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ORIGINAL ARTICLE

Effects of volatile anesthetics on circadian rhythm in mice: a comparative study of sevoflurane, desflurane, and isoflurane

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Abstract

Purpose Volatile anesthetics affect the circadian rhythm of mammals, although the effects of different types of anesthetics are unclear. Here, we anesthetized mice using several volatile anesthetics at two different times during the day. Our objective was to compare the effects of these anesthetics on circadian rhythm.

Methods Male adult C57BL/6 J mice were divided into eight groups (n = 8 each) based on the anesthetic (sevoflurane, desflurane, isoflurane, or no anesthesia) and anesthesia time (Zeitgeber time [ZT] 6–12 or ZT18–24). Mice were anesthetized for 6 h using a 0.5 minimum alveolar concentration (MAC) dose under constant dark conditions. The difference between the start of the active phase before and after anesthesia was measured as a phase shift. Clock genes were measured by polymerase chain reaction in suprachiasmatic nucleus (SCN) samples removed from mouse brain after anesthesia (n = 8–9 each).

Results Phase shift after anesthesia at ZT6–12 using sevoflurane (− 0.49 h) was smaller compared with desflurane (− 1.1 h) and isoflurane (− 1.4 h) ($p < 0.05$). *Clock* mRNA (ZT6–12, $p < 0.05$) and *Per2* mRNA (ZT18–24, $p < 0.05$) expression were different between the groups after anesthesia.

Conclusion 0.5 MAC sevoflurane anesthesia administered during the late inactive to early active phase has less impact on the phase shift of circadian rhythm than desflurane and isoflurane. This may be due to differences in the effects of volatile anesthetics on the expression of clock genes in the SCN, the master clock of the circadian rhythm.

Keywords Volatile anesthesia · Perioperative care · Circadian rhythm

Introduction

The circadian rhythm is a cycle of approximately 24 h that appears in physiological activities, the sleep cycle, body temperature, immune system, and blood hormone levels [1]. Recent studies have shown that anesthetics affect the circadian rhythm. Anesthesia with desflurane or isoflurane shifts the phase of rodent behavioral rhythms, and this effect varies with the time of day when the anesthetic was administered [2, 3]. Another study suggested that its effects also vary depending on the anesthesia duration and concentration [4]. Sevoflurane anesthesia alters the expression of a clock gene Period2 (*Per2*) in the suprachiasmatic nucleus (SCN) [5]. Intravenous anesthetics such as propofol and various benzodiazepines also affect mammalian circadian rhythms [6, 7]. However, it is unclear how different volatile anesthetics affect the circadian rhythm because previous studies often use different experimental conditions. There are some clinical applications of circadian medicine to perioperative care. For example, Montaigne et al. state that patients undergoing heart surgery in the afternoon have a lower perioperative myocardial injury than in the morning [8]. However, circadian medicine is hardly applied in perioperative care because the effect of perioperative interventions (including anesthesia) on circadian rhythm is unclear. Clarifying the impact of general anesthetics on circadian rhythm will be helpful for future clinical trials.

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The SCN, which comprises a pair of small nerve nuclei in the hypothalamus, is the master clock of the mammalian circadian rhythm. The oscillator that creates a 24-h cycle is thought to be a transcriptional translation feedback loop (TTFL) consisting of multiple clock genes in SCN neurons [9]. The SCN provides an approximately 24-h cycle for organs via neural and other output pathways [10]. The TTFL contains a central mechanism called a core loop. *Clock*, Period (*Per*), basic helix-loop-helix ARNT like 1 (*Bmal*), and Cryptochrome (*Cry*) are clock genes that comprise the core loop. Volatile anesthetics alter clock gene expression, but the effect of each anesthetic on these genes remains unknown [11, 12].

In the present study, we anesthetized mice using sevoflurane, desflurane, or isoflurane, the most common volatile anesthetics. Our objective was to compare rest/activity rhythm phase shifts after anesthesia. Effects other than those of the anesthetics were eliminated using the same experimental conditions for all groups. We also measured changes in clock gene expression (*Clock*, *Bmal*, *Per2*, and *Cry1*) in the SCN after anesthesia using quantitative polymerase chain reaction (PCR).

Materials and methods

Animals

All experiments were approved by the Animal Care Committee at Hamamatsu University School of Medicine Animal Care Facility (Protocol Number: 2020055). We used male C57BL/6 J mice (SLC, Hamamatsu, Japan) that were 8–12 weeks old and weighed 20–24 g. There were 144 mice used in this study. Mice were housed in individual cages and acclimatized for at least ten days under light and dark conditions (LD). The onset of the dark period was set at ZT12 and the onset of the light period at ZT0. ZT is a chronobiological term that defines 24 h entrained mostly by the light phase; mice are nocturnal animals; therefore, ZT12 corresponds to the start of the active phase. All mice were at a constant room temperature (24 ± 2 °C) and humidity (50–70%) throughout the experimental period. Mice were randomly allocated to each anesthetic group when divided into individual cages. In the behavioral experiments, mice were also acclimated to running on the running wheel under LD conditions. Food and water were allowed ad libitum throughout the experiment.

