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Circadian Disruption of Body Core Temperature and Rest–Activity Rhythms after General (Propofol) Anesthesia in Rats

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Background: **General anesthesia is commonly associated with sleep disorders, fatigue, drowsiness, and mood alterations in patients. The authors examined whether general (propofol) anesthesia can impact the circadian temporal structure by disturbing circadian rest–activity and body temperature rhythms under normal light–dark conditions (light–dark 12:12 h) in rats.**

Methods: **A group of rats was anesthetized with propofol, and another was injected with 10% Intralipid, which was used as a control lipidic solution. The authors examined six groups of rats according to the Zeitgeber time of intraperitoneal administration (ZT6, ZT10, ZT16) and the substance injected (propofol or Intralipid).**

Results: **On the day after anesthesia, propofol induced a significant 60- to 80-min phase advance of both rest–activity and body temperature rhythms. A significant 45- to 60-min phase advance of body temperature and a significant 20-min phase advance of rest–activity were still observed on the second day after anesthesia. The amplitudes of both rest–activity and body temperature rhythms were decreased on the first and second days after anesthesia. The 24-h mean rest–activity rhythm was decreased on the day after anesthesia, whereas the 24-h mean body temperature rhythm was not modified.**

Conclusion: **The results demonstrate the disturbing effects of propofol anesthesia on the circadian time structure in rats under normal light conditions.**

CLINICAL research on postoperative circadian rhythms in patients suggests that both general anesthetics and surgery can disturb the circadian time structure *via* multifactorial mechanisms.[1,2] General anesthesia can be described as a pharmacologic state involving amnesia, immobility, unconsciousness, and analgesia.[3] The aim of anesthesia is to create a state of sensory deprivation, to induce a lack of motor reaction to stimuli, and to obtain explicit amnesia. General anesthesia seems to be a particular state of consciousness that shares some common neuronal mechanisms with sleep.[4,5]

Previous studies showed that propofol disturbed the circadian rest–activity rhythm in rats in constant darkness.[6] Indeed, when rats were anesthetized with propofol near (±2 h) the rest–activity transition point, propofol induced a 1-h phase advance of the rest–activity rhythm. However, this desynchronizing effect was observed in specific laboratory conditions (free-running rhythms, constant darkness). The issue with experiments performed in constant darkness is that there are far from environmental conditions present in human clinical practice. Therefore, even if propofol can disturb the circadian time structure in laboratory conditions of constant darkness, its effects could be masked by the presence of light, which is the most powerful synchronizer.

Circadian rhythms (period close to 24 h) are regulated in mammals by a main circadian clock in the suprachiasmatic nuclei of the anterior hypothalamus. Input pathways (*e.g.*, light, social synchronizers) connect the circadian clock to the external environment, and output pathways transfer circadian rhythmicity to physiologic, behavioral, and biochemical parameters of the organism.[7,8]

Our hypothesis was that general anesthesia could act as an external factor that could disturb the circadian time structure. The aim of our study was to examine the effects of general (propofol) anesthesia on rest–activity and body temperature rhythms in normal light conditions (12 h light–12 h dark) in rats. It is important to study whether general anesthesia effects observed in constant darkness persists under normal light conditions or disappears because of the powerful resynchronizing effect of the light on the circadian structure. The disturbing effects of general anesthesia on circadian rest–activity and core body temperature rhythms might theoretically have large consequences in humans and sustain postoperative wake–sleep disorders.

The effects of propofol anesthesia on rest–activity and body temperature rhythms were assessed by telemetry, a method widely used for the continuous measurement of body temperature and locomotor activity in rats.[9]

Materials and Methods

Animals

Forty male Wistar rats (Janvier, Le Genest-St-Isle, France), 5 weeks old and weighing 170–195 g at the beginning of the experiment, were studied. Rats were housed in individual cages, with food and water available *ad libitum*, and were maintained in a chronobiologic animal facility (Enceinte autonome d'animalerie, Ref. A 110-SP-6; ESI Flufrance, Arcueil, France). The chronobiologic facility was equipped with equidistant, sound-

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Received from the Department of Biochemistry and Molecular Biology, Faculty of Medicine Pierre and Marie Curie, INSERM U713, Paris, France. Submitted for publication July 22, 2008. Accepted for publication January 15, 2009. Supported by a doctoral fellowship from the Institut de Médecine Aérospatiale du Service de Santé des Armées (to Dr. Dispersyn), Brétigny-sur-Orge, France.

