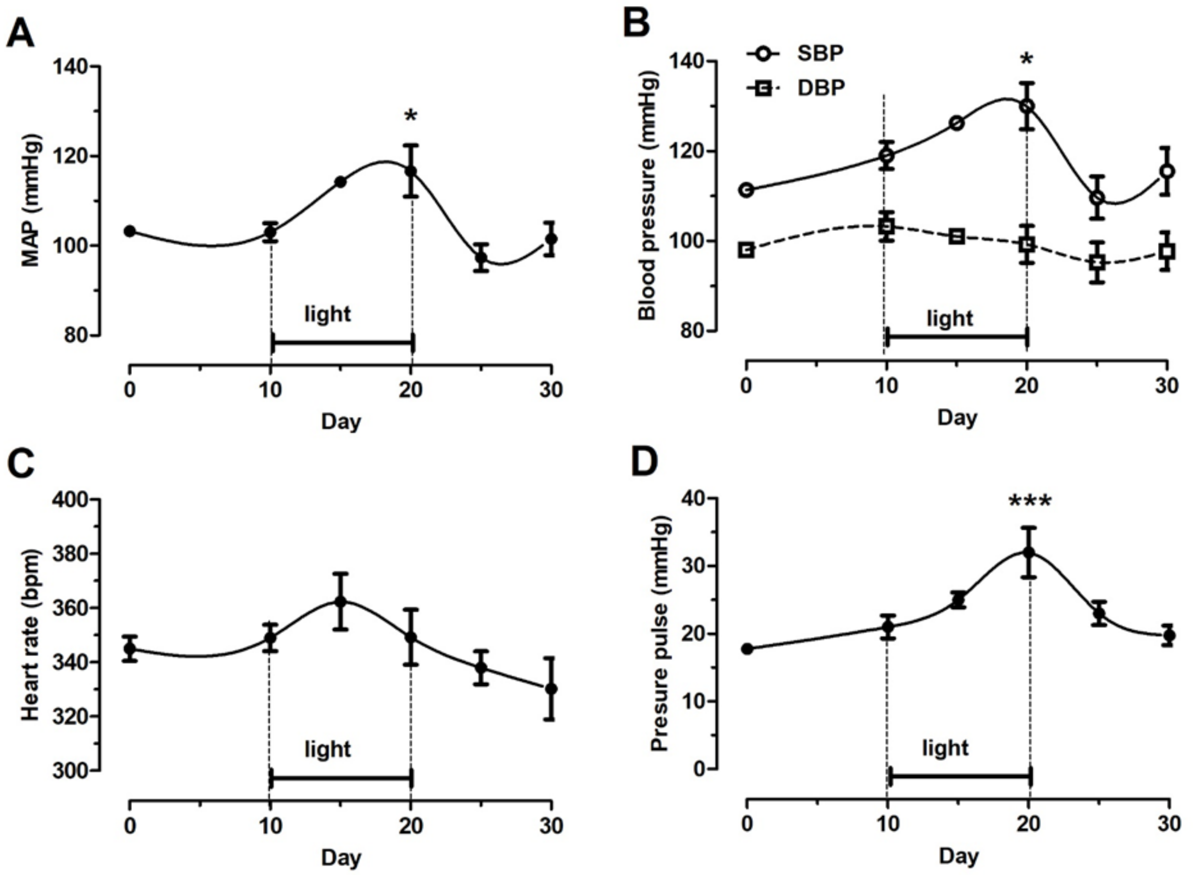

Fig. 2: Effect of continuous exposure to light on cardiovascular parameters in female rats. Mean arterial blood pressure (MAP) (**A**), Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) (**B**), Pressure pulse (**C**), and Heart Rate (**D**) were measured in the same cohort of rat for 30 days. The animals were in continuous exposure to light for 10 days. Data represent the Standard Error of the Mean (SEM) of 4 independent experiments. *P<0.05 and ***P<0.001 versus day zero.