Rest/activity rhythm phase shift measurements

Sixty-four mice were divided into eight groups (n = 8 in each group) based on the volatile anesthetic to be administered (sevoflurane, desflurane, isoflurane, or no anesthesia) and the time of day of anesthesia (ZT6–12 or ZT18–24). These times were selected based upon a previous study in which the circadian rhythm phase shifted the most after anesthesia during ZT6–12, with ZT18–24 being the opposite phase [3]. Details of the rest/activity rhythm phase shift measurement method are described in our previous study [3]. Briefly, mice were acclimated for at least ten days as described above, and the first experimental day (day 1) started with constant dark conditions (DD) in order to eliminate the light stimuli effect. Anesthesia was performed at ZT6–12 or ZT18–24 on day 5. Behavioral measurements continued until day 11, when the experiment was terminated. The phase shift was calculated as the difference between the expected start time of the active phase on day 6 (predicted from the circadian period of days 1–4), and the actual start time of the active phase (Fig. 1). In addition, we evaluated the circadian period of the LD term, the DD term before and after anesthesia, and circadian time (CT) of anesthesia, which is the subjective time at the end of anesthesia. CT is a chronobiological term representing the start time of the subjective light phase as CT0, with a day cycle being CT0 to 24. The evaluator (RI) was blinded to the groupings.

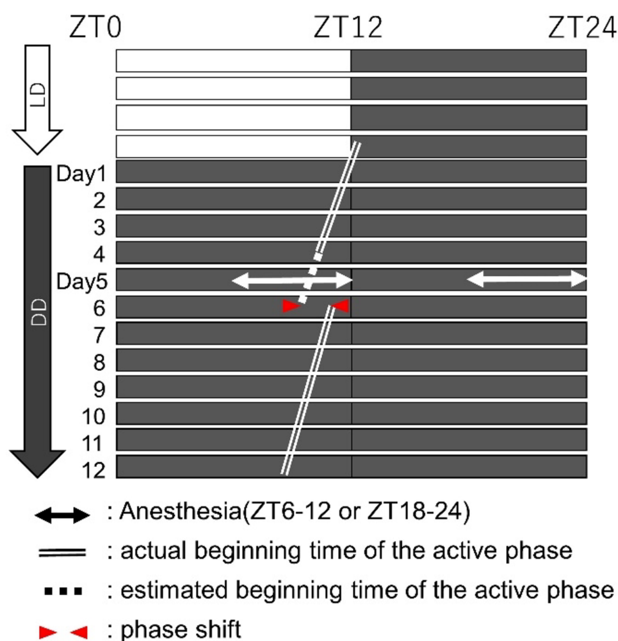

Fig. 1 Measurement of rest/activity rhythm and phase shift after anesthesia Anesthesia was performed at ZT6–12 or ZT18–24 on Day 5 with each anesthetic. Double lines indicate the beginning of the active phase. The phase shift was calculated as the difference between the predicted start time of the active phase and the actual start time of the active phase on day 6. LD, light dark condition; DD, constant dark condition; ZT, Zeitgeber time

Anesthesia

Mice were transferred from each cage to the anesthesia chamber (volume of approximately 1 L). Each anesthetic gas was supplied using 50% oxygen at a velocity of 2 L/min. After 30 min, the gas flow velocity was reduced to 1 L/min. The anesthetic concentration in the chamber was constantly measured using an IntelliVue G5–M1019A (GE HealthCare, Chicago, IL, USA) to adjust the dose to 0.5 minimum alveolar concentration (MAC) (sevoflurane, 1.4%; desflurane, 3.8%; and isoflurane, 0.65%). The MAC was determined based on previous study results in C57BL/6 mice [13–15]. Mice were placed on a heating pad that was set at 35 °C during anesthesia to prevent hypothermia. Anesthesia was performed under dark conditions for 6 h at ZT18–24 or ZT6–12, and the mice were returned to their cages immediately after anesthesia. A dim red light was used for a short time when needed to transfer mice, which ensured that their circadian rhythm was not affected. In the no-anesthesia group, no anesthetic was used with only sham procedures (such as transferring mice to the chamber).

Physiological measurements

Twelve mice were divided into three groups ($n = 4$ in each group) based on the anesthetic that was used (sevoflurane, desflurane, or isoflurane). Mice were anesthetized under dark conditions at ZT6–12. Heart rate (/min), respiratory rate (/min), and body temperature (°C) were measured every 60 min under anesthesia. Immediately after the mice entered deep anesthesia, arterial blood was collected from the exposed carotid artery, and the mice were then euthanized. The pH, arterial partial pressure of oxygen (paO_2), arterial partial pressure of carbon dioxide ($paCO_2$), and lactate levels were analyzed using the i–stat1 Analyzer MN:300-G with a CG4 + cartridge (Abbott Laboratories, Chicago, IL, USA).

