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RESEARCH ARTICLE

Influences of Desflurane on the Central and Hepatic Circadian Clock Persist at 24 Hours Following Anaesthesia in Rats

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Abstract

Circadian rhythm disturbances caused by anaesthesia may be one of the important reasons of many disturbances following surgery. The effects of 6% desflurane on suprachiasmatic nucleus (SCN), liver, and pancreas PER2, CRY1, BMAL, and CLOCK circadian protein levels and serum concentrations of glucose, melatonin, cortisol 24 h following the administration of anaesthesia were determined in this study. 10–12-week-old Wistar albino male rats (n=12) were divided into two groups. Control group (C) was not exposed to desflurane, while the anaesthesia group (D) received desflurane at 6% concentration for 6 h between 07.00 and 13.00 a.m. and was euthanized 24 h after anaesthesia. Tissue samples were collected from both groups at the same time of the day (07.00 a.m.). Serum glucose, melatonin, cortisol and SCN, liver, pancreas CLOCK, BMAL1, PER2, CRY1 levels were determined using commercial kits. CLOCK and PER2 protein levels in SCN and BMAL1 protein concentration in liver decreased, while liver PER2 protein level increased at the 24th h following anaesthesia. Pancreas BMAL1, CLOCK, PER2, CRY1 protein levels were not affected by the anaesthesia protocol applied. No statistically significant alteration was observed in plasma glucose, cortisol and melatonin concentrations. Our findings show that, the circadian clock rhythm in SCN and liver are still affected at the 24th h following exposure to desflurane.

Keywords: Circadian rhythm, Clock proteins, Desflurane, Liver, SCN

Introduction

Determining how anaesthesia affects the circadian rhythm is not only of scientific interest but also of potential clinical importance. Patients often experience sleep disturbances after surgery. Disruption of circadian rhythm and related sleep disorders cause adverse effects on inflammation and immune function, as well as altered mood, behaviour and cognitive dysfunction [1-4]. For these reasons, it is clinically important to elucidate the mechanism of postoperative circadian rhythm disorders.

Twenty-four h physiological processes are controlled by the circadian rhythm through a transcriptional feedback loop of clock genes and proteins [5,6]. The circadian clock is made up of four basic components at the molecular level. In mammals, these are the genes brain and muscle ARNT-like 1 *(Bmal1),* circadian locomotor exit hood *(Clock),* Period 2 *(Per2),* and Cryptochrome 1 *(Cry1)* [7]. While CLOCK and BMAL proteins were demonstrated to promote the transcription of *Per2* and *Cry1*, PER2 and CRY1 proteins inhibit BMAL1:CLOCK transcriptional activity, thus limit their own transcription [8,9]. The protein-mediated disruptions in the circadian rhythm caused by anaesthesia may hinder post-operative recovery [10].

Neurons associated with molecular clocks are located in the ventral region of the suprachiasmatic nucleus (SCN). When photoreceptors of the eye detect light, NMDA receptors in SCN are activated by the retinohypothalamic pathway, resulting in transcription of *Per2* [8,11]. While the "master" of the circadian rhythm is the SCN, peripheral clocks in organs govern processes specific to that tissue in a particular tissue or cell type [12]. For example, the liver circadian clock regulates fasting glycaemic control and glucose clearance, while the pancreatic circadian clock regulates insulin secretion and glucose homeostasis [13].

Sevoflurane, isoflurane and desflurane are the most commonly used inhalation anaesthetics in clinical practice.

Circadian rhythm is affected after general anaesthesia with sevoflurane and isoflurane [14]. However, there is still a lack of information in the literature regarding the effects of desflurane anaesthesia on the circadian clock. Desflurane, one of the third generation inhaled anaesthetic drugs, is frequently preferred in clinical use for providing safe and effective anaesthesia [15] . Desflurane has a significantly lower blood solubility compared to other inhaler agents [8]. With low tissue solubility, there is an acceleration of induction and elimination and more precise control of alveolar desflurane concentration in anaesthesia maintenance. These properties contribute to the rapid onset of desflurane anaesthesia. Anaesthesiologists administer desflurane as a general anaesthetic to wake patients quickly after surgery. Based on these properties, desflurane may have a different effect on the circadian rhythm compared to other inhaled anaesthetics [16]. Human studies have suggested that there may be two separate toxic problems during the metabolism of desflurane. The first one is high current renal failure as a result of biotransformation to free fluoride ion; the second is that it can bind to hepatic tissue macromolecules and cause hepatotoxicity. Desflurane may also induce hepatic microsomal enzymes [16].

