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# Decreased emotional reactivity of rats exposed to repeated phase shifts of light–dark cycle

Monika Okuliarova *, Lubos Molcan, Michal Zeman

*Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic*

## HIGHLIGHTS

- Shifts of light–dark cycle decreased emotional reactivity of rats.
- Rats exposed to repeated shifts displayed increased locomotion and exploration.
- Natural aversion of rats to brightly lit areas was reduced under shifted regime.
- Exposure to phase shifts potentiated the sympathovagal reactivity in rats.

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## ABSTRACT

Disturbed light–dark (LD) cycles are associated with circadian disruption of physiological and behavioural rhythms and in turn with an increased risk of disease development. However, direct causal links and underlying mechanisms leading to negative health consequences still need to be revealed. In the present study, we exposed male Wistar rats to repeated phase shifts of LD cycle and analysed their ability to cope with mild emotional stressors. In experiment 1, rats were submitted to either a regular 12:12 LD cycle (CTRL rats) or 8-h phase delay shifts applied every 2 days for 5 weeks (SHIFT rats). Subsequently, the behaviour was examined in the open-field, black–white box and elevated plus maze tests. In experiment 2, changes in blood pressure (BP), heart rate (HR) as well as the activity of autonomic nervous system were measured in telemeterised rats in response to open-field and black–white box tests before and after 5-week exposure to shifted LD regime. Locomotor activity was consistently higher in SHIFT than CTRL rats in in the open-field and black–white box tests. Interestingly, in the elevated plus maze, SHIFT rats displayed increased risk assessment and decreased grooming compared to CTRL rats. Anxiety measures were affected only in the black–white box, where SHIFT rats displayed reduced anxiety-like behaviour compared to CTRL rats. Differences in behavioural reactivity between SHIFT and CTRL rats did not correspond with BP and HR changes. However, exposure to phase shifts increased the sympathovagal reactivity in the black–white box. Together, our results demonstrated that disturbed LD conditions decreased emotional reactivity of rats and affected their ability to cope with emotional stressors denoting an additional risk mechanism linking disrupted circadian organisation to adverse health effects.



## 1. Introduction

The daily light–dark (LD) cycle is the most important environmental signal that entrains endogenous circadian rhythms of physiological and behavioural processes to the exact 24-hour rhythm throughout all living organisms. The internal synchrony is coordinated by the master circadian pacemaker in the suprachiasmatic nuclei (SCN) of the hypothalamus and peripheral oscillators in other tissues and organs [1]. Altered timing of the external entraining cue results in misalignment between internal circadian clocks and external environment causing a disruption of phase relationships at different levels of circadian organisation [2,3]. This phenomenon has become closely related to the modern lifestyle and is increasingly encountered especially by rotating shift workers, night workers and frequent travellers across time zones [4]. Subsequently, impaired circadian organisation can lead to negative health consequences and contribute to a higher incidence of diseases in shift workers [5,6]. Epidemiological studies have indicated that shift work is associated with disturbed sleep–wake cycle [7], increased risk of cardiovascular diseases [8], several types of malignancies [9,10] and psychological disorders [11] although the significance of these links is still disputable and varies among studies.

To further understand causality and underlying mechanisms linking circadian disruption and shift work to adverse health effects, animal

* Corresponding author at: Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Ilkovičova 6, 842 15, Bratislava, Slovak Republic.
*E-mail addresses:* okuliarova@fns.uniba.sk (M. Okuliarova), molcan@fns.uniba.sk (L. Molcan), mzeman@fns.uniba.sk (M. Zeman).

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studies have been performed [12]. Frequently explored experimental models of circadian disruption, which are mainly focused on the SCN, employ exposure of rodents to constant light [13], altered length of LD period [14] or repeated shifts in LD cycle [2,15]. Disturbed circadian oscillations can induce causal pathways leading to an increased risk of disease development through physiological, psychosocial and behavioural mechanisms [16]. In rodents, physiological effects of altered light conditions are implicated in metabolic functions, especially in glucose metabolism [12]. Behavioural consequences of these experimental manipulations are primarily documented by changed rhythms of locomotor activity [17,18]. Moreover, exposure to constant light results in deficient cognitive functions [19], reduced anxiety-like responses and increased depressive-like behaviours [20]. Similarly, hamsters submitted to phase shifts of LD cycle displayed impaired cognitive skills [21]. In mice, fast rotating shifts of LD cycle produced long-term neurobehavioural consequences such as hyperlocomotion and anxiety-like behaviour in an open-field test [18].

On the other hand, some findings showed that altered timing of light exposure does not have to directly trigger pathological processes but it can increase susceptibility of organisms to challenging factors [22] or negative heath consequence may depend on genetic predisposition [17]. Likewise, in our study with rats, rotating shifts of LD cycle did not elevate absolute levels of blood pressure (BP) and heart rate (HR) [23] but the treatment changed sensitivity of the animals to sympathetic stimulation as shown by higher BP response after norepinephrine administration in comparison with controls [24]. Since effective coping with stress represents an adaptive way to restore homeostasis in healthy individuals, the excessively high response to challenge may in the long term potentiate adverse effects. Thus, an augmented stress response can represent a mechanism, which mediates effects of circadian desynchrony resulting from disturbed LD conditions on disease development.