SCN sample collection

Sixty-eight mice were divided into eight groups ($n = 8$–9 in each group) based on the type of volatile anesthetic that was administered (sevoflurane, desflurane, isoflurane, or non-anesthesia) and the time of day of anesthesia (ZT18–24 or ZT6–12). Details of the SCN sample collection method are described in our previous study [3]. Briefly, after acclimation, DD conditions were initiated, and mice were anesthetized on day 1. The mice were anesthetized on day 1 to minimize variations of sampling timing on subjective time. Mice were euthanized by cervical dislocation immediately after 6 h of anesthesia. The brain was promptly resected and placed into RNA protect (Qiagen, Venlo, the Netherlands) and then sliced to a thickness of 1.5 mm using a brain slicer (Muromachi Kikai, Tokyo, Japan). Brain tissue containing the bilateral SCN was resected as a small block (2 mm per side). The SCN samples were placed into a collection tube with RNA protect (150 µL) and stored at 4 °C.

Quantitative real-time polymerase chain reaction

Details of this method are described in our previous study [3]. Briefly, total RNA was extracted from the resected SCN samples using the RNeasy Mini Kit (Qiagen). Then reverse transcription was performed using the QuantiTect Reverse Transcription kit (Qiagen) to synthesize cDNA. The QuantiTect SYBR Green PCR kit (Qiagen) was used as the fluorescent dye, and the StepOnePlus Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) was used for quantitative real-time PCR. The $2^{-\Delta\Delta CT}$ (–delta delta CT) method was used to quantify gene expression levels. The target clock gene primers were as follows: *Clock* forward primer, 5′–AGCTGCATATTGCCGTTGTAATTG–3′ and *Clock* reverse primer, 5′–CTGAGTGAAGGCATGCTG GTG–3′; *Bmal* forward primer, 5′–ACGACATAGGACACC TCGCAGA–3′ and *Bmal* reverse primer, 5′–TCCTTGGTC CACGGGTTCA–3′; *Per2* forward primer, 5′–ATCAGCCAT GTTGCCGTGTC–3′ and *Per2* reverse primer, 5′–CGTGCT CAGTGGCTGCTTTC–3′; *Cry1* forward primer, 5′–GGA TCCACCATTTAGCCAGACAC–3′ and *Cry1* reverse primer, 5′–CATTTATGCTCCAATCTGCATCAAG–3′. The internal control gene primers were as follows: beta-actin (*Actb*) forward primer, 5′–CATCCGTAAAGACCT CTATGCCAAC–3′ and *Actb* reverse primer, 5′–ATGGAG CCACCGATCCACA–3′.The protocol for quantitative PCR consisted of three stages. The holding stage kept the temperature at 95 °C for 15 min; the cycling stage repeated 40 cycles of 94 °C for 15 s and then 58 °C for 1 min; and the melt curve stage was 95 °C for 15 s and 60 °C for 1 min, followed by increasing the temperature in increments of 0.3 °C to 95 °C for 15 s.

Statistical analysis

The sample size was calculated to detect rest/activity rhythm phase shift differences between the sevoflurane and desflurane groups. A power analysis was performed using G*Power (version 3.19)18 with power ($1 - \beta$) set at 0.8 and $\alpha = 0.05$; this indicated that seven per group was sufficient to reach statistical significance ($p < 0.05$). In a previous study, the mean phase shift was –1.9 h (standard deviation [SD], 1.1) after desflurane anesthesia [3]. In our preliminary experiment, the mean phase shift was –0.29 h (SD, 0.7) after sevoflurane anesthesia.

The sample size required to detect clock gene mRNA expression differences after anesthesia between the sevoflurane and desflurane groups was estimated. A power analysis was performed using G*Power (version 3.19) [18], with

power (1 − β) set at 0.8 and α = 0.05; this indicated that nine mice per group was sufficient to reach statistical significance ($p < 0.05$). There was no appropriate preliminary study, and we estimated an effect size of d = 1.5 (large effect).

Data are presented as the median (interquartile range [IQR]). The phase shift, gene expression, physiological variables, and blood–gas results were analyzed using the Kruskal–Wallis test. If there was a statistical difference in the Kruskal–Wallis test, the Steel–Dwass test was used to determine the difference between groups. All data obtained were used for statistical analysis. All statistical analyses were calculated using Bell Curve for Excel (version 3.21, Social Survey Research Information Co., Tokyo, Japan), and p values < 0.05 were considered statistically significant.

