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Dim light at night attenuates circadian rhythms in the cardiovascular system and suppresses melatonin in rats

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ABSTRACT

Aims: Cardiovascular parameters exhibit significant 24-h variability, which is coordinated by the suprachiasmatic nucleus (SCN), and light/dark cycles control SCN activity. We aimed to study the effects of light at night (ALAN; 1–2 lx) on cardiovascular system control in normotensive rats.

Main methods: Heart rate (HR) and blood pressure (BP) were measured by telemetry during five weeks of ALAN exposure. From beat-to-beat telemetry data, we evaluated spontaneous baroreflex sensitivity (sBRS). After 2 (A2) and 5 (A5) weeks of ALAN, plasma melatonin concentrations and the response of BP and HR to norepinephrine administration were measured. The expression of endothelial nitric oxide synthase (eNOS) and endothelin-1 was determined in the aorta. Spontaneous exploratory behaviour was evaluated in an open-field test.

Key findings: ALAN significantly suppressed the 24-h variability in the HR, BP, and sBRS after A2, although the parameters were partially restored after A5. The daily variability in the BP response to norepinephrine was reduced after A2 and restored after A5. ALAN increased the BP response to norepinephrine compared to the control after A5. Increased eNOS expression was found in arteries after A2 but not A5. Endothelin-1 expression was not affected by ALAN. Plasma melatonin levels were suppressed after A2 and A5. Spontaneous exploratory behaviour was reduced.

Significance: ALAN decreased plasma melatonin and the 24-h variability in the haemodynamic parameters and increased the BP response to norepinephrine. A low intensity ALAN can suppress circadian control of the cardiovascular system with negative consequences on the anticipation of a load.

1. Introduction

Artificial light at night (ALAN) is ubiquitous in industrial life but can negatively influence the physiological processes and behaviour of animals and humans [1,2]. Even at low intensities, ALAN can affect the central circadian oscillator, disturbing the transcription-translation feedback loop and reducing the amplitude of the 24-h oscillations in clock genes [3,4].

Light stimulates the suprachiasmatic nucleus (SCN) via the retinohypothalamic tract in both diurnal and nocturnal animals. The rhythmic environmental signals entrain the internal transcription-translation negative feedback loop of clock genes in the SCN [5] and the information is transferred via different output pathways from the SCN to peripheral organs. In this way, melatonin biosynthesis [6,7], behaviour [8], and autonomic regulations including the baroreflex responses [9] can be regulated.

Melatonin is rhythmically synthesised in the pineal gland with high levels during the night and low during the day. It is considered as a direct output of the central circadian clock and entrains circadian processes in peripheral organs. Moreover, melatonin acts via receptor-mediated and receptor-independent pathways to affect a number of physiological processes, including the vascular contractility and blood pressure (BP) [10]. Moreover, vascular smooth muscle cell contraction is controlled by different signalling molecules. For example, nitric oxide, produced by endothelial nitric oxide synthase (eNOS), decreases vascular contractility [11] while endothelin-1 is the most potent long-lasting vasoconstrictor [12]. Interestingly, clock genes are involved in the regulation of eNOS signalling and endothelin-1 expression [13–16].

Circadian rhythmicity is important for cardiovascular health because the intact cardiovascular rhythms help to predict periods of activity during the day and prepare for related physiological demands [17]. Haemodynamic parameters and the incidence of cardiovascular events demonstrate significant 24-h variability which is synchronised with environmental cycles by the SCN [18–20]. Lesions of the SCN

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[21], mutations in clock genes [22], and shifts in the light/dark regime [23,24] significantly alter the 24-h variability in haemodynamic parameters. Long-term disruption of 24-h variability increases the risk of myocardial infarction in shift workers [25]. Therefore, the functioning of the major circadian pacemaker as well as a regular light/dark regime are important conditions for the significant 24-h variations in the cardiovascular system.

Consequences of low-intensity light at night (1–5 lx), which is typical for urban/suburban areas [26], have been studied preferentially in relation to food intake, body weight and metabolism [27]. Until now, there is only limited knowledge about the effects of ALAN on the circadian control of the cardiovascular system. Therefore, the aim of our work was to study the effects of five-week ALAN exposure (1–2 lx) on cardiovascular system control in mature rats. Rats are nocturnal animals and heart rate (HR) and BP peak during the dark phase in contrast with diurnal humans. We predict, that exposure to ALAN during the dark-time can change the circadian variability in both diurnal and nocturnal species. To outline underlying mechanisms how ALAN affects circadian variability in the cardiovascular system, we analysed 1) the day/night differences in plasma melatonin concentrations, which mediate circadian rhythmicity, 2) expression of vasorelaxation (eNOS) and vasoconstriction (endothelin-1) factors, 3) the cardiovascular response to norepinephrine administration and 4) behaviour in an open-field test.

2. Material and methods

2.1. Animals

Male Wistar rats (WT; $n = 36$, age: 18 weeks, body weight: 326 ± 3 g) were obtained from a breeding station at the Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences (Dobrá Voda, Slovak Republic). Animals were kept in plastic cages with food and water ad libitum under a stable 12:12 light/dark (LD) regime (lights on at 10:00 = ZT0), with a controlled room temperature (21 ± 2 °C) and humidity (55 ± 10%). Rats were kept on this LD regime for 4 weeks before the experiment started. The experiments were approved by the Ethical Committee for the Care and Use of Laboratory Animals at the Comenius University in Bratislava, Slovak Republic and the State Veterinary Authority of Slovak Republic. The animal studies are reported in compliance with the ARRIVE guidelines [28,29].