Melatonin has important physiological functions such as regulation of the circadian rhythm and the reproductive axis [17]. Melatonin secretion is controlled by the SCN, which regulates the day/night cycle. The administration of anaesthesia regardless of surgery may affect the circadian rhythm of melatonin [18]. Another hormone affected by anaesthesia is the cortisol. When melatonin receptors in the adrenal gland are stimulated with physiological doses, ACTH-mediated cortisol formation is suppressed [19]. The balance between these two hormones is important for health.

In the light of above information, we aimed to investigate SCN, liver and pancreas PER2, CRY1, BMAL and CLOCK protein levels and serum concentrations of glucose, melatonin, cortisol 24 h following the administration of 6% desflurane in 6 L min-1 100% oxygen to rats for 6 h. It is anticipated that the results of the current study may provide important information about how long the effects of desflurane, a widely used anaesthetic in the clinic, on the circadian rhythm and hormonal balance may last.

Material and Methods

Ethical Approval

All experimental procedures were conducted in accordance with institutional animal care guidelines of Pamukkale University and approved by the local Animal Experiments Ethics Committee (PAUHADYEK-2021/33, 24.08.2021-07).

Animals

200-250 g, 10-12-week-old Wistar albino male rats (n=12, Pamukkale University Experimental Animal Unit) were used. The animals were housed in a temperature and humidity controlled (22-23°C, 50±5%) room under a 12 h light-dark cycle. Standard diet food and water were available ad libitum.

Study Design and Anaesthesia Protocol

Rats were divided into two groups (n=6 per group). For adaptation, the animals were placed in the anaesthetic chamber approximately 1 h, for 4 days before beginning the experiments. Control group (C) was not exposed to desflurane but was placed in the anaesthetic chamber, then transferred back to the cages and was euthanized at 07.00 a.m. The anaesthesia exposure group (D) received desflurane at 6% concentration in 6 L min-1% 100% oxygen for 6 h in a transparent anaesthesia box between 07.00-13.00 a.m. and was euthanized 24 h after anaesthesia at 07.00 a.m. Tissue samples were collected from both groups at the same time of the day (07.00 a.m.) in order to avoid the original circadian variation in clock gene expressions. After anaesthesia, the fresh gas flow was decreased to 1 L/min, then rats were transferred to their cages and kept at normal conditions (bright in daytime, dark at night) of the experimental animals unit of the university. In order to maintain their normal body temperature, the chambers housing the mice were placed on a heat sheet. The anaesthesia procedures were performed under dark conditions, and under a dim red light that did not affect the circadian rhythm.

Blood Sample and SCN, Liver and Pancreas Collection

After the experiment, rats were euthanized with an overdose of sodium pentobarbital. SCN, liver and pancreas samples were collected without anaesthesia (C group) and 24 h after exposure to desflurane (D group). The blood samples obtained from the tail vein of all rats at 07.00 a.m. were centrifuged on the same day (1100×g, 20 min). SCN, liver and pancreas tissues were minced and homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) using a glass homogenizer on ice. The homogenates were then centrifuged for 5 min at 5000×g to get the supernatant. Serum, SCN, liver and pancreas samples were stored at -80°C until experimental analysis.

Determining Glucose, Melatonin, Cortisol, CLOCK, BMAL1, PER2 and CRY1 Levels

Serum glucose (Cat.No E1623Ra, BT Lab), melatonin (Cat.No E0601Ra, BT Lab), cortisol (Cat.No E0828Ra, BT Lab) and SCN, liver, pancreas CLOCK (Cat.No E2920Ra, BT Lab), BMAL1 (Cat.No E2919Ra, BT Lab), PER2 (Cat. No E2917Ra, BT Lab), CRY1 (Cat.No E2918Ra, BT Lab), levels were determined using commercial ELISA kits.