Results

Rest/activity rhythm phase shift after anesthesia

Sevoflurane anesthesia shifted the rest/activity rhythm less than desflurane or isoflurane anesthesia. Behavioral disturbances and changes in the rest/activity rhythm phase on the following day were not observed in the control group mice. Representative actogram for each group is presented in Fig. 2. The phase shift after anesthesia at ZT6–12 was different between the anesthetics (sevoflurane group, − 0.49 h [IQR, − 0.63 to − 0.29], n = 8; desflurane group, − 1.1 h [IQR, −1.3 to −0.79], n = 8; isoflurane group, -1.4 h [IQR, −1.6 to -1.2], n = 8; control group, -0.17 h [IQR, −0.27 to 0.15], n = 7 [one sample was lost because of measuring device failure]; p < 0.001). The post hoc test revealed a significant difference between the sevoflurane and desflurane groups (−0.49 vs. −1.1 h, p = 0.048) and between the sevoflurane and isoflurane groups (−0.49 vs. −1.4 h, p = 0.006; Fig. 3a). The phase shift after anesthesia at ZT18–24 was small in all groups, and a difference was not observed (sevoflurane group, 0.17 h [IQR, −0.72 to 0.58], n = 8; desflurane group, −0.26 h [IQR, −0.75 to −0.85], n = 8; isoflurane group, 0.33 h [IQR, 0.27 to 0.37], n = 8; control group, −0.08 h [IQR, −0.56 to 0.72], n = 7 [one sample was lost because of measuring device failure]; p = 0.92) (Fig. 3b). The circadian periods of rest/activity rhythm in each group are shown in Table 1. All actograms of each mouse are shown in Supplementary Figures S1.

Physiological measurements

Body temperature, heart rate, and respiratory rate decreased slightly during anesthesia, but no differences were observed between different anesthetic groups (p > 0.05) (Fig. 4).

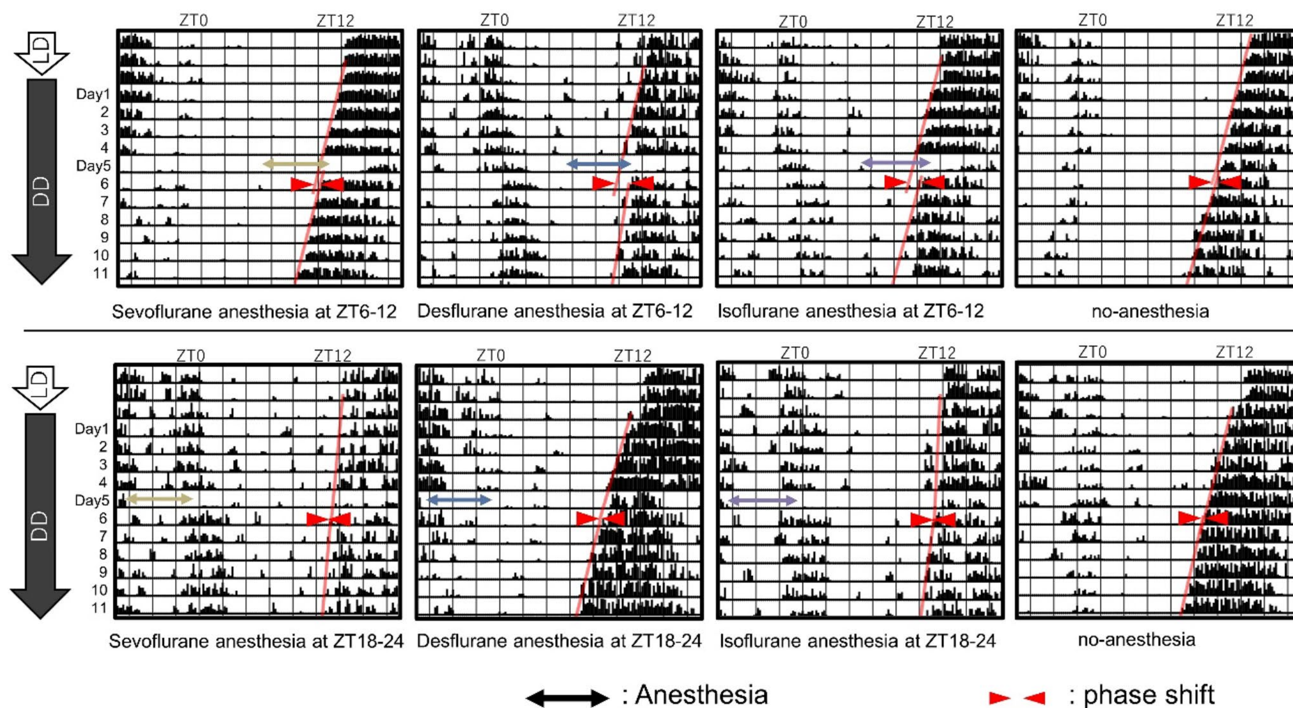

Fig. 2 Representative actograms**.** Representative actograms of mice anesthetized at ZT6-12 and ZT18-24. A portion of the double-plot actogram is shown. The count of running wheel rotations obtained from each mouse was determined in 10-min intervals to generate a bar graph. All double-plot actograms of each mouse are shown in Supplementary Figure S1