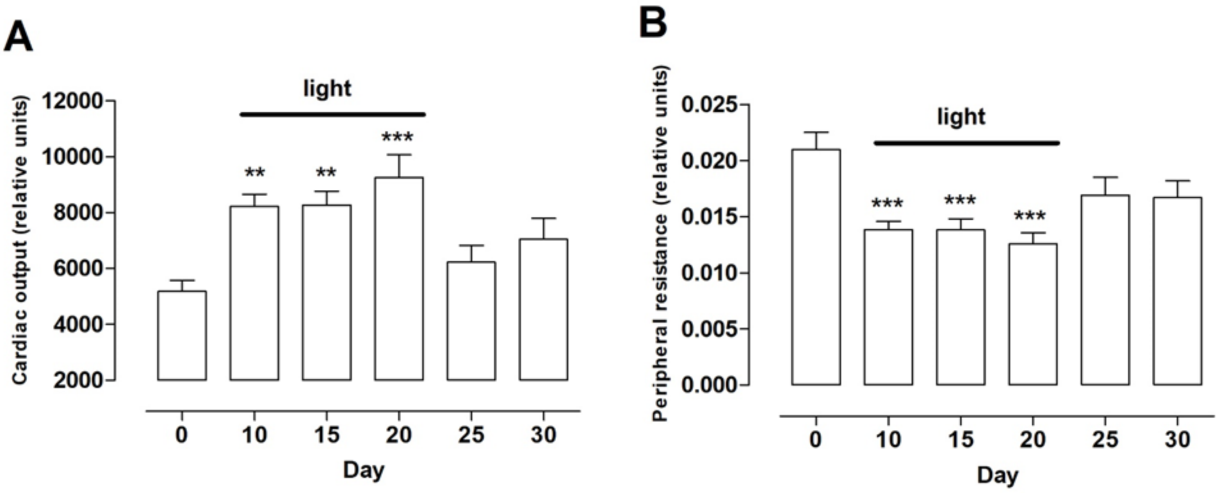

Fig. 3: Continuous exposure to light for 24 h increases the cardiac output (**A**) and reduces the peripheral resistance in female rats (**B**). Data represent the Standard Error of the Mean (SEM) of 4 independent experiments. **P<0.01 and ***P<0.001 versus day zero.

mmHg at 20 min; P<0.05, n = 4; Fig. 2B). Heart rate (HR) did not significantly change during the exposure to light (Fig. 2C). However, the pulse pressure (PP) increased at day 20 on exposure to light (18±1 mmHg control vs. 32±4 mmHg at 20 min; P<0.05, n = 4; Fig. 2D).

We calculated the cardiac output (CO) and peripheral resistance (PR) using the formulas described earlier in Material and Methods section. As shown in Fig. 3A and Fig. 3B, CO increased significantly (P<0.001), while the PR decreased significantly (P<0.001) during the continuous light exposure. Both cardiac parameters recovered to normal values after the stimulus ceased.

Effect of continuous exposure to light on spectral components of Heart rate variability (HRV): We determined HRV as a LF/HF ratio under continuous exposure to light (Fig. 4). Although we did not find a significant increase of HR by light, the LF/HF ratio increased significantly during continuous exposure to light compared to baseline (before exposure to light).