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proof, temperature-controlled (21° ± 1.0°C) compartments, each having independent light–dark cycles. All of the research procedures were performed in accordance with the National Institutes of Health principles of laboratory animal care, French National Laws, and standards and ethics for animal biologic rhythm research.[10] This study, including care of the animals involved, was conducted according to the official edict presented by the French Ministry of Agriculture (Paris, France) and the recommendations of the Declaration of Helsinki. These experiments were conducted in an authorized laboratory and under the supervision of an authorized researcher (Y.T.).

Experimental Procedures

Rats were housed in individual cages and maintained under a 12-h light–12-h dark cycle 4 weeks before the beginning of drug injection. Radiotelemetric recorders were surgically implanted under aseptic conditions, 3 weeks before the start of drug injection. Rats were lightly anesthetized by an intraperitoneal administration of diazepam (5 mg/kg Valium; Roche, Neuilly-sur-Seine, France), followed by ketamine (80 mg/kg Ketalar; Parke-Davis, Fresnes, France). Each rat was fitted with an intraabdominal radiotelemetric implant (model TA10TA-F40; Data Sciences Int., Saint Paul, MN) to allow for the automatic recording of body temperature and general locomotor activity every 10 min throughout a 24-h period, using the Dataquest 4.0 data acquisition software (Data Sciences Int.). During the experiment, radio waves from the implant were collected *via* a radar receiver (PHYSIOTEL Receiver, model RPC-1; Data Sciences Int.) that was placed beneath the cage. General locomotor activity and body temperature were continuously recorded for 2 weeks before drug injections. Propofol (10 mg/ml; Fresenius Kabi, Sèvres, France) was injected intraperitoneally at a dose of 120 mg/kg. Intralipid, 10%, was used as a control lipidic solution (10 ml/kg; Fresenius Kabi). The duration of general propofol anesthesia was between 25 and 30 min, and all rats were responsive 30 min after propofol administration. The dose of propofol was an anesthetic dose and not a sedative one, in accord with previous published data obtained in rats.[11,12] Either propofol or Intralipid for control animals was administered at three different Zeitgeber times (ZTs): ZT6, ZT10, and ZT16. ZT0 represented the beginning of the rest period (light onset), and ZT12 represented the beginning of the activity period (light offset). Therefore, rats were injected in the middle of their rest period (ZT6), 2 h before the beginning of activity (ZT10), and 4 h after the beginning of the activity period (ZT16) (fig. 1).We used in each Zeitgeber time group 12–14 rats assigned as followed: 14 rats at ZT6, 12 rats at ZT10, and 14 rats at ZT16. In all three groups, half of the rats received propofol administration, and the other half received Intralipid injection. After the injections, general locomotor activity and temperature were recorded for a period of 13 days.

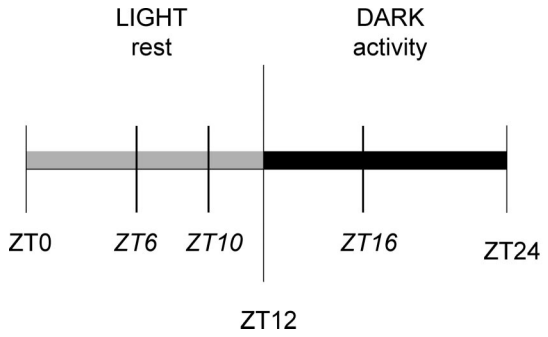

Fig. 1. Drug administration. Six groups of rats corresponding to three schedules of intraperitoneal injection (Zeitgeber time [ZT]) were created: ZT6 (n = 14), ZT10 (n = 12), and ZT16 (n = 14). Within each of the three ZT groups, half of the rats were injected with propofol and the other half were injected with Intralipid.

Data Analysis

A circadian rhythm analysis was performed by fitting a cosinor function to the data using the Chronos-Fit program data analysis (Zuther and Lemmer, Institute of Pharmacology and Toxicology Faculty of Clinical Medicine, Mannheim, Germany), an extended version of Win-ABPM-Fit. This program fits a cosine curve to the measured data points and calculates the midline estimating statistic of rhythm (MESOR; a rhythm-adjusted 24 h mean), amplitude (half of peak-to-trough of rhythmic change), and acrophase (peak time of the rhythm). These circadian parameters were determined 5 days before the day of injection (baseline) and compared with each of the 5 days after injection (D1, D2, D3, D4, D5).