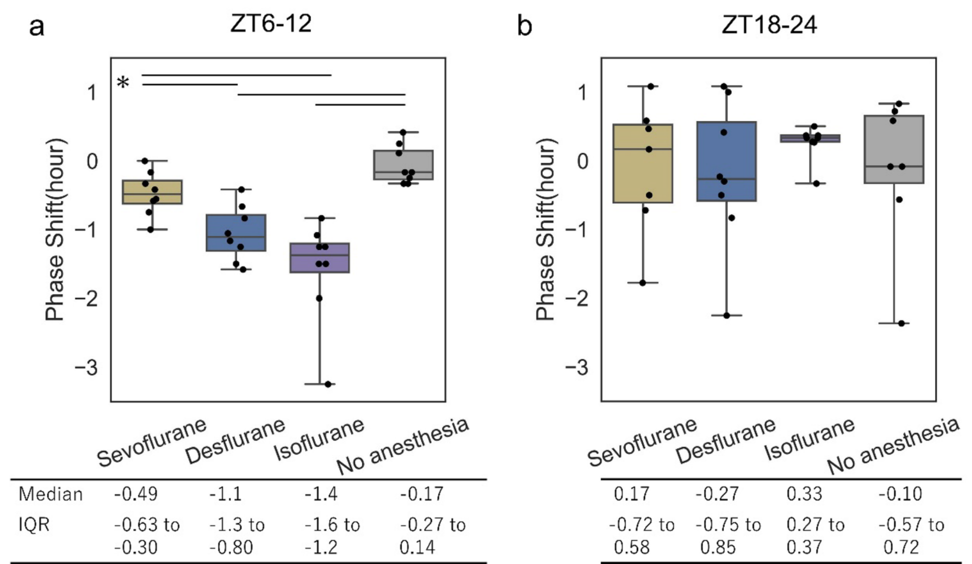

Fig. 3 The phase shift after anesthesia**.** The phase shift after anesthesia with different volatile anesthetics at ZT6–12 (**a**) or ZT18–24 (**b**). Boxes indicate the interquartile range. The line inside the box represents the median of the dataset. Whiskers extend from the box to the smallest and largest observations. Individual measurement values are plotted. After anesthesia at ZT6–12, the phase shift differed in different anesthetic groups (*$p < 0.05$). Mice in the sevoflurane group showed the smallest phase shift. There was no difference between anesthetics in phase shift after anesthesia at ZT18–24 between anesthetics ($p = 0.92$)

Table 1 Circadian period and circadian time of anesthesia

Circadian period (h)	Sevoflurane	Desflurane	Isoflurane	No Anesthesia
Anesthesia time ZT6–12				
LD	24.0(24.99–24.04)	24.0(23.99–24.01)	24.04(23.99–24.04)	23.98(23.96–24.0)
DD day 1–4	23.69(23.57–23.74)*	23.46(23.11–23.59)	23.15(22.83–23.47)*	23.48(23.4–23.55)
CT of anesthesia	13.56(13.3–14.14)*	14.71(14.03–16.46)	16.27(14.66–17.83)*	14.6(14.27–15.02)
DD day 6–	23.65(23.53–23.78)	23.65(23.39–23.76)	23.62(23.46–23.74)	23.38(23.28–23.44)
Anesthesia time ZT18–24				
LD	24.0(23.98–24.02)	23.98(23.96–24.0)	23.96(23.96–24.01)	24.0(23.98–24.0)
DD day 1–4	23.54(23.03–23.6)	23.47(23.3–23.5)	23.54(23.43–23.64)	23.71(23.46–23.85)
CT of anesthesia	2.29(1.98–4.86)	2.67(2.5–3.51)	2.29(1.81–2.84)	1.46(0.73–2.69)
DD day 6–	23.83(23.62–23.9)	23.73(23.59–23.75)	23.83(23.77–23.84)	23.75(23.71–23.79)

Median (interquartile range) are shown. *$p = 0.048$, sevoflurane vs. isoflurane. *LD* light and dark conditions, *DD* constant dark condition, *CT* circadian time, *ZT* Zeitgeber time