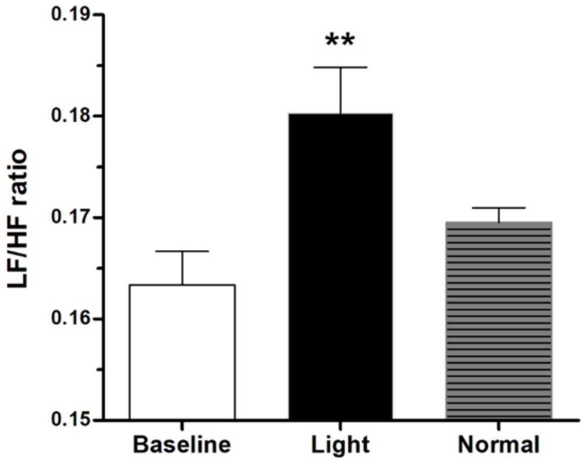

Fig. 4: Effect of continuous exposure to light on sympathovagal balance of the ECG in normotensive female rats. Heart Rate Variability (HRV) of the ECG was expressed as LF/HF ratio (LF, low frequency; HF, high frequency). The ECG and blood pressure measurements were simultaneously in the same cohort of rats. Mean (SEM) of 4 independent experiments. **p<0.01 versus control.

DISCUSSION

This study investigated the effects of a continuous exposure to light on the increase of the blood pressure in normotensive female rats, so as to understand the role of the plasma oestrogen levels and the sympathovagal response.

It is well known that vaginal smears of rats are directly associated with the female sexual hormones (Wied & Davis, 1959; Mbegbu et al., 2020). We found that animals exposed to continuous light showed a persistent estrus, and once the stimulus ceased, the estrous cycle returned to normal. Our results agreed with previous studies which showed that circaquadridian rhythms are disrupted as the continuous exposure light in rats (Takeo, 1984; Benova, 2021). This study also found that the plasma level of oestrogen (17-estradiol) remains high during the continuous light exposure for 20 days, while secretions of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and progesterone are inhibited (Takeo, 1984). However, the continuous light exposure for 100 days increases the LH and decreases FSH levels and leads to polycystic ovaries in rodents (Shaaban *et al*., 2018).

Oestrogen is involved in regulating blood pressure in different ways. Previous studies found that oestrogen produces vasodilatory effect and modulates the sympathetic tone (Caroccia *et al*., 2016), or the renin-angiotensin-aldosterone system (RAAS) (Rossi *et al*., 2019). A study showed that continuous exposure of light decreased the melatonin production and increased the blood pressure in rats (Simko *et al*., 2014). This study showed that the continuous exposure to light significantly increased the MAP and SBP. Other authors reported that the bright light (12/12 h light/dark) caused changes in the rhythmic parameters of blood pressure and HR in normotensive rats (Blagonravov, 2019). Since the pulse pressure (PP) is directly associated with cardiac contractility (Dart and Kingwell, 2001), the significant increase in PP by the continuous exposure to light suggests that the increase in MAP is due, in part, to increased contractility.

HRV is considered a parameter for sympathovagal balance, which regulates the HR (Stein *et al*., 1994). Although we did not find a significant increase of HR by light, the LF/HF ratio increased significantly during continuous exposure to light compared to baseline (before exposure to light). An increased sympathetic activity, coupled with an attenuation of the heart rate baroreflex control, is proposed to be affected by the increased / continuous light exposure. (Cifuentes *et al*., 2016), and a possible increase in the risk of malignant arrhythmias (Benova, 2021).

It is important to note that increase in PP (stroke volume) and CO (cardiac output) are limited by increase in afterload (Li *et al*., 1998). Higher sympathetic activity (autonomic nervous system; SNA) causes an increase in afterload, leading to a lower PP (stroke volume) and elevating myocardial contractibility (Li *et al*., 1998). Therefore, the decrease PR (peripheral resistance) observed could be associated with an increase of CO.

Conclusions: The continuous exposure to light increases the blood pressure in normotensive female rats, in part, by increasing the cardiac output. This effect is mainly because of the increase of sympathovagal activity, leading to increase stroke volume, cardiac contractility and decrease in peripheral resistance (Li *et al*., 1998).

Authors contribution: JLM got the main idea; JLM and JP designed the experiments; JP and CRN performed the experiments; JP analyzed the data; JLM, OY, MR, JE, AP, RV and LJ wrote the draft of the article. JP, CRN and JLM revised this last version of the manuscript.

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