Statistical Analysis

Statistical analysis on acrophase, MESOR, and amplitude were performed on raw data (table 1). The statistical analyses were performed using the software SYSTAT version 8.0 (Systat Software Inc., San Jose, CA). For clarity, data were calculated as percentage from the baseline and phase shift for acrophase, amplitude, and MESOR, respectively (figs. 2–7). For each parameter examined (acrophase, amplitude, MESOR), multivariate analyses of variance for repeated measures were performed on raw data (between factors: Zeitgeber time [ZT6, ZT10, ZT16], treatment [propofol *vs.* Intralipid]; within factor: days [baseline, D0, D1, D2, D3, D4, D5]). *Post hoc* analyses on the phase shift of acrophase, as well as the absolute values of MESOR and amplitude, were performed using a *t* test with Bonferroni correction, which compared the propofol and Intralipid groups, on D1–D5.

Results

Acrophase of Rest–Activity Rhythm

We observed a phase advance of rest–activity acrophase during the 48 h after the administration of

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Table 1. Results of Cosinor Analysis

	ZT6		ZT10		ZT16	
	Propofol	Intralipid	Propofol	Intralipid	Propofol	Intralipid
Rest–activity						
Acrophase						
D ref	17.40 ± 09	17.26 ± 10	17.30 ± 03	17.43 ± 06	17.33 ± 05	17.41 ± 06
D0	16.28 ± 14	17.15 ± 11	15.10 ± 18	17.52 ± 05	16.01 ± 13	17.54 ± 08
D1	16.29 ± 07	17.18 ± 10	16.37 ± 06	17.44 ± 05	16.37 ± 06	17.45 ± 09
D2	17.13 ± 12	17.22 ± 10	16.59 ± 09	17.41 ± 07	17.14 ± 10	17.46 ± 06
D3	17.30 ± 13	17.22 ± 08	17.26 ± 05	17.42 ± 06	17.28 ± 04	17.46 ± 07
D4	17.33 ± 09	17.17 ± 07	17.27 ± 04	17.44 ± 08	17.32 ± 05	17.44 ± 07
D5	17.44 ± 10	17.20 ± 08	17.30 ± 05	17.45 ± 07	17.35 ± 05	17.43 ± 08
Amplitude						
D ref	2.12 ± 0.26	2.21 ± 0.13	1.95 ± 0.14	2.54 ± 0.24	2.80 ± 0.36	2.50 ± 0.13
D0	1.36 ± 0.21	2.10 ± 0.14	0.98 ± 0.05	2.46 ± 0.23	1.93 ± 0.24	2.48 ± 0.16
D1	1.89 ± 0.24	2.21 ± 0.12	1.44 ± 0.13	2.56 ± 0.24	2.33 ± 0.24	2.50 ± 0.15
D2	1.97 ± 0.22	2.22 ± 0.12	1.78 ± 0.08	2.59 ± 0.23	2.60 ± 0.33	2.51 ± 0.14
D3	2.04 ± 0.22	2.19 ± 0.12	1.95 ± 0.16	2.56 ± 0.24	2.67 ± 0.32	2.50 ± 0.14
D4	2.07 ± 0.24	2.23 ± 0.12	1.98 ± 0.14	2.54 ± 0.24	2.70 ± 0.32	2.51 ± 0.14
D5	2.11 ± 0.26	2.21 ± 0.13	1.97 ± 0.14	2.54 ± 0.23	2.75 ± 0.33	2.51 ± 0.13
MESOR						
D ref	3.10 ± 0.26	2.55 ± 0.24	2.79 ± 0.28	3.47 ± 0.39	3.46 ± 0.39	3.15 ± 0.29
D0	2.54 ± 0.21	2.39 ± 0.23	1.85 ± 0.28	3.51 ± 0.37	2.71 ± 0.23	3.16 ± 0.30
D1	2.71 ± 0.30	2.57 ± 0.23	2.31 ± 0.31	3.49 ± 0.39	2.57 ± 0.34	3.16 ± 0.30
D2	2.85 ± 0.28	2.56 ± 0.24	2.63 ± 0.27	3.55 ± 0.38	2.93 ± 0.29	3.12 ± 0.27
D3	2.95 ± 0.26	2.57 ± 0.24	2.74 ± 0.25	3.46 ± 0.40	3.14 ± 0.33	3.11 ± 0.27
D4	3.01 ± 0.25	2.59 ± 0.25	2.75 ± 0.26	3.44 ± 0.39	3.26 ± 0.40	3.12 ± 0.27
D5	3.04 ± 0.26	2.55 ± 0.23	2.78 ± 0.27	3.43 ± 0.39	3.33 ± 0.40	3.16 ± 0.28
Temperature						
Acrophase						
D ref	17.08 ± 14	16.41 ± 15	16.47 ± 12	17.24 ± 04	16.54 ± 12	17.22 ± 10
D0	15.25 ± 14	16.10 ± 15	14.31 ± 75	17.14 ± 07	13.51 ± 38	17.26 ± 09
D1	16.06 ± 15	16.31 ± 12	15.19 ± 19	17.29 ± 05	15.43 ± 07	17.26 ± 08
D2	16.22 ± 15	16.35 ± 15	15.57 ± 15	17.25 ± 06	15.55 ± 09	17.24 ± 10
D3	16.50 ± 14	16.42 ± 16	16.28 ± 15	17.21 ± 06	16.39 ± 13	17.25 ± 09
D4	17.00 ± 14	16.42 ± 15	16.37 ± 14	17.24 ± 06	16.47 ± 12	17.27 ± 11
D5	17.07 ± 13	16.43 ± 14	16.47 ± 12	17.25 ± 05	16.54 ± 12	17.25 ± 10
Amplitude						
D ref	0.51 ± 0.03	0.52 ± 0.02	0.58 ± 0.04	0.51 ± 0.03	0.64 ± 0.04	0.49 ± 0.06
D0	0.36 ± 0.04	0.47 ± 0.02	0.44 ± 0.02	0.49 ± 0.03	0.39 ± 0.05	0.48 ± 0.06
D1	0.34 ± 0.04	0.51 ± 0.02	0.25 ± 0.02	0.51 ± 0.03	0.37 ± 0.03	0.49 ± 0.06
D2	0.41 ± 0.02	0.54 ± 0.03	0.41 ± 0.02	0.53 ± 0.03	0.47 ± 0.03	0.49 ± 0.04
D3	0.51 ± 0.02	0.55 ± 0.02	0.61 ± 0.02	0.53 ± 0.02	0.60 ± 0.05	0.49 ± 0.04
D4	0.53 ± 0.02	0.53 ± 0.03	0.59 ± 0.04	0.51 ± 0.03	0.62 ± 0.04	0.50 ± 0.05
D5	0.53 ± 0.03	0.55 ± 0.03	0.59 ± 0.03	0.53 ± 0.03	0.63 ± 0.04	0.50 ± 0.06
MESOR						
D ref	37.20 ± 0.06	37.24 ± 0.07	37.04 ± 0.05	37.28 ± 0.11	37.02 ± 0.05	37.11 ± 0.03
D0	37.01 ± 0.02	37.07 ± 0.07	36.8 ± 0.10	37.06 ± 0.05	36.83 ± 0.09	37.11 ± 0.04
D1	37.22 ± 0.07	37.22 ± 0.08	37.14 ± 0.04	37.32 ± 0.11	37.14 ± 0.05	37.18 ± 0.04
D2	37.25 ± 0.06	37.24 ± 0.07	37.07 ± 0.04	37.29 ± 0.11	37.04 ± 0.06	37.11 ± 0.02
D3	37.25 ± 0.07	37.20 ± 0.07	37.04 ± 0.03	37.24 ± 0.10	36.99 ± 0.06	37.11 ± 0.02
D4	37.18 ± 0.05	37.19 ± 0.08	37.03 ± 0.05	37.25 ± 0.11	37.01 ± 0.04	37.10 ± 0.03
D5	37.22 ± 0.04	37.22 ± 0.07	37.09 ± 0.07	37.27 ± 0.10	37.03 ± 0.05	37.12 ± 0.02