2.2. Experimental design

Rats were randomly allocated to the control and ALAN groups. Control rats were exposed to the regular 12:12 LD regime (L: 150 lx; D: 0 lx) and the ALAN group was exposed to the 12:12 light/dim light regime (L: 150 lx; dim light: 1–2 lx) for 5 weeks (Fig. 1). During this period, the behaviour of the rats was analysed in an open-field test and blood samples were taken from the rats for melatonin analysis as described below. Rats from each group were sacrificed by decapitation under isoflurane anaesthesia during the first half of the light phase at the end of week 2 (control: $n = 5$, ALAN: $n = 6$) and week 5 (control: $n = 8$, ALAN: $n = 10$). The samples of thoracic aorta were quickly removed and immediately frozen in liquid nitrogen and stored at −80 °C until further processing. A group of rats ($n = 7$) was implanted with telemetry transmitters and monitored for systolic BP and HR over 1 week (control week) in the control lightning regime and then over 5 weeks in the ALAN regime.

2.3. Heart rate and blood pressure telemetry measurement

Telemetry transmitters (HD-S10; Data Sciences International, St. Paul, MN, USA) were implanted in the abdominal aorta under isoflurane anaesthesia induced with 4% isoflurane in 100% oxygen and maintained with 1.5–2% isoflurane in 100% oxygen. Immediately after the surgery, rats were treated subcutaneously with ampicillin (100 mg/kg; BB Pharma a.s., Prague, Czech Republic) and tramadol (15 mg/kg; Tramal, Stada, Bad Vilbel, Germany). Two weeks after the surgery, when distinct circadian rhythms of BP and HR were present, animals were included in the study. Due to radio signal interference, telemetry-monitored rats were housed individually in transparent polycarbonate cages placed on the receiver during the experiments. Telemetry data were acquired with a sampling rate of 500 Hz continuously over a 5-min recording period, once per 15 min during the control week and weeks 2 and 5 of ALAN. From the original telemetry data (continuous 5-min segments measured once per 15 min), spontaneous baroreflex sensitivity (sBRS) was estimated as the α-index [30].

2.4. Pressor and HR response to norepinephrine

At the end of the control week, week 2, and week 5 of ALAN, telemetry-monitored rats were administered subcutaneously with an adrenergic agonist, norepinephrine (200 μg/kg, arterenol bitartrate hydrate; Calbiochem, La Jolla. CA, USA) 3 h before the lights were turned off (ZT09) and 3 h before the lights were turned on (ZT21) as described previously [24]. Telemetry data (continuous 275-s segments, sampling frequency 500 Hz) were acquired every ten minutes. Pressor and HR responses to norepinephrine were expressed as the difference between the stimulated and basal values (delta). The basal values were defined separately for each rat as the value from the two-hour segment before norepinephrine administration. The area under the curve was calculated from a 90-min norepinephrine response using a trapezoidal rule.

2.5. Western blot

Total protein lysates of the thoracic aortas were mixed with a protease inhibitor and stored at −80 °C until analysis. The protein concentrations were determined using a BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL, USA). The desired amount of proteins (15 μg) were separated on 10% reducing sodium dodecyl sulfate-polyacrylamide gels and transferred to a Hybond® ECL™ nitrocellulose membrane (Sigma-Aldrich, Steinheim, Germany). The presence of proteins on the membranes was confirmed by Ponceau S. Membranes were incubated overnight in phosphate buffered saline containing 0.1% (v/v) Tween 20 (PBST; MP Biomedicals, Eschwege, Germany) and 1% (w/v; in case of eNOS detection) or 5% (w/v; in case of endothelin-1 and α-smooth muscle actin detection) bovine serum albumin (Biowest, Nuaillé, France) to block nonspecific binding. The next day, the membranes were incubated with primary antibodies: mouse monoclonal anti-eNOS (ab76198; Abcam, Cambridge, United Kingdom; diluted 1:1000; incubated 1.5 h at room temperature); rabbit polyclonal anti-endothelin-1 (ab117757, Abcam, diluted 1:5000, incubated 1.5 h at room temperature); and mouse monoclonal anti-smooth muscle actin (A2547 Sigma, diluted 1:5000, incubated 1 h at room temperature). After washing with PBST, membranes were incubated with appropriate horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA, diluted 1:5000) for 1 h at room temperature. Blots were washed and chemiluminescence was detected using Clarity™ Western ECL Substrate (BioRad, Hercules, CA, USA) and Hyperfilms ECL (GE Healthcare Europe GmbH, Freiburg, Germany). The expression of eNOS and endothelin-1 was normalised to that of α-smooth muscle actin.

2.6. Melatonin analysis

To analyse plasma melatonin levels, rats from both control and ALAN groups were repeatedly blood sampled at ZT09 and ZT21 during week 2 (control: $n = 8$, ALAN: $n = 9$) and week 5 (control: $n = 7$, ALAN: n = 7) of the ALAN treatment. Rats were anaesthetised with

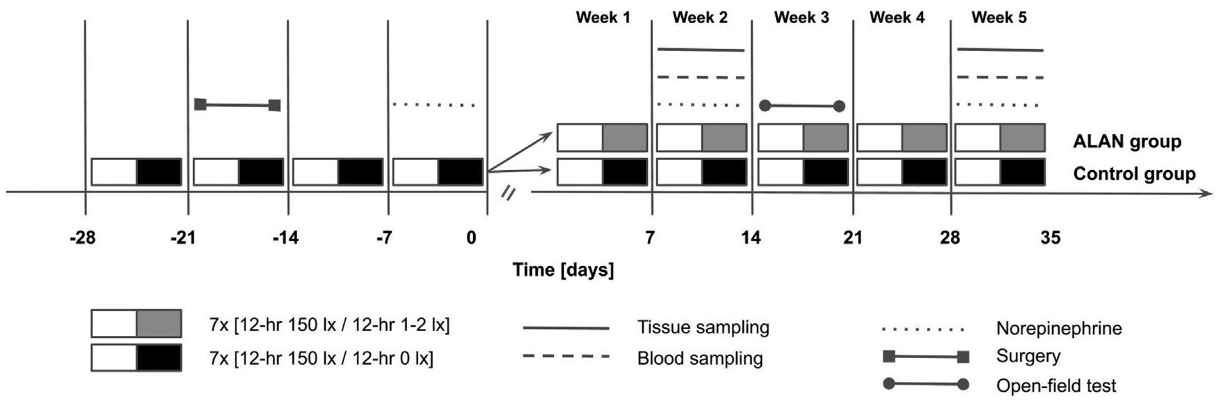

Fig. 1. The design of the experiment and time schedule of procedures used in the study. Rats were kept on 12:12 light/dark (LD) regime for 4 weeks before the experiment started (day 0). Before the experiment started, rats were randomly assigned to the control and ALAN (artificial light at night) groups. Control rats were exposed to the regular 12:12 LD regime (L: 150 lx; D: 0 lx) and the ALAN group was exposed to the 12:12 light/dim light regime (L: 150 lx; dim light: 1–2 lx) for 5 weeks.

