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Original Article

Day-Time Isoflurane Administration Suppresses Circadian Gene Expressions in Both the Brain and a Peripheral Organ, Liver

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Abstract

Objective: The aim of this study is to investigate the effects of light and administration time of isoflurane on circadian gene expression in the brains and liver tissues of rats kept in light-dark cycle.

Methods: Seventy two 15-days-old rats pups were divided into four groups. All animals were exposed to 1.5% concentration of isoflurane or to 6 L min^{-1} O_2 for six hours between Zeitgeber Time (ZT) 0-ZT06 (day-time administration) or ZT12-ZT18 (night-time administration). Rats were sacrificed after six hours of anaesthesia with four-hour time intervals. Total RNA was isolated from brains and liver tissues. Circadian gene expression was examined using quantitative real-time Reverse transcription polymerase chain reaction (RT-PCR).

Results: BMAL1, CLOCK, PER2 and CRY2 gene expression levels were markedly suppressed after day-time anaesthesia in the both brain and liver, but night-time administration caused only temporary suppression of gene expression.

Conclusion: The effect of isoflurane on the circadian clock is time-dependent, and administered isoflurane anaesthesia at night had minimal effect on clock gene expression. Additionally, when the treated animals were kept in a regular light-dark cycle, isoflurane-induced phase shift was not observed, possibly because of the light.

Keywords: Anaesthesia, circadian rhythm, clock genes, isoflurane

Introduction

In mammals, the circadian clock influences nearly all aspects of physiology and behaviour, including sleep-wake cycles, cardiovascular activity, the endocrine system, body temperature, renal activity, physiology of the gastrointestinal tract and hepatic metabolism (1, 2). In mammals, the suprachiasmatic nucleus (SCN), named as central pacemaker, receives signals from the environment and coordinates the oscillating activity of peripheral clocks. The most dominant synchronizer is sunlight. The activation of melanopsin receptors by the light induces proteins like CLOCK, BMAL1, REV-ERB and PERIOD1. Peripheral tissues also have a circadian rhythm like brain. Circadian rhythm is affected by various factors including daylight, temperature, feeding and social interactions.

Abolished circadian rhythm is common in intensive care unit (ICU) patient, who treated with sedatives. Following general anaesthesia, people are often confused about the time of day and experience sleep disruption and fatigue. Although the reasons for sleep deprivation appear to be multi-focal, these symptoms may be caused by general anaesthesia affecting the circadian clock. It is thought that restoration of the circadian clock after anaesthesia can decrease the risk insomnia, confusion or post-operative delirium (3). The time-dependent effects of anaesthesia on the circadian clocks of various animals have been described before. Several studies indicated that general anaesthesia regulates the expression of many genes in the brain and other organs such as blood, heart and lung (4-6). Kobayashi et al. (7) showed that the expression of several circadian genes including PER2 (period homolog 2) was suppressed during inhalation of sevoflurane for two hours and six hours in rat whole brain. Yoshida et al. (8) found that propofol administration significantly changes the expression of circadian genes in rat whole brain, and the influence of intravenous anaesthesia also persists for 24 hours

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after awakening from anaesthesia. Cheeseman et al. (9) investigated the effect of the time of day of general anaesthesia administration and circadian gene expression profile in the brain of a honeybee (*Apis mellifera*). They found that this effect is dependent on the time of day of administration, and night-time anaesthesia did not shift the clock. They concluded that general anaesthesia during the day caused a persistent and marked shift of the clock, effectively inducing 'jet lag' and causing impaired time perception. Challet et al. (10) reported that 30 min propofol administration causes a one-hour time shift in circadian rhythms (activity and temperature) in rats which were kept in a dark-dark (DD) cycle during the study period.

Here, we aimed to investigate the effects of light and administration time of isoflurane on circadian gene expression in the brain and a peripheral organ (the liver). In contrast to previously published reports, administered animals were kept in 12-12 light-dark (LD) cycle to speculate about the effect of the light. The absense of isoflurane induced phase shift in brain and liver tissues might be due to light exposure in regular LD cycle after anaesthesia.