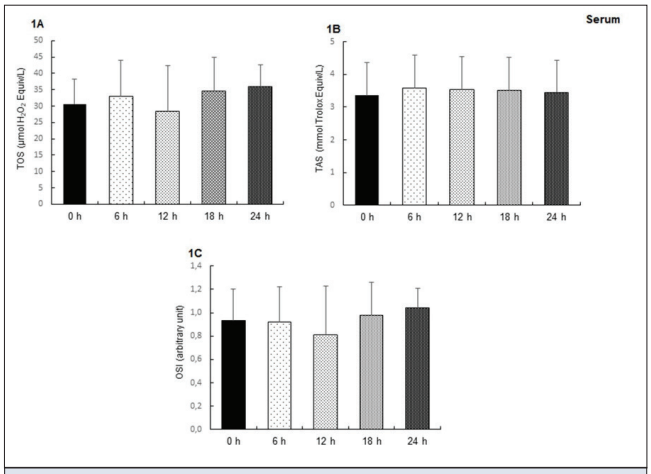

Fig. 1. Serum glucose (A), cortisol (B) and melatonin (C) levels of the groups. C: Control, D: Desfluran group. Values are expressed as mean ± SD. :P<0.05, difference from C

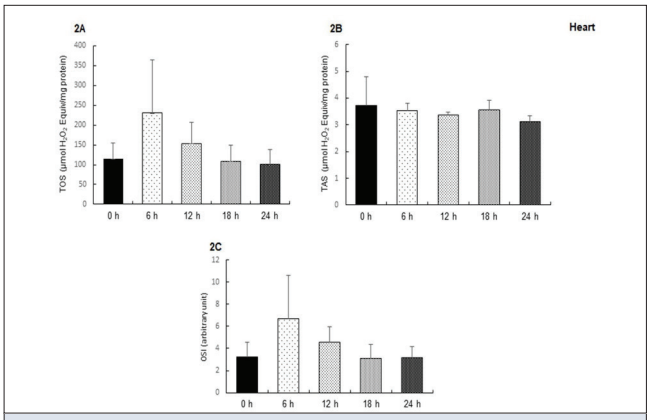

Fig. 2. SCN BMAL1 (A), CLOCK (B), PER2 (C), CRY1 (D) protein levels of groups. C: Control, D: Desfluran group, SCN: Suprachiasmatic nucleus, BMAL1: brain and muscle ARNT-like 1, CLOCK: Circadian Locomoter Output Cycles Protein Kaput, PER2: PERIOD-2, CRY1: Cryptochrome-1. Values are expressed as mean ± SD. :P<0.05, difference from C

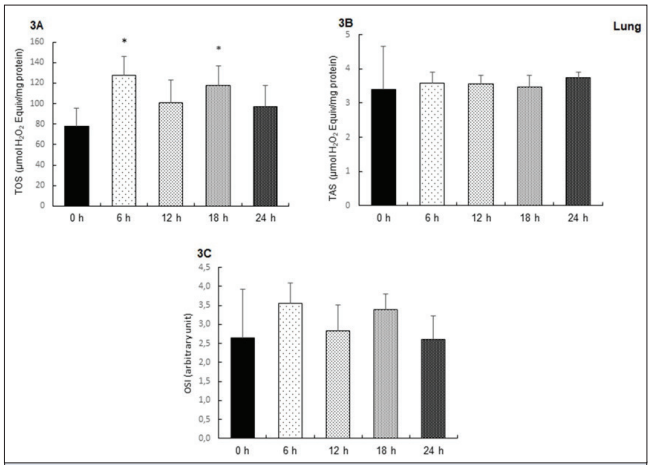

Fig. 3. Liver BMAL1 (A), CLOCK (B), PER2 (C), CRY1 (D) protein levels of groups. C: Control, D: Desfluran group, BMAL1: brain and muscle ARNT-like 1, CLOCK: Circadian Locomoter Output Cycles Protein Kaput, PER2: PERIOD-2, CRY1: Cryptochrome-1. Values are expressed as mean ± SD. :P<0.05, difference from C