isoflurane and blood was collected from the tail vein into heparinised tubes. The plasma was separated by centrifugation (2500 *g*, 10 min, 4 °C) and stored until the melatonin analysis. Melatonin concentrations were quantified with a radioimmunoassay [31] using a melatonin antibody raised in a sheep (Stockgrand Ltd., Guildford, UK) and an [*O*-methyl-^{3}H]-labelled melatonin tracer (PerkinElmer, USA, specific activity 3.07 TBq/mmol). All samples were measured in two assays which had inter-assay and intra-assay variation coefficients lower than 10%. The assay sensitivity was 0.5 pg of melatonin per tube.

2.7. Open-field test

During week 3 of the experiment, control ($n = 6$) and ALAN (n = 6) rats were tested for their spontaneous exploratory behaviour (locomotor and olfactory activity), emotionality and anxiety-like behaviour in an open-field test using a system that continuously records of the kinetic activity of laboratory rodents (Conducta, Experimetria Ltd., Budapest, Hungary). The testing chamber consisted of a dark plastic box (48 × 48 × 40 cm) with a floor divided into 25 squares and built-in infrared beam lights (16 diodes at 16 mm distance from each other in three lines) for recording the animal's movement. During the second half of the light phase, each rat was put into the centre of the testing chamber and subsequently monitored for 20 min. Locomotor activity, expressed as distance travelled in cm, time spent in vertical movement, and immobility time, were exported from the Conducta system. Self-grooming, sniffing (walls, floor), urination and defecation were manually registered from video recordings. Anxiety-like behaviour was evaluated based on total activity (the sum of the number of the horizontal and vertical beam breaks) in the outer and inner zones of the testing chambers.

2.8. Data processing and statistical analysis

Twelve-hour averages of HR and systolic BP were calculated for the light and dark phases during the control week, and for the light and dim light phases during weeks 2 and 5 of the ALAN regime using proprietary telemetry software Dataquest A.R.T. 4.31 (Data Sciences International, St. Paul, MN, USA). Data for BR, HR, sBRS, protein levels of eNOS and endothelin-1, and behavioural parameters were compared between the control and ALAN groups using Student's *t*-tests. The pressor and HR response to norepinephrine was expressed as the area under the curve and the effect of ALAN was evaluated using a two-way repeated ANOVA (factors: ZT, week) followed by Tukey post hoc tests. The time of maximal BP increase after norepinephrine administration was analysed using a Wilcoxon Signed Rank test. The data for plasma melatonin levels were evaluated with a repeated ANOVA (within-group factor: ZT; between-group factor: regime) and Tukey post hoc tests. Differences were considered statistically significant at $p < 0.05$. Statistical evaluations were performed using the statistical package R, version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). Data are presented and visualised as the arithmetic mean ± standard error of the mean (SEM).

3. Results

3.1. Effects of ALAN on heart rate, blood pressure, and sBRS

During the control week, we observed significantly higher HR in the dark than in the light phase (395 ± 8 bpm vs. 337 ± 7 bpm, $p < 0.001$). After 2 and 5 weeks of ALAN, the dark-light difference in HR significantly decreased compared to the control LD week ($p < 0.01$; Table 1). This decrease was caused by a decline in HR during the dim light phase in ALAN compared to the dark phase in the control regime ($p < 0.001$; Fig. 2). The heart rate did not change due to ALAN during the light phases (Fig. 2, Table 1).

During the control week, systolic BP was significantly higher in the dark than in the light phase (129 ± 1 mm Hg vs. 123 ± 2 mm Hg, $p < 0.001$; Fig. 2). The dark-light difference in systolic BP significantly decreased after 2 weeks of ALAN compared to that of the control week ($p < 0.05$; Table 1) but was restored in rats exposed to ALAN for

Table 1
Twelve-hour averages and dark-light differences of heart rate and systolic blood pressure of telemetry monitored rats ($n = 7$) exposed to the control light/dark regime (control week) followed by five weeks of 12:12 light/dim light (ALAN). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to the control week.

	Heart rate (beats/min)			Systolic blood pressure (mm Hg)		
	Light	Dark	Dark-Light	Light	Dark	Dark-Light
Control week	337 ± 7	395 ± 8	58 ± 3	123 ± 2	129 ± 1	6 ± 1
ALAN, week 2	337 ± 9	369 ± 8 (***)	32 ± 4 (**)	124 ± 2	127 ± 1 (0.051)	3 ± 1 (*)
ALAN, week 5	328 ± 7	361 ± 8 (***)	33 ± 4 (**)	121 ± 2	126 ± 2 (**)	5 ± 1