Methods

Animals and anaesthesia administration

After approval from the ethics committee of Dokuz Eylul University (approval number: 77-2010), 72 15-days-old rat pups, grown and adapted to a 12-12 LD cycle, light is on at 7:00 a.m. (Zeitgeber time =0 [ZT0]) and light is off at 7:00 p.m. (Zeitgeber Time=12 [ZT12]), after births until the experiments, were included the study. We studied in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All efforts to reduce the number of animals and their suffering were made. All rat pups were kept with their mothers and fed ad libitum until the experiments. At the end of anaesthesia, when the pups recovered from anaesthesia, they were put in their mother's cage.

Rats were randomised into control and anaesthesia groups as follows.

Group I (day-time isoflurane-treated) (n=18): Rat pups were exposed to anaesthesia (1.5% concentration of isoflurane [Forane, Abbott Lab, Istanbul, Turkey] for six hours) between 7:00 a.m. and 1:00 p.m. (between ZT0 and ZT06).

Group II (untreated controls) (n=18): Rat pups were exposed to 6 L min^{-1} O_2 between 7:00 a.m. and 1:00 p.m. (between ZT0 and ZT06).

Group III (night-time isoflurane-treated) (n=18): Rat pups were exposed to anaesthesia (1.5% concentration of isoflurane for six hours) between 7:00 p.m. and 1:00 a.m. (between ZT12 and ZT18).

Group IV (untreated controls) (n=18): Rat pups were exposed to 6 L min^{-1} O_2 between 7:00 p.m. and 1:00 a.m. (between ZT12 and ZT18).

Anaesthesia apparatus, induction and maintenance

Separate glass jars for every single rat were used as an anaesthesia apparatus. The volume of each jar was 450 mL. Jars had gas-in and gas-out systems. Inspired oxygen and volatile agent concentrations were monitored (Anesthesia Gas Monitoring 1304, Brüel & Kjær Sound & Vibration Measurement A/S Nærum, Denmark) and kept constant. Respiratory effort and skin colour were also inspected during the study period. All jars were placed in a water bath with a constant temperature of 37°C.

Sample preparation and quantitative real-time RT-PCR

Rats were sacrificed after six hours of anaesthesia with four-hour time intervals (0, 4, 8, 12, 16, 20 and 24 hours after treatment), two rats from each group at each time interval, and the whole brain and liver were rapidly removed. The whole brain and liver were immediately frozen in liquid nitrogen. During the night hours, the lights were not on and all of the procedures were done in the dark with red light. It has been shown that red light does not alter the circadian rhythm of rats (11).

Total RNA was extracted by quickly homogenising each tissue using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The concentration of total RNA was measured by ND-2000 Nanodrop spectrophotometer (Thermo Fisher Scientific Wilmington, DE, USA), and RNA quality was confirmed by agarose gel electrophoresis. One microgram of each total RNA was reverse transcribed with hexanucleotide random primers. Quantitative real-time PCR was used to investigate the expression of CRY2, PER2, clock and BMAL1 genes using the previously published primers (12). The amplification of the glyceraldehyde-3-phosphate dehydrogenase (Gapdh) gene was used as an internal control. A previous report confirmed that Gapdh was a suitable endogenous control (8). All samples were run in triplicate. Student's t-test was used to compare gene expression levels in investigated time points. The anaesthesia-administrated tissue value was compared with untreated tissue in each time points.

Results

All rats completed the study. CRY2, PER2, BMAL1 and clock genes were investigated in rat brains and liver tissues. We have found that day-time administration of isoflurane caused statistically significant suppression of clock gene expression levels investigated time points in brain tissues ($p<0.05$). The circadian gene (CRY2, PER2, BMAL1 and clock genes) expression levels in the liver after day-time isoflurane administration at the same time points were also significantly suppressed ($p<0.05$). We also showed that night-time administration of isoflurane caused only temporary suppression of gene levels (Figures 1 and 2).

Additionally, we did not observe a phase shift in circadian gene expression after day- or night-time isoflurane anaesthesia.

Discussion

The effect of isoflurane administration on the circadian expression pattern of core clock genes (CRY2, PER2, BMAL1 and clock) was investigated in rat brains and liver tissues. Rats were treated with isoflurane between ZT0 and ZT6 (day-time administration) or between ZT12 and ZT18 (night-time administration). Rats were sacrificed after 6 hours of anaesthesia with four-hour time intervals. The relative levels of each gene were normalised to the corresponding Gapdh gene levels. We found that isoflurane administration during the day-time