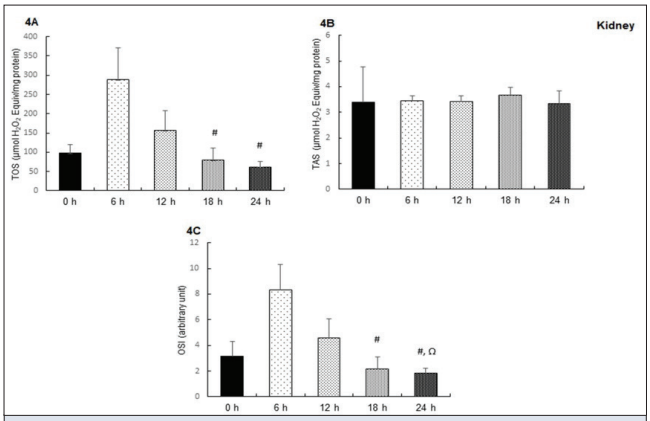

Fig. 4. Pancreas BMAL1 (A), CLOCK (B), PER2 (C), CRY1 (D) protein levels of groups. C: Control, D: Desfluran group, BMAL1: brain and muscle ARNT-like 1, CLOCK: Circadian Locomoter Output Cycles Protein Kaput, PER2: PERIOD-2, CRY1: Cryptochrome-1. Values are expressed as mean ± SD

Statistical Analysis

As a result of the power analysis we performed, assuming that we could obtain a lower power, it was calculated that a power of 80% at a confidence level of 95% could be obtained if at least 12 rats (at least 6 rats for each group) were included in the study and the effect size was d=0.69. All calculations and power analysis were performed by the G-power program (version 3.1.9.2. Heinrich-Heine-Universitat. Duesseldorf. Germany). All statistical analyses were performed using SPSS 25.0 (IBM SPSS Statistics 25 software (Armonk, NY: IBM Corp.). Continuous variables were defined by the mean ± standard deviation. Shapiro Wilk tests were used for determination of normal distribution. For nonparametric test assumptions were provided, Kruskal Wallis Variance Analysis (post hoc: Mann Whitney U test with Bonferroni Correction) were used when parametric test assumptions were not provided. The level of statistical significance was set at P≤0.05.

Results

Fig. 1 demonstrates that serum glucose, cortisol and melatonin levels were not altered significantly by the desflurane applied herein. Anaesthesia induced statistically significant decrements in SCN CLOCK, PER *(Fig. 2)* and liver BMAL1 protein levels, whereas it caused increment in liver PER protein concentration *(Fig. 3)* 24 h after the application compared to control (P<0.05). On the other hand, no statistically significant alteration was determined in pancreas BMAL1, CLOCK, PER2, CRY1 protein levels following desflurane anaesthesia *(Fig. 4)*.

Discussion

Desflurane is one of the most commonly used modern inhaled anaesthetic agents [20]. It provides safer and effective

anaesthesia in clinical use. Since uptake, distribution and elimination of desflurane are faster than similar drugs, it allows rapid changes in the depth of anaesthesia. Its solubility in blood and tissue is lower than the other halogenated anaesthetics [21]. We have administered desflurane at a concentration of 6% in accordance with the dose commonly used in surgeries at our hospital. Desflurane has the least *in vivo* metabolism among inhaled halogenated anaesthetics. However, all halogenated inhaled anaesthetics such as sevoflurane, desflurane and isoflurane may cause metabolic hepatocellular damage. Very serious but few cases of post-anaesthesia hepatic and renal injury have been reported [21]. In the present study, the protein levels which play role in the circadian rhythm (BMAL1, CLOCK, PER2, CRY1) at 07.00 a.m. in the non-anaesthetized group were compared with the same protein levels at 07.00 a.m., 24 h after 6% desflurane anaesthesia. Our results demonstrated that CLOCK and PER2 protein levels in SCN as well as BMAL1 protein concentration in liver decreased, while liver PER2 protein level increased at the 24th h following anaesthesia compared to control group. Pancreas BMAL1, CLOCK, PER2, CRY1 protein levels were not affected by the anaesthesia protocol applied. Similarly, no statistically significant alteration was observed in plasma glucose, cortisol and melatonin concentrations 24 h after desflurane anaesthesia.