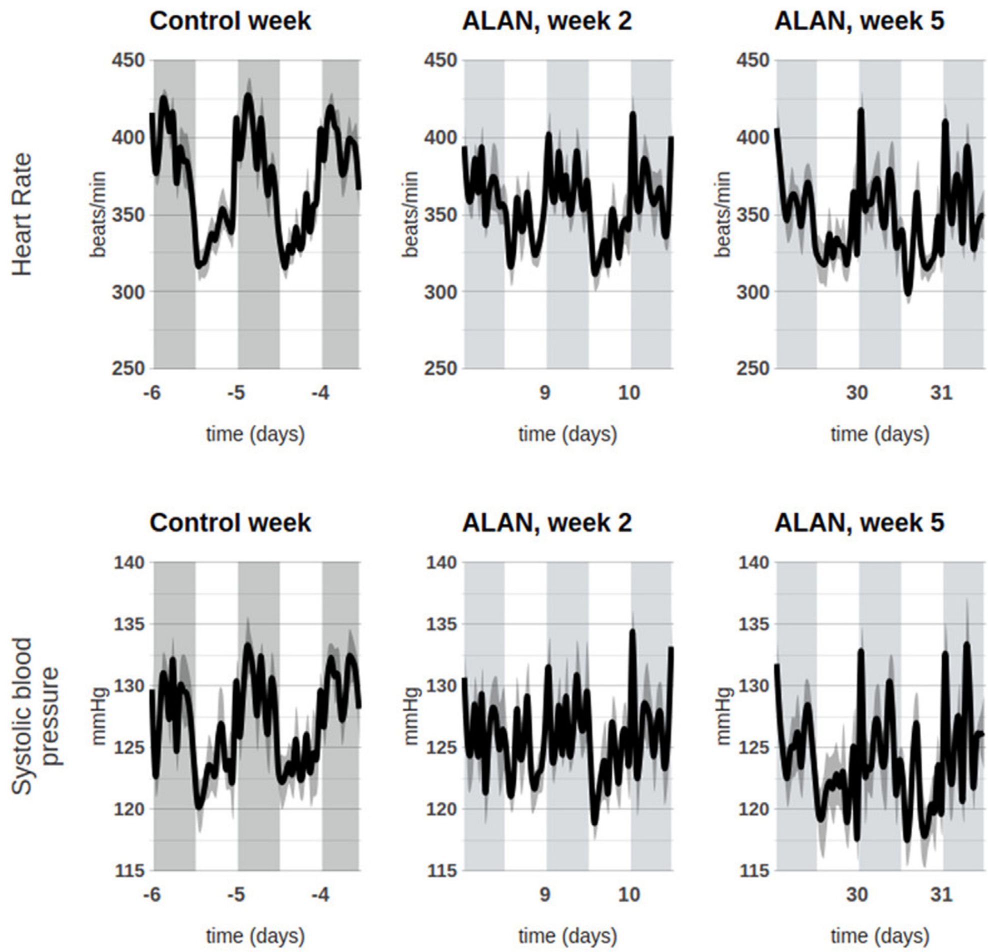

Fig. 2. Heart rate and systolic blood pressure in rats exposed to the control light/dark regime (control week) and dim light at night (ALAN) for five weeks. The white and grey bars represent the light and dark/dim light phases, respectively. Individual hourly averages of telemetry data are calculated and represented as mean ± SEM for $n = 7$.

5 weeks (Table 1). However, systolic BP decreased in the dim light phase after both 2 ($p = 0.051$) and 5 weeks of ALAN ($p < 0.01$) compared to the dark phase in the control week (Fig. 2, Table 1). No changes in systolic BP were observed during the light phase over the 5 weeks of ALAN. Diastolic BP followed a similar pattern as systolic BP (data not shown).

During the control week, sBRS was significantly higher in the light than the dark phase of the day ($p < 0.001$). The dark-light difference in sBRS decreased after exposure to ALAN compared to that of the control week ($p < 0.05$ and $p < 0.01$ for weeks 2 and 5 of ALAN, respectively; Fig. 3B). The loss of the dark-light difference was caused by increased sBRS during the dim light phase, which was significant after 5 weeks of ALAN compared to the control week ($p < 0.05$; Fig. 3A).

3.2. Effects of ALAN on the pressor and HR response to norepinephrine

The pressor and HR response to norepinephrine was significantly higher in the light than the dark phase in the control regime (the area under the curve: 288 ± 18 vs. 216 ± 22, $p < 0.001$ for BP and

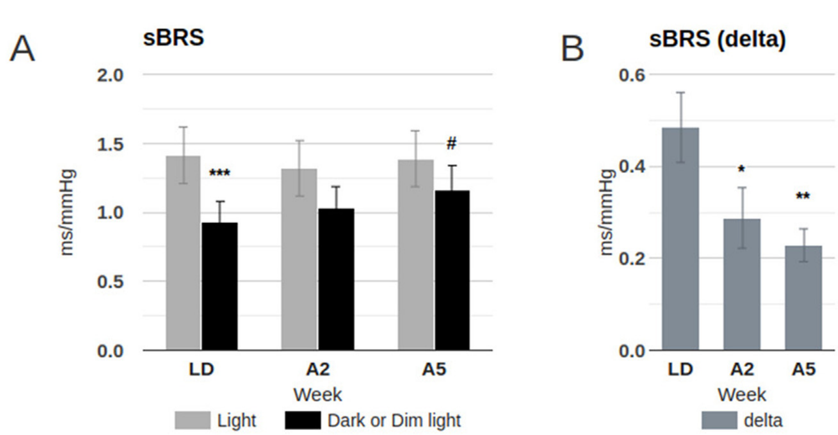

Fig. 3. A) Spontaneous baroreflex sensitivity (sBRS) in rats (n = 7) exposed to the control light/dark (LD) regime for a week and to dim light at night for 2 (A2) and 5 (A5) weeks. B) Delta represents the difference in sBRS between dark and light phases during LD or dim light and light phases at A2 and A5. *$p < 0.05$, **$p < 0.01$ A2 and A5 vs. LD for delta; ***$p < 0.001$ dark vs. light phase during the LD week; #$p < 0.05$ A5 dim light vs. LD dark phase. Data are presented as mean ± SEM.