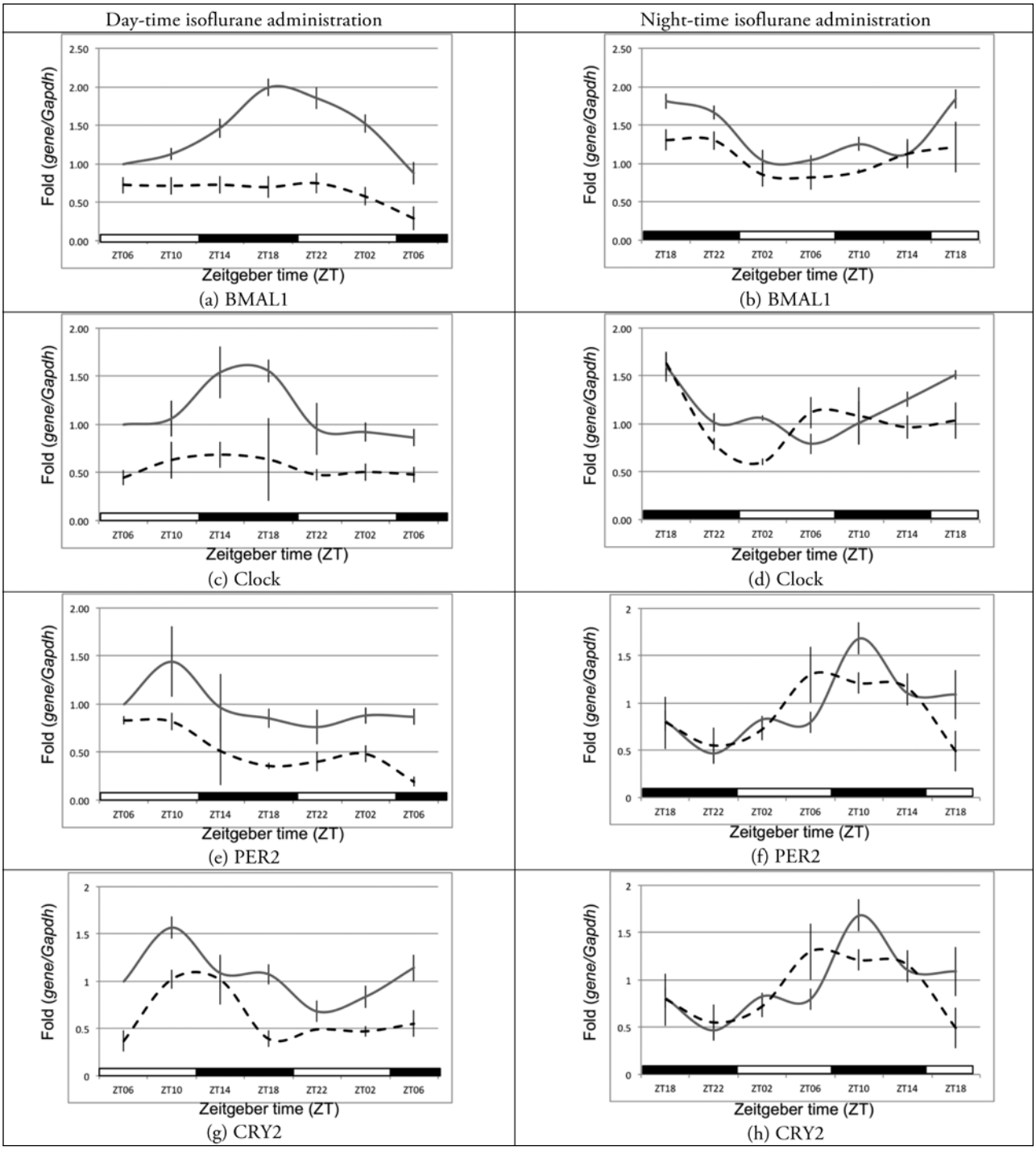

Figure 1. Effects of isoflurane on the expression of circadian genes in rat brains. Rats were sacrificed after 6 hours of anaesthesia with four-hour time intervals (0, 4, 8, 12, 16, 20 and 24 hours after treatment). Data are shown as mean ± standard deviation of triplicate samples. The relative levels of each gene were normalised to the corresponding Gapdh gene levels. The ZT6 and Z18 value of the untreated group was set to 1 in day-time-treated and night-time-treated groups, respectively

ZT: Zeitgeber time; ZT0: light on; ZT12: light off for rat under a 12-12 LD cycle; line: untreated groups; line: isoflurane-administered groups.

causes a significantly persistent and marked suppression expression of the circadian genes ($p<0.05$), whereas night-time administration caused only temporary suppression of gene expression in both brain and liver tissues. Isoflurane administration did not shift the circadian clock in investigated tissues of animals that were kept under the LD cycle.