The relationship between melatonin and cortisol is especially important in terms of the effects of both hormones on the immune system. General anaesthesia is a sleep-wake state thought to alter circadian rhythms. Different studies have shown conflicting effects of surgery and anaesthesia on melatonin levels which is controlled by the SCN [10,22]. Ozer et al.[23] demonstrated that, 5.7% desflurane administration for six hours during the day and at night does not alter plasma melatonin levels in blood samples collected immediately after anaesthesia in 15 day old rats. Here, we have demonstrated for the first time that, plasma melatonin levels were not changed 24 h after 6% desflurane inhalation. The fact that samples were collected from both groups at the same time of the day (07.00 a.m.) in order to avoid the original circadian variation may have played role in these results. Unfortunately, we could not determine possible time-dependent alterations in melatonin, cortisol and glucose levels in response to desflurane in the current study. Changes in these parameters might have occurred during the 24-hour period, but these alterations might have disappeared when we sampled blood at the 24th hour. Supporting this suggestion, in humans, following 1.5 h of anaesthesia, the elevated cortisol level was reduced to baseline after 24 h [24]. Unchanged plasma cortisol and glucose levels 24 h after exposure to desflurane in healthy rats without any surgical procedure may indicate that, the stress level of our rats was low. Similar to our results, Dikmen et al.[25] stated that, both desflurane and sevoflurane can be used safely during acute hyperglycemic state, because of their non-increasing blood glucose influences.

The presence of common clock and entrainment mechanisms for nocturnal and diurnal species was asserted [26]. The circadian rhythm involves the central clock in the SCN and peripheral clocks in other tissues which regulate local tissue-specific physiological functions [27]. Light entrains the SCN through retinohypothalamic pathway; whereas other stimuli as nutrition, temperature, stress reset the peripheral clocks, and entrainment depends on the timing of stimulation [28]. The core clock system, which co-ordinates the activity of peripheral clocks is made up of a feedback loop whereby BMAL1 and CLOCK induce the expression of *Per2* and *Cry1* genes. Later, the PER-CRY heterodimer is known to repress the transcription of *Clock* and *Bmal1* with negative feedback mechanism [29]. An additional loop consists the ROR and Rev-Erb factors, also regulated by BMAL1-CLOCK complex [30].

Volatile anaesthetics exert their specific actions at the molecular level, with proteins rather than lipids [31]. Imai et al.[5] demonstrated that, 4% desflurane applied for 6 h induces a phase shift in the circadian rhythm being largest in ZT6-12. *Bmal* and *Cry1* expressions were elevated, whereas *Clock* expression was decreased in ZT12 in SCN. *Per2* expression was also increased from ZT2 to ZT8 and decreased later on. These authors collected their last SCN sample at ZT20 following anaesthesia and examined expressions of circadian rhythm genes. The reflection of gene expression alterations on protein levels may take several hours [26]. The innovation our data brings to the literature is that, CLOCK protein level still remains lower in SCN at the 24th h following 6% desflurane anaesthesia in rats. Additionally in line with the results of the above mentioned study, PER2 protein is also decreased in SCN at that time thereby causing a phase shift in the circadian rhythm of mice. This issue should be taken into consideration while using desflurane anaesthesia.

Most tissues of the body exhibit circadian oscillations through mechanisms involving both core clock genes common to all and numerous other tissue-specific genes that cooperate with each other and many circadian epigenetic modifiers are known to function in a tissue-specific manner [32]. We also investigated the effects of 6% desflurane on liver and pancreas circadian clock proteins and have shown for the first time that, while desflurane did not affect pancreas levels of these proteins, liver BMAL1 protein is supressed and PER2 is elevated 24 h after anaesthesia. This finding should be kept in mind when dealing with patients with insulin resistance since it was demonstrated that liver-specific deletion of BMAL1 is known to result in a blunted sensitivity