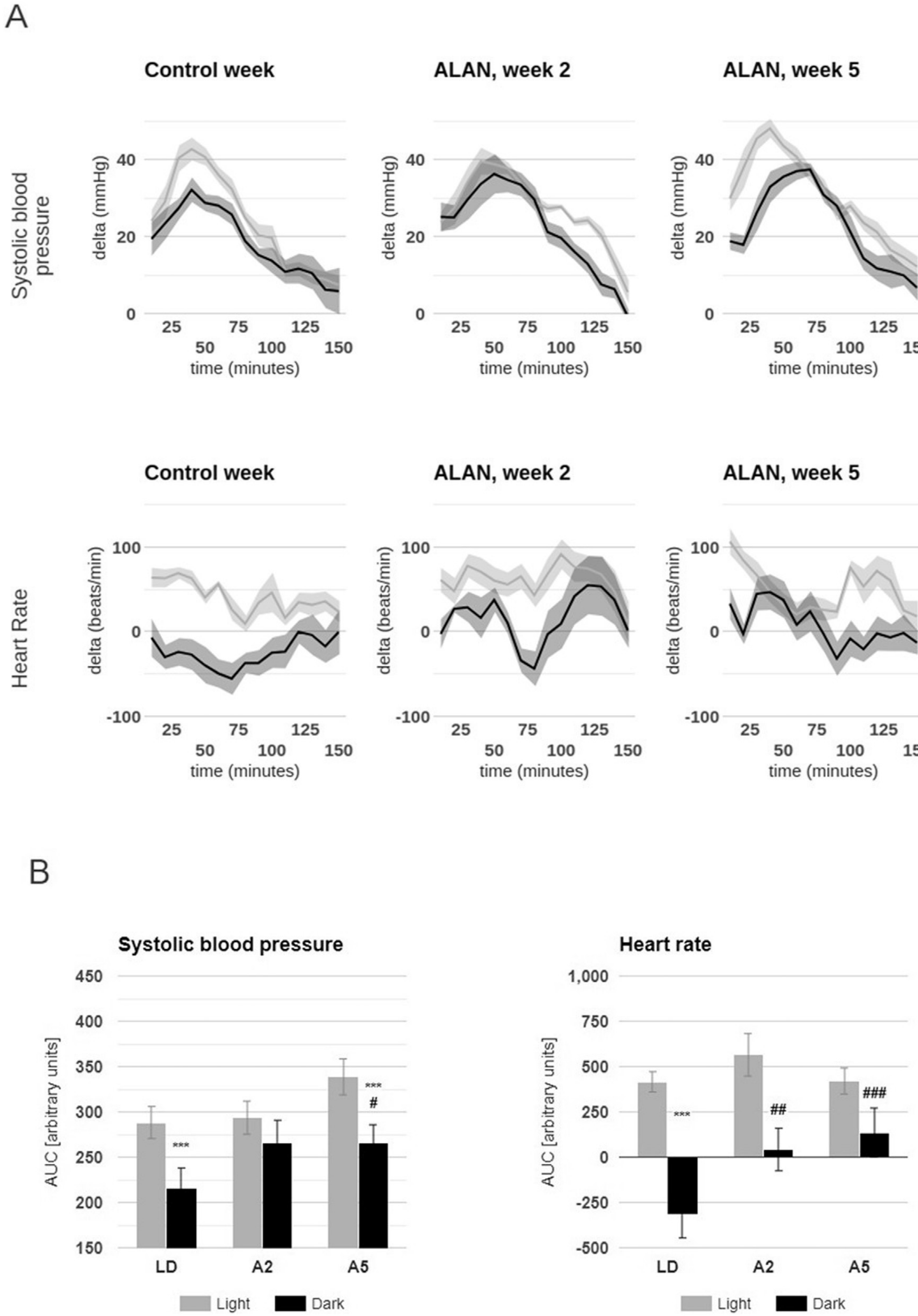

Fig. 4. The pressor and heart rate response to norepinephrine in rats exposed to the control light/dark (LD) regime for one week and to dim light at night (ALAN) for 2 (A2) and 5 (A5) weeks. A) Dynamic changes in the haemodynamic parameters are presented as mean ± SEM. B) The area under the curve was calculated from a 90 min norepinephrine response using the trapezoidal rule and is expressed as mean ± SEM for the light/dark or light/dim light at night phase. $^{***}p < 0.001$ dark vs. light phases during the LD week or dim light vs. light phases during week A5; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ A2 or A5 dim light vs. LD dark phase.

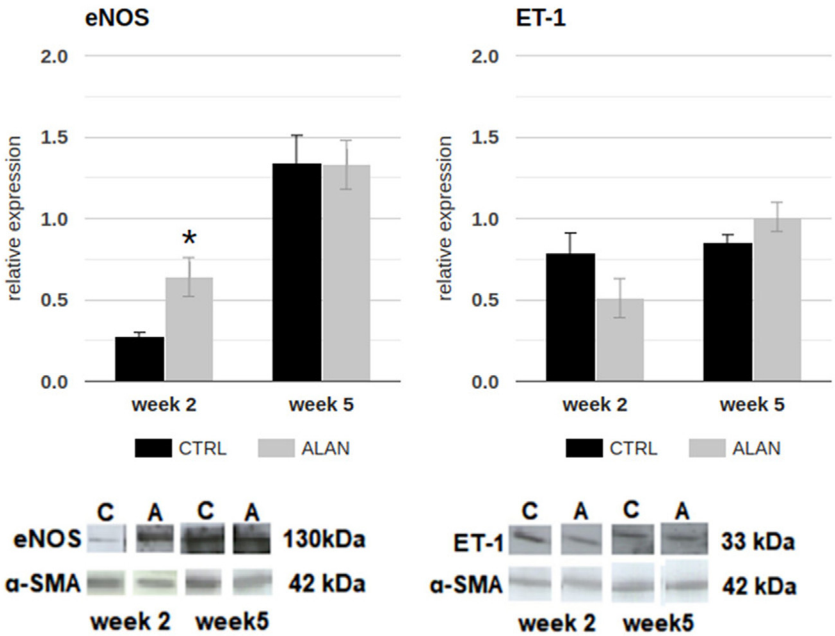

Fig. 5. Relative expression of endothelial nitric oxide synthase (eNOS) and endothelin-1 (ET-1) in the thoracic aortas of rats exposed either to the control light/dark regime (CTRL, C, $n = 5$ and 6) or dim light (1–2 lx) during the whole dark phase (ALAN, A, $n = 6$ and 7) for 2 and 5 weeks. Data are normalised to α-smooth muscle actin (α-SMA) and presented as mean ± SEM. $^{*}p < 0.05$ ALAN vs. CTRL rats at week 2.