Several studies investigated the effect of the administration of anaesthetics on circadian gene expression in mammalian organisms (mice and rats) (7, 13-15). All these studies investigated circadian gene expression 2, 6 or 24 hours after administration. They did not investigate the circadian gene expression pattern. They found that anaesthesia administra-

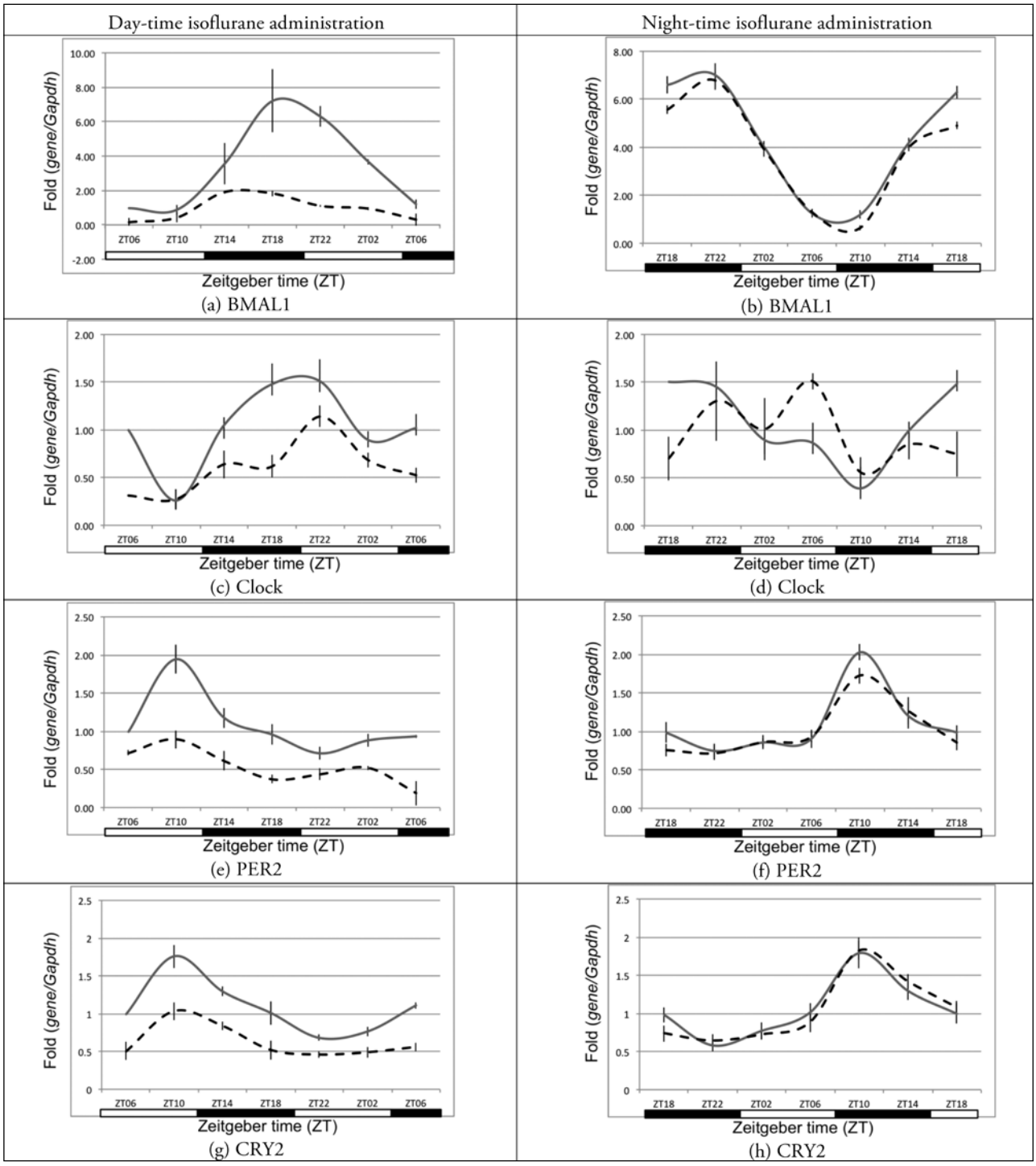

Figure 2. Effects of isoflurane on the expression of circadian genes in rat livers. Rats were sacrificed after six hours of anaesthesia with four-hour time intervals (0, 4, 8, 12, 16, 20 and 24 hours after treatment). Data are shown as mean ± standard deviation of triplicate samples. The relative levels of each gene were normalised to the corresponding Gapdh gene levels. The ZT6 and Z18 value of the untreated group was set to 1 in day-time-treated and night-time-treated groups, respectively