to insulin [13]. DNA microarray study revealed that, approximately 10% of the sampled liver genome is under circadian control [33]. The regulated genes include the ones encoding cytoskeletal elements and enzymes involved in carbohydrate metabolism emphasizing the importance of liver circadian rhythm on feeding and digestion. Moreover it was demonstrated that, in the case of *mPer2* and *mBmal1*, the anti-phasic relationship observed in the SCN is retained in the liver, with a slightly delayed timing for both clusters [26]. Our resuts lead to the interpretation that following desflurane exposure, elevated liver PER2 protein levels may have caused inhibition of BMAL1 by negative feedback mechanism.

Halogenated ethers may have hepatic and renal side effects via transformation to toxic metabolites [34]. Rare instances of acute liver injury have been reported with all halogenated agents. Cytochrome P450 CYP2El seems to be the specific P450 isoform largely responsible for the defluorination of isoflurane. Although desflurane metabolism is approximately 10% of isoflurane, it is suggested that desflurane may also undergo metabolism by a route similar to that of isoflurane [35]. However, we have observed that liver CRY1 protein levels were not altered at 24th h following desflurane exposure. When all circadian protein findings of the current study are evaluated together, it appears that 6% desflurane administration affects the levels of these proteins not only in the SCN but also in the liver for at least 24 h.

The present study may be considered as a preliminary one giving clues about the prevention and treatment of postoperative complications related to circadian rhythms following desflurane inhalation. Our results demonstrate that, the circadian clock rhythm is affected in SCN and liver for at least 24 h following desflurane anaesthesia. Examination of the effects of desflurane only at the 24th h, the fact that the intraday influences and later effects were not studied constitute the most important limitations of this study. Another limitation is that although the chambers housing the mice were placed on a heat sheet in order to maintain their normal body temperature, the body temperatures of the rats were not notified during the experimental procedure. When the influences of desflurane on circadian protein levels disappears and the time-dependent effects of this anaesthetic on other peripheral clocks as well as the mechanisms of these influences have not been clarified yet. Investigation of the circadian clock genes/proteins in response to desflurane anaesthesia in a variety of clinical situations is also necessary in order to clarify whether desflurane offers significant advantages over the other anaesthetic drugs.

Declarations

Availability of Data and Materials: The authors declare that data supporting the study findings are also available from the corresponding author (I. H. A.) on reasonable request.

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Conflict of Interest: The authors declare that there is no conflict of interest in publishing this article.

Author Contributions: I.H.A., O.K.E.: Conceptualization, methodology, data curation, investigation, resources, project administration; M.B.K.: Methodology, writing, review, and editing. All authors read and approved the final manuscript.

References

1. Logan RW, McClung CA: Rhythms of life: Circadian disruption and brain disorders across the lifespan. *Nat Rev Neurosci*, 20 (1): 49-65, 2019. DOI: 10.1038/s41583-018-0088-y

2. Teichman EM, O'riordan KJ, Gahan CG, Dinan TG, Cryan JF: When rhythms meet the blues: Circadian ınteractions with the microbiota-gut-brain axis. *Cell Metab*, 31 (3): 448-471, 2020. DOI: 10.1016/j.cmet.2020.02.008

3. Walker WH, Walton JC, DeVries AC, Nelson RJ: Circadian rhythm disruption and mental health. *Transl Psychiatry,* 10 (1):28, 2020. DOI: 10.1038/s41398-020-0694-0

4. Zou H, Zhou H, Yan R, Yao Z, Lu Q: Chronotype, circadian rhythm, and psychiatric disorders: Recent evidence and potential mechanisms. *Front Neurosci*, 16:811771, 2022. DOI: 10.3389/fnins.2022.811771

5. Imai R, Makino H, Katoh T, Kimura T, Kurita T: Desflurane anaesthesia shifts the circadian rhythm phase depending on the time of day of anaesthesia. *Sci Rep,* 10 (1):18273, 2020. DOI: 10.1038/s41598-020-75434-6