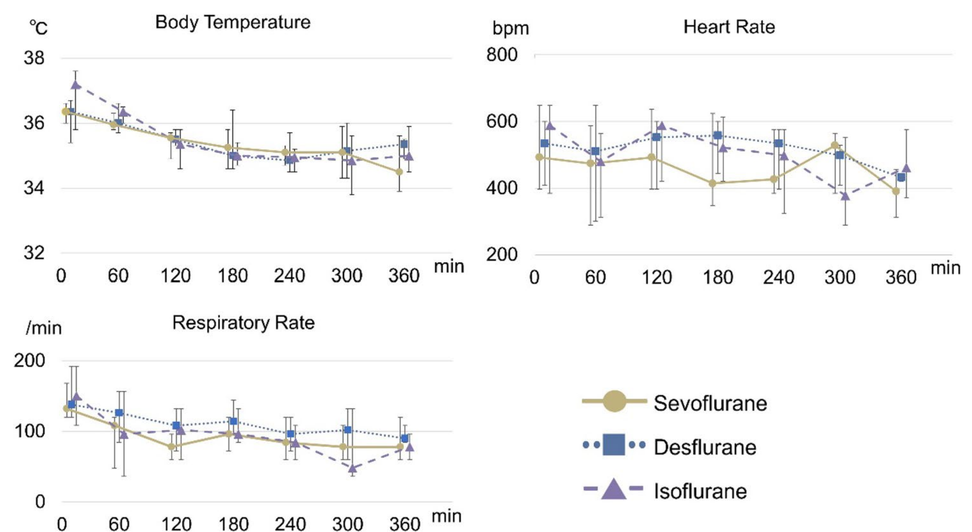

Fig. 4 Physiological variables of mice during anesthesia. Physiological variables of mice during six hours of anesthesia are indicated as line graphs with interquartile ranges. Heart rate, body temperature, and respiratory rate during anesthesia decreased to some extent with all anesthetics. There were no differences in any of the parameters between the different volatile anesthetics ($p > 0.05$)

Arterial blood–gas analysis was performed immediately after 6 h of anesthesia (n = 4). There was no significant difference in pH, paO2, paCO2, or lactate levels at the end of anesthesia between the groups (p > 0.05) (Table 2).

Clock gene expression in the SCN after anesthesia

The mRNA expression ratios of clock genes in the SCN after anesthesia were measured as relative expression levels using a reference sample of the non-anesthesia group collected at the same time of day. *Clock* mRNA expression after anesthesia at ZT6–12 differed between the anesthetics (p = 0.044). The *Clock* mRNA expression ratio was 1.4 (IQR, 0.68 to 3.86) (n = 9) after sevoflurane anesthesia, 0.61 (IQR, 0.48 to 0.79) (n = 9) after desflurane anesthesia, and 0.81 (IQR 0.67 to 1.7) (n = 8, one sample was lost because of sampling failure) after isoflurane anesthesia (Fig. 4a). The *Bmal*, *Per2*, and *Cry1* expression ratios were not different between anesthetics after administration at ZT6–12 (p > 0.05). *Per2* mRNA expression after anesthesia at ZT18–24 differed between the anesthetics (p = 0.031). The *Per2* mRNA expression ratio was 1.12 (IQR, 0.49 to 1.34) (n = 8) after sevoflurane anesthesia, 0.34 (IQR, 0.19 to 0.40) (n = 8) after desflurane anesthesia, and 0.26 (IQR, 0.14 to 0.62) (n = 8) after isoflurane anesthesia (Fig. 5b). The *Clock*, *Bmal*, and *Cry1* mRNA expression ratio was not different between anesthetics after administration at ZT18–24 (p > 0.05).

Discussion

We found that the phase shift after sevoflurane anesthesia (− 0.49 h) at ZT6–12 was smaller than that of desflurane (− 1.1 h) and isoflurane (− 1.4 h) anesthesia (Fig. 3a). This

Table 2 Arterial blood–gas analysis

	pH	paO_2(mmHg)	pCO_2(mmHg)	Lactate(mmol/l)
Sevoflurane	7.33(7.30–7.50)	258(250–272)	49(39.0–60.0)	1.3 (1.1–1.4)
Desflurane	7.39(7.31–7.50)	246(212–274)	42.8(33.0–53.1)	2.0(1.8–2.2)
Isoflurane	7.42(7.36–7.45)	247(218–274)	44.8(42.6–46.6)	1.6(1.4–2.0)

