Abstract. This study investigated whether continuous light exposure (CLE) results in behavioural disturbances in rats and whether melatonin can modify these potential changes. Four groups of 3-month-old Wistar rats were treated as follows for six weeks: control, melatonin, CLE, and CLE with melatonin. CLE increased systolic blood pressure and melatonin reduced it. No changes in behavioural patterns by CLE were observed. In the controls, melatonin reduced both exploration and locomotion but these parameters remained uninfluenced in the CLE. We conclude that melatonin exerted a different impact on behaviour in controls and in the CLE group.

Key words: Melatonin — Behaviour — Continuous light — Hypertension

Artificial light at night is a source of light pollution that may result in the disruption of circadian rhythms and the suppression of nocturnal melatonin production by the pineal gland. Circadian disruption has been implicated in multiple psychiatric disorders like anxiety, bipolar disorder or schizophrenia (Smolensky et al. 2015). In experimental conditions, the exposure of animals to continuous light mimics the exposure of humans to artificial lighting (Simko et al. 2013). Continuous illumination is associated with a number of structural and functional alterations of the pineal gland (Fujisaka et al. 2011; Litvinenko et al. 2011) including the suppression of the production and the release of melatonin, downregulating thus the nocturnal surge of melatonin levels (Simko et al. 2013). Melatonin deficiency results in sleep disturbances (Xie et al. 2017), neurodegenerative disorders (Wu et al. 2003), and other pathological conditions such as hypertension development (Simko et al. 2014a). Eventually, these alterations could result in behavioural disorders.

The aim of this experiment was to show whether continuous light exposure induces behavioural disturbances in rats and whether melatonin administration could modify these potential alterations.

All experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No 85/23, revised 1985). Male adult (three-month-old) Wistar rats (Dobra Voda, Slovakia) were randomly divided into four groups (n = 10 per group): age-matched control (Wistar) rats (LD), rats treated with melatonin (10 mg/kg/day; Sigma Chemical Co., Deisenhofen, Germany; LD+Mel), rats exposed to 24 h/day continuous light (LL) and rats exposed to 24 h/day continuous light treated with melatonin (10 mg/kg/day; LL+Mel). Melatonin was dissolved in drinking water and its concentration was adjusted to daily water consumption to ensure the correct dosage. Melatonin containing solutions were protected from light by using black bottles. Rats were kept in individual cages.
at 22–24°C (the temperature was maintained at constant level by air conditioning system) and fed with a regular pellet diet ad libitum. Systolic blood pressure (SBP) was measured each week by noninvasive tail-cuff plethysmography (Hugo-Sachs Elektronik, Freiburg, Germany). In the home cages, the light intensity was 500 lux to simulate the office workplace conditions, while during the open field testing it was reduced to 150 lux to adapt light conditions used in other laboratories during open field testing (Aubrecht et al. 2013; Okularova et al. 2016).

During the six weeks of the experiment, the behavioural parameters were measured by open field test each week to determine locomotion and exploration. The open field test consisted of a device in the shape of a 1 × 1 m square with sidewalls 0.5 m in height and virtually divided into 16 squares. Each animal was placed in the central part, and was permitted to freely explore the field for 5 minutes. The total horizontal activity (number of squares crossed) and vertical activity (number of instances of rearing up on hind legs) were monitored. The results were expressed as the percentage change relative to the scores of the first week. Results are expressed as mean ± standard error of mean (SEM). For statistical analysis a one-way, two-tailed analysis of variance (ANOVA) and the Bonferroni post-hoc test were used for statistical analysis of behaviour. The differences were considered significant if the value p < 0.05.

After six weeks of treatment, SBP was 125 ± 0.79 mmHg in the control group and was increased by 29% (p < 0.05) in the LL group. SBP decreased significantly by melatonin treatment (by 15%, p < 0.05) compared to the LL group (Figure 1).