6. Wang D, Huang Y, Wang X, Chen X, Li J, Zhang S, Wu J, Liu D, Ma D, Mei W: Circadian differences in emergence from volatile anaesthesia in mice: Involvement of the locus coeruleus noradrenergic system. *Br J Anaesth*, 125 (4): 548-559, 2020. DOI: 10.1016/j.bja.2020.07.012

7. Zheng B, Albrecht U, Kaasik K, Sage M, Lu W: Nonredundant roles of the mPer1 and mPer2 genes in the mammalian circadian clock. *Cell*, 105 (5): 683-694, 2001. DOI: 10.1016/s0092-8674(01)00380-4

8. Poulsen RC, Warman GR, Sleigh J, Ludin NM, Cheeseman JF: How does general anaesthesia affect the circadian clock? *Sleep Med Rev*, 37, 35-44, 2018. DOI: 10.1016/j.smrv.2016.12.002

9. Voss LJ, García PS, Hentschke H, Banks MI: Understanding the effects of general anesthetics on cortical network activity using *ex vivo* preparations. *Anesthesiology*, 130 (6): 1049-1063, 2019. DOI: 10.1097/ALN.0000000000002554

10. Cheeseman JF, Winnebeck EC, Millar CD, Kirkland LS, Sleigh J: General anaesthesia alters time perception by phase shifting the circadian clock. *Proc Natl Acad Sci U S A*, 109 (18): 7061-7066, 2012. DOI: 10.1073/pnas.1201734109

11. Tischkau SA, Mitchell JW, Tyan SH, Buchanan GF, Gillette MU: Ca^{2+}/cAMP response element-binding protein (CREB)-dependent activation of *Per1* is required for light-induced signaling in the suprachiasmatic nucleus circadian clock. *J Biol Chem*, 278 (2): 718-723, 2003. DOI: 10.1074/jbc.M209241200

12. Shostak A, Meyer-Kovac J, Oster H: Circadian regulation of lipid mobilization in white adipose tissues. *Diabetes,* 62 (7): 2195-2203, 2013. DOI: 10.2337/db12-1449

13. Lamia KA, Storch KF, Weitz CJ: Physiological significance of a peripheral tissue circadian clock. *Proc Natl Acad Sci U S A,* 105 (39): 15172-15177, 2008. DOI: 10.1073/pnas.0806717105

14. Ocmen E, Erdost HA, Duru LS, Akan P, Cimrin D: Effect of day/night administration of three different inhalational anesthetics on melatonin levels in rats. *Kaohsiung J Med Sci,* 32 (6): 302-305, 2016. DOI: 10.1016/j.kjms.2016.04.016

15. Akbudak IH, Kilic Erkek O, Bor Kucukatay M: Desflurane 6% ınhalation ınhibits erythrocyte deformability and alters oxidative stress in rat lung and kidney in a time-dependent manner. *Kafkas Univ Vet Fak Derg*, 29 (3): 273-279, 2023. DOI: 10.9775/kvfd.2023.29165

16. Brown BR and Frink EJ: Biodegradation and organ toxicity of new volatile anesthetics. *Curr Opin Anesthesiol,* 6 (4): 644-647, 1993.

17. Dispersyn G, Touitou Y, Coste O, Jouffroy L, Lleu JC: Desynchronization of daily rest-activity rhythm in the days following light propofol anaesthesia for colonoscopy. *Clin Pharmacol Ther,* 85 (1): 51-55, 2009. DOI: 10.1038/clpt.2008.179

18. Mihara T, Kikuchi T, Kamiya Y, Koga M, Uchimoto K: Day or night administration of ketamine and pentobarbital differentially affect circadian rhythms of pineal melatonin secretion and locomotor activity in rats. *Anesth Analg*, 115 (4): 805-813, 2012. DOI: 10.1213/ANE.0b013e3182632bcb

19. Kim MH, Lee KY, Bae SJ, Jo M, Cho JS: Intraoperative dexmedetomidine attenuates stress responses in patients undergoing major spine surgery. *Minerva Anestesiol,* 85 (5): 468-477, 2019. DOI: 10.23736/S0375-9393.18.12992-0