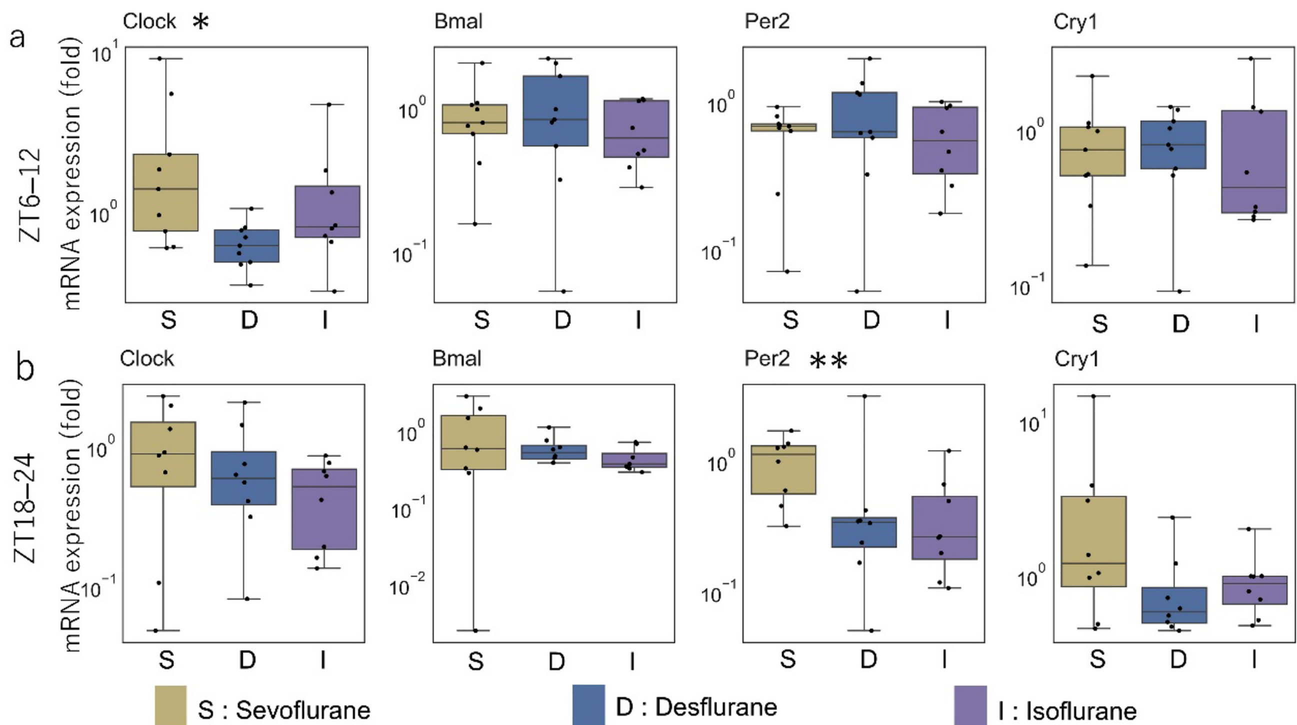

Fig. 5 Relative expression levels of clock genes in the SCN. Boxes and whiskers indicate interquartile ranges and the smallest and largest values, respectively. The line inside each box represents the median of the dataset. The relative expression level was calculated using the no-anesthesia group samples (represented as 1) collected at the same time as the reference samples. All individual values are plotted. Clock gene expression was different for *Clock* after ZT6–12 anesthesia (*p = 0.044) (**a**) and *Per2* after ZT18–24 anesthesia (**p = 0.031) (**b**) with different anesthetics

study is the first to clarify the different effect of each volatile anesthetics (sevoflurane, desflurane and isoflurane) on circadian rhythms in mammals by administering three types of volatile anesthetics under the same experimental conditions. In previous studies, the phase shift after sevoflurane anesthesia under free-running conditions was not significant (1.5 MAC, 8 h, 08:00–16:00, conducted in rats [17]; or − 0.2 to − 0.5 h (0.9 MAC, 4 h, various timepoints, conducted in mice [5]). In our results, the phase shift after sevoflurane anesthesia was small and similar to these past studies, with no significant difference compared with the control group ($p > 0.05$). In contrast, in the desflurane group, the phase shift after anesthesia was large, similar to our previous study conducted in mice (− 1.7 h phase shift, 0.5 MAC, 6 h, ZT6–12, [3]). There was a significant difference between the desflurane and sevoflurane groups. The phase shift after anesthesia in the isoflurane group was significantly larger than the sevoflurane group, and also compared with previous studies (0 to − 2 h phase shift, 1.4 MAC, 6 h, various times, conducted in bees [18]; and 0.23 to − 0.77 h phase shift,1.4 MAC, 6 h, various times, conducted in mice [2]). This may be due to a significantly shorter circadian period before anesthesia in the isoflurane group than in the sevoflurane group, resulting in a difference in subjective time of anesthesia (i.e., CT of anesthesia). In this study, the interventions were performed simultaneously in all four groups to allow comparisons and ensure that the conditions other than anesthetic were as similar as possible. Therefore, parameters arising from individual differences (such as the circadian period) could not be equalized between groups due to the study design.

The phase shift after anesthesia at ZT18–24 did not differ between anesthetics (Fig. 3b). The phase shift after desflurane and isoflurane anesthesia is known to vary with the time of day of anesthesia [2, 3]. In this study, a large phase shift was observed after anesthesia at ZT6–12, and a small phase shift was observed after anesthesia at ZT18–24 (desflurane, − 1.1 vs. − 0.27 h, $p = 0.03$; isoflurane − 1.4 vs. 0.33 h, $p < 0.001$). However, for sevoflurane, the phase shift after anesthesia at ZT18–24 was as small as that of ZT6–12 (− 0.49 vs. 0.17 h, $p = 0.25$).