416 ± 56 vs. -316 ± 130, $p < 0.001$ for HR; Fig. 4A, B). After 2 ALAN weeks, we observed a loss of dark-light differences in the pressor response to norepinephrine ($p = 0.999$). However, these dark-light differences were restored after 5 weeks of ALAN ($p < 0.001$). In line with these changes, the pressor response to norepinephrine was significantly enhanced during the dim light phase ($p < 0.05$) compared to that of the dark phase and tended to be higher during the light phase ($p = 0.09$) after 5 weeks of ALAN than during the control week (Fig. 4B). Interestingly, after 5 weeks of ALAN, the peak pressor response to norepinephrine was significantly delayed during the dim light phase compared to the dark phase during the control week (61 ± 5 min vs 37 ± 5 min, $p < 0.05$; Fig. 4A). In contrast to the BP, the dark-light differences in the HR response to norepinephrine were not restored after 5 weeks of ALAN. We found an increased HR response during the dim light phase at both week 2 ($p < 0.01$) and week 5 ($p < 0.001$) of ALAN compared to that of the dark phase during the control week (Fig. 4B).

3.3. Effects of ALAN on eNOS and endothelin-1 in the aorta

The protein levels of eNOS were significantly higher in rats exposed to ALAN for 2 ($p < 0.05$) but not 5 ($p = 0.128$) weeks than in control rats (Fig. 5A). No difference in the protein level of endothelin-1 was found between the ALAN and control groups either after 2 ($p = 0.141$) or 5 ($p = 0.151$) weeks of ALAN (Fig. 5B).

3.4. Effects of ALAN on plasma melatonin

The exposure of rats to ALAN significantly affected plasma melatonin levels (interaction ZT vs. regime: $F_{(1,15)} = 34.60$, $p < 0.001$ and $F_{(1,12)} = 27.91$, $p < 0.001$ during weeks 2 and 5, respectively; Fig. 6). In control rats, plasma melatonin levels were higher at ZT21 than at ZT09 ($p < 0.001$ and $p < 0.01$ during weeks 2 and 5, respectively), whereas no difference between ZT09 and ZT21 was found in rats exposed to ALAN during both weeks 2 and 5 (Fig. 6). As a consequence, the ALAN group displayed lower melatonin concentrations than control rats at ZT21 ($p < 0.001$) and no difference between the groups was recorded at ZT09 during both weeks 2 and 5 (Fig. 6).

3.5. Effects of ALAN on behaviour in the open-field test

Differences between the ALAN and control rats were found in the locomotor and olfactory components of exploratory behaviour. Rats exposed to ALAN displayed a tendency to travel a shorter distance ($p = 0.065$; Fig. 7A), showed significantly less vertical activity ($p < 0.05$; Fig. 7B), a tendency to spend more time immobile

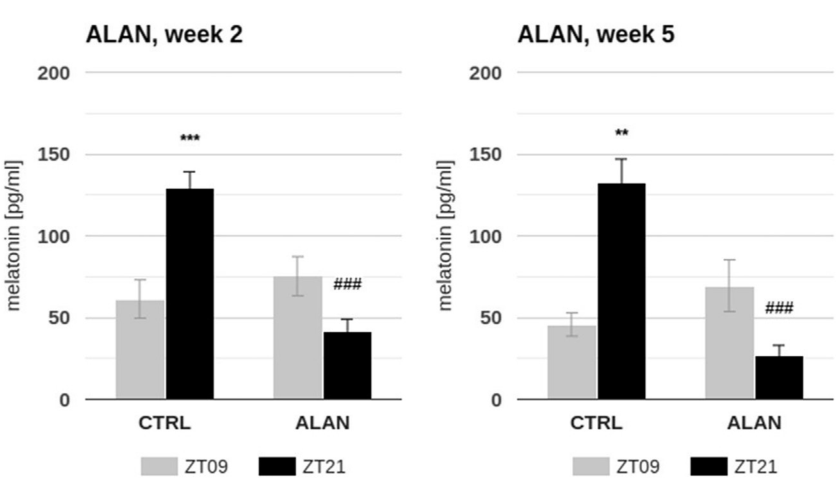

Fig. 6. Plasma melatonin concentrations (mean ± SEM) in male rats exposed either to the control light/dark regime (CTRL, $n = 8$ and 7) or dim light (1–2 lx) during the whole dark phase (ALAN, $n = 9$ and 7) for 2 and 5 weeks. Data are presented as means ± SEM. ZT00 = light on. $^{**}p < 0.01$, $^{***}p < 0.001$ ZT09 vs. ZT21 in CTRL group; $^{###}p < 0.001$ ALAN vs. CTRL group at ZT21.