The present study extends our previous results, which demonstrated that chronic phase advance and delay shifts disturb circadian system functioning based on changed temporal organisation of locomotor activity, cardiovascular parameters and clock gene expression [23,25]. In the current study, we analysed effects of repeated phase shifts of LD cycle on behaviour and the activity of autonomic nervous system (ANS) in response to different behavioural tests. We applied 8-h phase delay shifts every 2 days for 5 weeks to male Wistar rats and examined their emotional reactivity in the open-field, black–white box and elevated plus maze tests, which are considered to be mild stressors of novelty, light areas and open spaces, respectively. Besides behavioural measures of emotional reactivity, we evaluated stress-related changes of autonomic regulation of cardiovascular response by analysing HR and BP variability and baroreflex sensitivity.

## 2. Material and methods

### 2.1. Animals

Adult male Wistar rats were obtained from a breeding station at the Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences (Dobra Voda, Slovak Republic) at the age of 8–10 weeks. Rats were housed in groups of four animals in plastic cages at an ambient temperature of 21 ± 2 °C and humidity of 55 ± 10%. Food and water were provided *ad libitum*. Rats were kept under a LD cycle of 12L:12D (lights on 06:00; light intensity 150 lx) for more than 3 weeks before the experimental procedure 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.

### 2.2. Experiment 1

Sixteen rats (256 ± 5 g at the beginning of the experiment) were randomly assigned to one of two groups. The first group (SHIFT rats) was exposed to phase delay shifted schedule of their LD cycle with 8 h longer dark phase every 2 days over 5 weeks (Fig. 1). The second group (CTRL rats) was exposed to a regular LD cycle of 12L:12D over the same 5-week period. During week 5, behaviour of rats from both groups was quantified in the open-field, black–white box and elevated plus maze tests.

### 2.3. Experiment 2

Five rats (305 ± 5 g at the beginning of the experiment) were surgically implanted with a radiotelemetry transmitter TA11PA-C40 (Data Science International, St. Paul, Minnesota, USA) into the abdominal aorta just above its bifurcation [23,26]. The catheter was stabilised to the aorta with tissue glue (DSI, USA) and secured with a cellulose patch (DSI, USA). Rats were anaesthetised with an intraperitoneal injection of a mixture of ketamine (75 mg/kg, Calypsol, Gedeon Richter, Hungary) and xylazine (10 mg/kg, Rometar 2%, Bioveta, Czech Republic). Following the surgery, animals were housed individually in their home cages under a regular LD cycle and they were allowed to recover for 2 weeks before being included in the experiment. Cardiovascular response of rats to open-field and black–white box tests was analysed under the control LD conditions (CTRL regime) and then after 5 weeks of exposure to the shifted light conditions as described above (SHIFT regime).

### 2.4. Behavioural tests

All tests were performed in the second half of the light phase and each rat was tested only in one test per day in following order: open-field test, black–white box test and elevated plus maze test (Fig. 1). SHIFT rats were always tested during the second light period after a prolonged dark phase. We included a representative actogram of heart rate in rats exposed to 8-h delay shifts of LD cycle for 4 weeks (Fig. 2). Light intensity in the test room was the same as in the animal's keeping rooms. All behaviours were recorded by a video camera and quantified using video-tracking software Any-maze (Stoelting Co., USA).

In the open-field test, measures of motor activity, exploration and anxiety-like behaviour were analysed. Open-field test was performed in a wooden box consisting of a black square floor (50 × 50 cm) enclosed with white-painted side walls (35 cm high). Each tested rat was placed in the centre of open-field arena and its activity was recorded for 20 min. A peripheral and a central zone (25 × 25 cm) were virtually defined by the software. Total distance travelled, percentage of distance travelled in a central zone, time spent freezing, time spent rearing and grooming were measured. Defecation was recorded at the end of the test as the number of faecal boli. The arena was cleaned before each test with water. During the test, one SHIFT rat jumped out of the open-filed box and was excluded from the analysis.

The black–white box was used to test anxiety-like behaviour on the basis of the rat's natural preference for dark spaces. The apparatus of black–white box was made of wood and divided into two compartments. The bigger one (30 × 30 cm) was covered with white self-sealing paper and the smaller one (30 × 20 cm) was covered with black self-sealing paper. Ambient illumination was 800 lx and 80 lx inside the white and the black compartments, respectively. The two compartments were connected by a small opening of 7 × 7 cm in the cross wall. Each tested rat was placed in the centre of the white compartment facing the opening and its behaviour was recorded for 5 min. Videotapes were scored for the initial latency to escape from the white compartment, the latency to re-enter white compartment, time spent in white compartment, the number of transitions between compartments and time spent rearing and grooming. Defecation was recorded at the end of the test as the number of faecal boli.

In the elevated plus maze, the rat's natural fear of open spaces was used to measure anxiety-like behaviour as its preference for the closed arms. The wooden-made apparatus of elevated plus maze consisted of two open arms (50 × 10) that were perpendicular to two closed arms (50 × 10 × 40 cm) and intersected by a central platform (10 × 10 cm). The whole apparatus