During six weeks of treatment, neither horizontal nor vertical activities of rats were altered by continuous light exposure (Figure 2A, 2B). In the control animals, melatonin reduced both horizontal (by 63%, p < 0.05) and vertical activity (by 71%, p < 0.05) (Figure 2C,D). In rats exposed to continuous light, melatonin had no effect on horizontal and vertical activity (Figure 2E,F).

Melatonin is predominantly released during the dark-phase of the day (Klein and Weller 1970). In addition to its specific receptor-induced effects, melatonin has a number of direct, pleiotropic actions such as antioxidative, anti-inflammatory and antiproliferative effects (Reiter et al. 2016; Dominguez-Rodriguez et al. 2017), which protect the heart, vasculature (Simko and Paulis 2007; Simko and Pechanova 2009; Simko et al. 2016; Repova-Bednarova et al. 2013) and other systems (Aziriova et al. 2014; Hrenak et al. 2015; Opie et al. 2016).

Inappropriate nocturnal illumination can disrupt the circadian rhythms, which may be harmful due to the inhibition of the nocturnal melatonin secretion. Shift or night work was shown to be associated with an increased risk of variable pathological conditions such as cancer, cardiovascular disturbances, diabetes and obesity development (Touitou et al. 2017). In experimental animals, it has been repeatedly shown that chronic continuous light exposure results in systolic hypertension development and left ventricular fibrotic remodelling (Simko et al. 2010, 2014b). In our experiment, the continuous light exposure was not accompanied by significant changes in locomotion or exploration. However, the literal data are quite controversial in this area. Some authors suggest that chronic exposure to continuous light leads to anxious and depressive behaviour in rats (Roman and Karlsson 2013; Tapia-Osorio et al. 2013), while others revealed reduced anxiety-like behaviour in rats as an adaptation to stress (Fonken et al. 2009). These data differences might have been determined by genetic variances of laboratory animals, the duration and intensity of continuous light exposure, as well as by variable methodological approaches. In this experiment, melatonin reduced both locomotion and exploration in the control rats; however, it had no significant effect on the behaviour in rats exposed to continuous light. The different impact of melatonin in rats in the control and continuous light-exposed groups might have been determined by its potentially different levels. Apparently, the control rats (that had normal levels of melatonin) may have reached a high, non-physiological, level by melatonin treatment resulting in a sedative effect as observed previously (Jan et al. 2011). On the other hand, in chronic illumination obviously associated with melatonin deficiency (Simko et al. 2013), the melatonin level might have been only partially corrected by chronic melatonin treatment. Furthermore, based on our findings, it may be supposed that SBP alterations did not

### Figure 1. The effect of continuous light exposure (LL) and melatonin (LD+Mel) administration on systolic blood pressure (SBP) during 6 weeks of treatment. LD, controls; LL+Mel, rats exposed to continuous light and treated with melatonin. * p < 0.05 vs. LD, # p < 0.05 vs. LL.
contribute to behavioural patterns in continuous illumination or during treatment with melatonin.

We conclude that continuous light exposure did not change the horizontal and vertical activities of rats. Melatonin exerted a different impact on behaviour in the control group and in the group exposed to continuous light.

Limitations: The repeated measurements of behavioural manifestations were done with the aim to reflect the poten-

Figure 2. The effect of continuous light exposure (LL) on horizontal (locomotion) (A) and vertical (exploration) (B) activity, the effect of melatonin (LD+Mel) administration on horizontal (C) and vertical (D) activity, and the effect of continuous light exposure and melatonin (LL+Mel) administration on horizontal (E) and vertical (F) activity. LD, controls. * $p < 0.05$ vs. LD.
tial effect of prolonged lightening and melatonin treatment on behaviour. Although the repeated measurements were done in a relatively long time gap of one week, the effect of habituation is not possible to be excluded.

Melatonin’s sedative effects might be expected to influence not only locomotor and exploratory behaviour but even more the anxiety-like behaviour; thus elevated plus maze might have bring interesting data. However, this investigation was beyond the scope of this experiment.

Conflicts of interest. The authors declare that they have no conflict of interest.

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