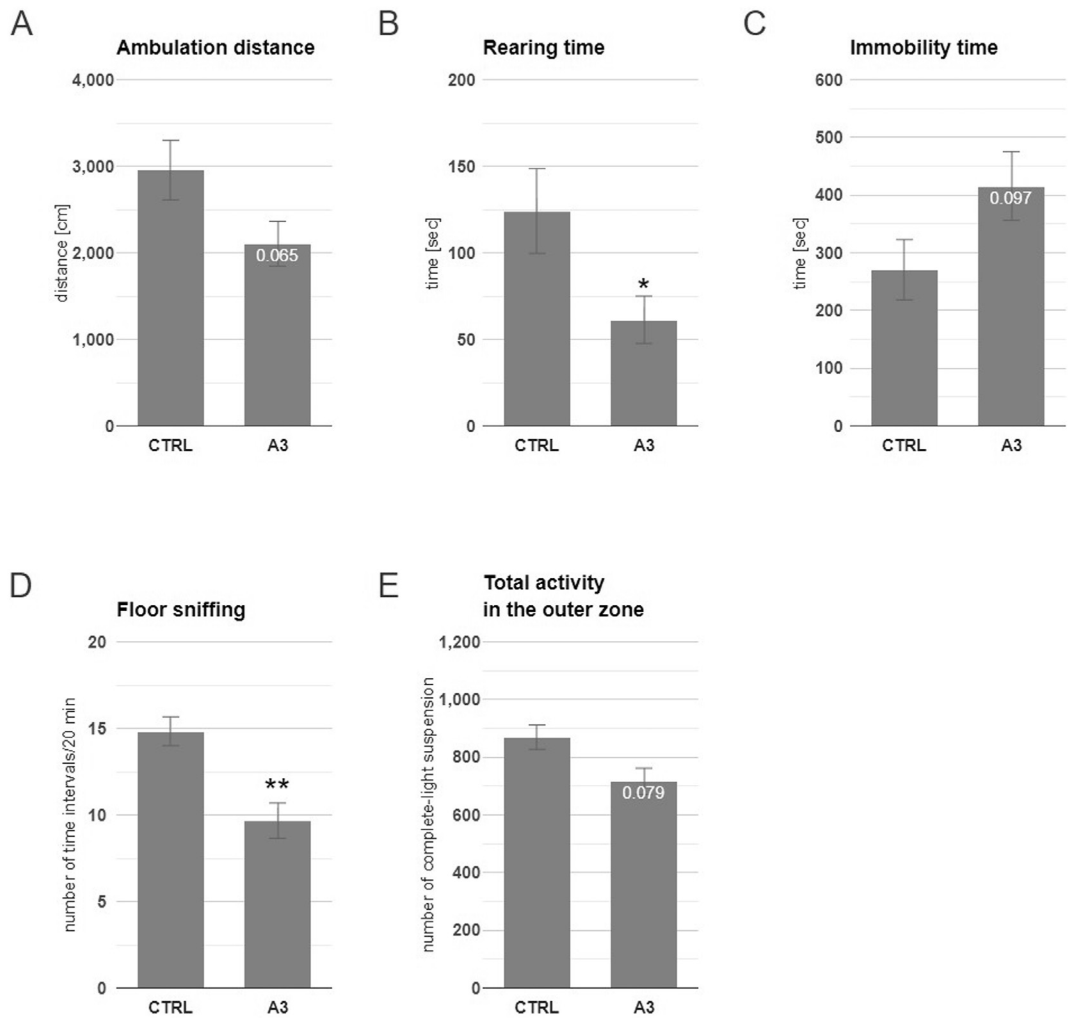

Fig. 7. Behavioural analysis of male rats exposed to dim light (1–2 lx) at night for three weeks (A3: n = 6) and control rats exposed to the standard light/dark regime (CTRL: n = 6) in the open-field test. Data are given as means ± SEM per 20 min. *$p < 0.05$, **$p < 0.01$.

($p = 0.097$; Fig. 7C), and a significantly lower occurrence of floor sniffing ($p < 0.001$; Fig. 7D). The total activity (horizontal plus vertical activity) in the outer zone was lower for rats from the ALAN group than control rats ($p < 0.05$; Fig. 7E), however, there were no differences in activity in the inner zone. No differences were found in self-grooming behaviour, urination, or defecation, which are considered indicators of emotionality.

4. Discussion

Epidemiological and experimental studies suggest that circadian misalignment can have negative consequences on the cardiovascular system [32]. Desynchronization of circadian rhythmicity can result from several factors and the ubiquitous presence of electrical lightning represent one of the most widespread environmental and health challenges. Indeed, a recent study with elderly people shows that low intensity ALAN can affect haemodynamic variables [33]. In our experiment, ALAN reduced the dark-light differences in systolic BP and HR due to a significant decrease in these variables during the dark phase. However, ALAN did not alter the absolute systolic BP or HR values during the light phase. A misalignment between the internal biological clock and the environment caused a fall in HR [23,34] and deteriorated heart function, including prolongation of PR and QT intervals, in nighttime animals [4]. Changes in the heart result from the altered activity of the autonomic nervous system and are independent of locomotor activity [4]. A decrease in HR and prolongation of the QT interval have also been observed in mice exposed to constant light or with food restricted to the passive (light) phase [4,35]. A direct connection between the SCN and heart is indicated by the finding that a pulse of light during the night reduced the HR in mice during the whole dark phase, not only at the time when the pulse was applied [4]. Light suppresses the frequency and endogenous amplitude of sleep-wake and temperature circadian rhythms [36] and circadian rhythmicity in overt rhythms might disappear as a result of a suppressed endogenous rhythmicity. Furthermore, ALAN attenuates the amplitude of clock gene expression in the hypothalamus and liver [3]. In our study, ALAN suppressed the circadian amplitude of the HR and BP and the decreased amplitude might reflect weakened or desynchronised rhythms in neurons of the SCN. The decreased circadian variability of BP has been observed in older people exposed to two ALAN nights [33]. From a clinical point of view, the reduced day/night difference in BP, in particular, an insufficient decrease during the night, is associated with an increased cardiovascular risk [37]. In contrary to elderly humans [33], we did not find the BP increase in our mature rats and cannot conclude if nocturnality, age or duration of ALAN exposure can explain the observed difference.

One of the most important regulatory loops in the cardiovascular system is the baroreflex control. As expected [9,30,38], we observed that the day/night dependent sensitivity of the baroreceptors during the control week was higher during the daytime. ALAN decreased the dark-light difference in the sensitivity of the baroreceptors due to an increased baroreflex sensitivity in dim light. This finding is in line with the SCN is being part of a homeostatic circuit adapting the BP response [39]. Therefore, the diminished phase-dependent sensitivity of the baroreceptors reflects, at least partially, changes in the central circadian control. Our results suggest the effect of ALAN on the central control of the cardiovascular system.