20. Zucco L, Santer P, Levy N, Hammer M, Grabitz SD, Nabel S, Ramachandran SK: A comparison of postoperative respiratory complications associated with the use of desflurane and sevoflurane: A single-centre cohort study. *Anaesthesia*, 76 (1): 36-44, 2021. DOI: 10.1111/anae.15203

21. Peyton PJ, Hendrickx J, Grouls RJE, Zundert AV, Wolf AD: End-tidal to arterial gradients and alveolar deadspace for anesthetic agents. *Anesthesiology,* 133, 534-547, 2020. DOI: 10.1097/ALN.0000000000003445

22. Naguib M, Gottumukkala V, Goldstein PA: Melatonin and anaesthesia: A clinical perspective. *J Pineal Res,* 42 (1): 12-21, 2007. DOI: 10.1111/j.1600-079X.2006.00384.x

23. Özer FD, Öçmen E, Akan P, Erdost HA, Korkut S: Effect of day and night desflurane anaesthesia on melatonin levels in rats. *Turk J Anaesthesiol Reanim*, 44 (4): 190-194, 2016. DOI: 10.5152/TJAR.2016.88609

24. Arruda NM, Braz LG, Nogueira FR, Souza KM, Aun AG: Gene and systemic inflammatory effects and neuroendocrine response in surgical patients anesthetized with desflurane-nitrous oxide or desflurane-nitrous oxide-free: A randomized trial. *Int Immunopharmacol*, 90:107163, 2021. DOI: 10.1016/j.intimp.2020.107163

25. Dikmen B, Arpaci AH, Kalayci D, Gunes I, Beskardes E: Are there any effects of Sevoflurane and Desflurane anaesthesia on blood glucose levels in acute hyperglycemic diabetic rats? *Bratisl Lek Listy*, 117 (6): 351-354, 2016. DOI: 10.4149/bll_2016_069

26. Hastings MH, Reddy AB, Garabette M, King VM, Chahad-Ehlers S: Expression of clock gene products in the suprachiasmatic nucleus in relation to circadian behaviour. *Novartis Found Symp*, 253, 203-222, 2003. DOI: 10.1002/0470090839.ch15

27. Bass J, Takahashi JS: Circadian integration of metabolism and energetics. *Science*, 330 (6009): 1349-1354, 2010. DOI: 10.1126/science.1195027

28. Tahara Y, Aoyama S, Shibata S: The mammalian circadian clock and its entrainment by stress and exercise. *J Physiol Sci*, 67 (1): 1-10, 2017. DOI: 10.1007/s12576-016-0450-7

29. Dibner C, Schibler U, Albrecht U: The mammalian circadian timing system: Organization and coordination of central and peripheral clocks. *Annu Rev Physiol*, 72, 517-549, 2009. DOI: 10.1146/annurev-physiol-021909-135821

30. Schiaffino S, Blaauw B, Dyar KA: The functional significance of the skeletal muscle clock: Lessons from *Bmal1* knockout models. *Skelet Muscle*, 6:33, 2016. DOI: 10.1186/s13395-016-0107-5

31. Franks NP, Lieb WR: Mapping of general anaesthetic target sites provides a molecular basis for cut off effects. *Nature,* 316 (6026): 349-351, 1985. DOI: 10.1038/316349a0

32. Merrow M, Spoelstra K, Roenneberg T: The circadian cycle: Daily rhythms from behaviour to genes. *EMBO Rep*, 6 (10): 930-935, 2005. DOI: 10.1038/sj.embor.7400541

33. Panda S, Hogenesch JB, Kay SA: Circadian rhythms from flies to human. *Nature*, 417, 329-335, 2002. DOI: 10.1038/417329a

34. Patel SS, Goa KL: Sevoflurane: A Review of its Pharmacodynamic and Pharmacokinetic Properties and its Clinical Use in General Anaesthesia. *Drugs*, 51 (4): 658-700, 1996. DOI: 10.2165/00003495-199651040-00009

35. Koblin DD, Weiskopf RB, Holmes MA, Konopka K, Rampil IJ: Metabolism of I-653 and isoflurane in swine. *Anesth Analg*, 68 (2): 147-149, 1989. DOI: 10.1213/00000539-198902000-00014