ZT: Zeitgeber time; ZT0: light on; ZT12: light off for rat under a 12-12 LD cycle; line: untreated groupsline: isoflurane-administered groups.

tion suppressed clock gene expression at investigated single time point. They did not comment about circadian gene expression pattern. The study performed by Cheeseman et al. (9) is the only study investigated circadian pattern of clock gene expression in isoflurane-administered honeybees and showed that the effect of isoflurane on the circadian clock is time-dependent and that isoflurane administration during night hours had minimal effect on circadian gene expression.

Unlike the above studies, the isoflurane-treated animals were kept in an LD cycle in our study. In contrast to Cheeseman et al. (9) and Challet et al. (10), isoflurane administration caused suppression of circadian gene expression but did not cause a shift in circadian pattern in investigated tissues. Cheeseman et al. (9) kept animals in a DD cycle after anaesthesia administration, and they found a phase shift in circadian gene expression. The different effect of anaesthesia on circadian gene expression in our study may be due to light in the LD cycle. Since the daily light exposure resets the phase of clock neurons in the suprachiasmatic nucleus (SCN), which, in turn, send multi-synaptic projections to other centres in the brain to synchronise the circadian clock throughout the body. Thus, the light may prevent the phase shift effect of anaesthesia administration in our study. Consistent with our study, a recent report found that when the six-hour isoflurane-administrated honey bees were kept in the light, behavioural analysis revealed that isoflurane-induced phase shift was disappeared with the light (16). Thus, light may provide a means of reducing isoflurane-induced phase shifts.

In this study, we also found that isoflurane anaesthesia can affect circadian gene expression in a peripheral organ consistent with brain. In contrast with our findings, Anzai et al. (17) failed to show the effect of sevoflurane on gene expression in peripheral organs, although they reported suppression in PER2 expression in SCN. The difference between these two studies may be arise from the difference in volatile agents or peripheral organs. To our knowledge, this is the first study investigating the effects of anaesthesia on circadian gene expression in a peripheral organ.

One of the limitations of this study is that we did not control the melatonin levels of the rats. One of the most remarkable effects of light is the melatonin production. In many studies, melatonin is used to monitor the light-induced circadian rhythm. However, in our previous study, we investigated the effect of isoflurane anaesthesia on melatonin levels and found that isoflurane administration increased plasma melatonin levels significantly in the day-time but did not affect at night (18, 19).

Human studies show alterations in melatonin levels after general anaesthesia and surgery (20-22). All of these studies discuss the effects of anaesthesia or pain related to surgery as the reason for sleep disorders or delirium. Disruption of circadian rhythms with administration of anaesthesia and surgery can result in post-operative sleep and cognitive disorders. Restoration of circadian rhythms after anaesthesia is critically important to prevent post-operative delirium, which is associated with disturbed circadian rhythms, which increases post-operative morbidity and mortality (3).

In this experimental study, we excluded the possible effects of surgery so that we could show the sole effect of isoflurane anaesthesia on circadian rhythms. We believe that the findings of the present study give some clues about the effect of the anaesthesia treatment in the patients since all patients are exposed to daily light in their rooms after general anaesthesia. It may be possible to alleviate the observed abolished circadian rhythm in the ICU by administering light during anaesthesia.

Conclusion

In this study, we have found that isoflurane administration did not shift the circadian clock in investigated tissues of animals that were kept under the LD cycle. Our findings showed that (1) inhaled isoflurane administration suppressed circadian gene expression in both the brain and liver, (2) administration time showed different suppression effect on the gene expression, (3) and isoflurane administration did not cause a shift in circadian gene expression in investigated tissues might be due to light exposure in regular LD cycle after anaesthesia.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Dokuz Eylül University School of Medicine (No: 77-2010).

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Conflict of Interest: No conflict of interest was declared by the authors.

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