During the control week, the basal systolic BP was higher during the nighttime than daytime but increased more during the light than the dark phase after norepinephrine administration. Moreover, we observed a significant day/night dependent response of BP and HR to norepinephrine administration. Two weeks of ALAN diminished the difference in BP response to norepinephrine. Similarly, reduced 24-h variability in the BP response to norepinephrine was observed in rats exposed to repeated phase shifts in photoperiod [24]. Norepinephrine is metabolised by monoamine oxidase A and its transcription is regulated by the circadian clock, namely *Per2*, which promotes expression of monoamine oxidase [40]. ALAN decreases the *Per2* rhythm amplitude [3,4,41] in the hypothalamus, reduces the expression of *Per1* and *Arntl* rhythms in the SCN [42], and might disrupt the adrenergic neurotransmission in the brain and also in the periphery. Reduced expression of *Per2* could lead to decreased expression of monoamine oxidase A and reduced degradation of norepinephrine, not only during the daytime, but also during dim light. This could attenuate the difference in the BP response after norepinephrine administration.

After five weeks of ALAN, the dark-light difference in the BP response to norepinephrine was restored but there was an enhanced response during both the daytime and nighttime compared to the control week. Moreover, a significant delay in the BP response during the nighttime was recorded. The basal BP significantly decreased after five weeks of ALAN. This probably prolonged the time after norepinephrine administration until the BP reached a sensitive point and activated the baroreflex centres, which subsequently led to a decrease in HR.

In addition to the central mechanisms of BP and HR regulation, BP is controlled by local mechanisms affecting the endothelium and smooth muscle cells [43]. In our study, we focused on the dominant vasorelaxation factor nitric oxide and the vasoconstrictor peptide endothelin-1. After two weeks of ALAN, we observed a significant increase in eNOS expression in the aorta. If ALAN causes chronodisruption of the autonomic regulation of BP, the increased eNOS expression could be a local compensatory mechanism. In mice with a deleted *Per2* gene, the expression of endothelial acetylcholine M3 receptors and eNOS was not changed [44], however, an impaired endothelial-dependent acetylcholine relaxation was observed [45]. Thus, disruption of the circadian clock resulted in a worsening of eNOS signalling [13,15]. We hypothesise that the observed increase in eNOS after two, but not five, weeks of ALAN could result from local compensation, for example, the impaired degradation of catecholamines in blood vessels. For the vasoconstriction factor endothelin-1, we did not observe a significant increase in vascular expression and it probably did not contribute significantly to BP control in these conditions.

The increased pressor response to norepinephrine after 5 weeks of the ALAN regime was consistent with the behavioural inhibition we found in the open-field test, which can be regarded as a mild emotional stressor of novelty. In the present study, rats exposed to ALAN displayed reduced motoric and olfactory components of exploratory activity compared to control rats. Indeed, the altered behavioural pattern has been reported as a consequence of aberrant light/dark conditions including phase shifts [46] and dim light at night [47]. The mechanisms underlying these effects are not well understood, however, they can be associated with either attenuated circadian control of sleep-wake behaviour [42] or direct effects on the brain circuitries controlling mood and emotions [41].

Plasma melatonin concentrations were suppressed after both 2 and 5 weeks of ALAN exposure. The results demonstrate that such low light intensity is able to decrease melatonin secretion and are in line with published data in rats [48]. The decreased melatonin production might result from the altered activity of the SCN since the circadian melatonin rhythm is the direct output of the central oscillator. Moreover, ALAN can directly inhibit melatonin biosynthesis since light suppresses the activity of arylalkylamine-*N*-acetyltransferase, the key enzyme in melatonin biosynthesis [49]. The decreased melatonin concentrations in our study suggest the attenuated circadian control of physiological processes, including rhythms in the cardiovascular system. Melatonin can have direct protective effects on the cardiovascular system [2] and suppressed melatonin production might represent a weakened protection of the system.

The limitation of our study is that rats are nocturnal animals in which HR and BP peak during the dark-time in contrast with diurnal species. Therefore, we cannot confidently translate absolute changes of BP and HR from rats to diurnal species, including humans. On the other hand, circadian dysregulation can similarly influence a daily variability of physiology and behaviour in both nocturnal and diurnal animals [33,50–52]. Moreover, this is a laboratory study performed with mature healthy males kept under optimal environmental conditions. It is possible that using older individuals with potential comorbidities and kept under suboptimal environmental conditions would result in more negative changes in the cardiovascular parameters. Therefore, the dose-dependent effects of ALAN and their link with variables such as age, sex, and strain should be explored in future studies.

5. Conclusions

A low-intensity ALAN (up to 2 lx) decreased 24-h variability in cardiovascular parameters and increased the BP response to norepinephrine. On the basis of the decreased melatonin levels and altered exploratory behaviour, we propose that the observed changes reflect at least partially impaired activity of the SCN and central control of the cardiovascular system. Dim light at night can attenuate the circadian control of the cardiovascular system with negative consequences on cardiovascular health because exposed individuals lose their ability to predict rhythmic changes in the environment and can inadequately respond to challenges.

Declaration of Competing Interest

The authors declare no conflicts of interest. The authors are solely responsible for the writing and content of the paper. The author's affiliation is as shown on the cover page.

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Author contribution to study

Lubos Molcan: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization; **Hana Sutovska:** Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing – Review & Editing, Visualization; **Monika Okuliarova:** Conceptualization, Validation, Formal Analysis, Investigation, Data Curation, Writing – Review & Editing, Visualization; **Tomas Senko:** Investigation, Data Curation, Writing – Review & Editing, Visualization; **Lucia Krskova:** Investigation, Writing – Review & Editing, Supervision; **Michal Zeman:** Conceptualization, Investigation, Resources, Data Curation, Writing – Original Draft, Writing – Review & Editing, Supervision, Project Administration, Funding Acquisition.

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