Under constant conditions, such as DD, which is blocked from environmental information (e.g., light, temperature, and sound), the rest activity rhythm is only determined by the circadian clock of each mouse. Therefore, in this experiment, anesthesia was performed at two scheduled times of day (ZT6–12 and ZT18–24), but each mouse was anesthetized at slightly different times of day for their own CT. Assuming a start time of an active phase of 7:00 in humans, the median end time of anesthesia was 9:35 for the ZT6–12 anesthesia group and 21:09 for the ZT18–24 anesthesia group. This means there is no difference between these anesthetics when anesthesia was conducted from evening to night. However, when anesthesia was conducted from dawn to morning, sevoflurane had the least effect on circadian rhythm in humans. However, this may be a misinterpretation because the mechanisms by which circadian rhythms determine nocturnal and diurnal behavior and their relationship to anesthesia are still unclear. Further basic and clinical studies are needed to clarify how anesthesia affects human circadian rhythms.

Clock mRNA expression differed between the anesthetics after ZT6–12 anesthesia ($p = 0.042$) (Fig. 5a). A previous study showed that desflurane anesthesia decreased *Clock* mRNA expression in the mouse SCN [3]. Isoflurane anesthesia also suppressed *Clock* mRNA expression in rat peripheral blood mononuclear cells [19]. We revealed that the impact on clock gene mRNA expression differs between volatile anesthetics for the first time. *Clock* is an important gene in the TTFL. CLOCK dimerizes with BMAL to activate transcription of other clock genes. The CLOCK–BMAL complex also regulates the transcription of thousands of genes that are directly linked to functions including metabolism, immune function and cell proliferation [9]. Thus, a more detailed study of volatile anesthetics on the effect of clock gene expression may improve perioperative complications.

Per2 mRNA expression in the SCN differed between the anesthetics after ZT6–12 anesthesia (Fig. 5b). *Per2* mRNA expression did not decrease in the sevoflurane group (1.12 [IQR, 0.49 to 1.34]). Sevoflurane was reported to reduce *Per2* mRNA expression by reducing CLOCK binding to the promoter region [11]. All volatile anesthetics were used at low concentrations of 0.5 MAC in this study, which may have caused inconsistencies, for example that *Per2* was not suppressed in the sevoflurane group compared with previous studies [5].

Differences in the anesthetic distribution in the brain may have affected these results. The distribution of volatile anesthetics in the brain begins in the cerebral cortex, and takes time to distribute to other brain areas [20, 21]. The distribution depends on the blood–gas partition coefficient and lipid solubility, and these parameters vary between sevoflurane, desflurane, and isoflurane. Therefore, even if we used volatile anesthetics with the same MAC, the SCN might have been exposed to anesthetics with different concentrations and times.

It is not known whether the same results will be observed in clinical studies targeted for patients after general anesthesia using these anesthetics. Because circadian rhythms are easily masked by environmental factors such as lighting conditions, food, and social activity. However, in a chronobiologically rigorously designed clinical study, differences in the effects on circadian rhythms caused by different volatile anesthetics might reveal variances in postoperative sleep disorders, cognitive impairment, delirium, and more. Our research findings could serve as a foundation

for future studies concerning anesthesia and postoperative complications.

There were several limitations to this study. First, the MAC was used to equalize anesthesia depth. MAC is the anesthetic concentration defined by immobilization in 50% of mice in response to tail clamp stimulation. MAC is thought to be the action of the spinal cord rather than that of the brain [24]. MAC awake, which is the anesthetic concentration defined by loss of the righting reflex in 50% of mice, might be more appropriate to equalize the depth of anesthesia. However, sevoflurane, isoflurane, and desflurane have similar MAC and MAC awake concentration ratios [25–27], and the anesthetic concentrations we used were almost the same as those of MAC awake. Second, blood lactate levels were statistically insignificant but slightly variable. Generally, these volatile anesthetics do not cause any variation in blood lactate levels under similar anesthetic conditions. Variable lactate levels might be related to experimental conditions and animal handling. In any case, the difference in lactate levels was minimal (0.7 mmol/L), so we assumed that the variation did not intrinsically affect our results. There was no difference between body temperature, heart rate, and respiratory rate during anesthesia and blood pH, paO_2, or $paCO_2$ levels at the end of anesthesia. Finally, the phase shifts calculated from the rest-activity rhythm in this study might be influenced by minor interventions other than anesthetic agents necessary for anesthesia (e.g., cage changes) or after-effects of transitioning from light–dark to constant dark condition, as well as nonspecific masking effects. However, these factors were consistent for both the anesthesia and non-anesthesia groups. Thus, we consider that these factors do not impact the main findings of this study.

In conclusion, 0.5 MAC sevoflurane anesthesia at the early active phase had less impact on circadian rest/activity rhythms and certain clock gene expression in the SCN than desflurane and isoflurane. Our study has clarified the relationship between anesthesia and circadian rhythm.

Declaration

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00540-023-03262-9.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by SS, RI and TK. The first draft of the manuscript was written by SS and RI and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The data that support the findings of this study are available from the corresponding autho, upon reasonable request.

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

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