Variations in parameters of rest–activity and body temperature rhythms for the three Zeitgeber times (ZTs) of administration, before propofol or Intralipid administration (D ref), on the day of administration (D0), and on the days after administration (D1, D2, D3, D4, D5). Performed by Chronos-Fit Software (Zuther and Lemmer, Institute of Pharmacology and Toxicology Faculty of Clinical Medicine, Mannheim, Germany). Values are given as mean ± SEM. Midline estimating statistic of rhythm (MESOR) and amplitude are expressed in degrees celsius (temperature) and counts (rest–activity). Acrophases are expressed as hour:minute and SEM in minutes (for rest–activity and temperature).

propofol anesthesia for all three Zeitgeber times, which was not detected after the administration of Intralipid (fig. 2 and table 1).

Analysis of variance on baseline acrophase showed no significant effect of Zeitgeber time ($F_{2,30} = 1.528$, $P = 0.233$) or treatment ($F_{1,30} = 0.005$, $P = 0.946$). Multivariate repeated-measure analysis (between factors: Zeitgeber time and treatment [propofol *vs.* Intralipid]; within factor: days [baseline, D0, D1, D2, D3, D4, D5]) on rest–activity rhythm acrophase showed significant effects of days ($F_{6,180} = 9.41$, $P < 10^{-4}$), Zeitgeber time ($F_{2,30} = 5.47$, $P = 0.009$), and treatment ($F_{1,30} = 6.9$, $P = 0.013$),

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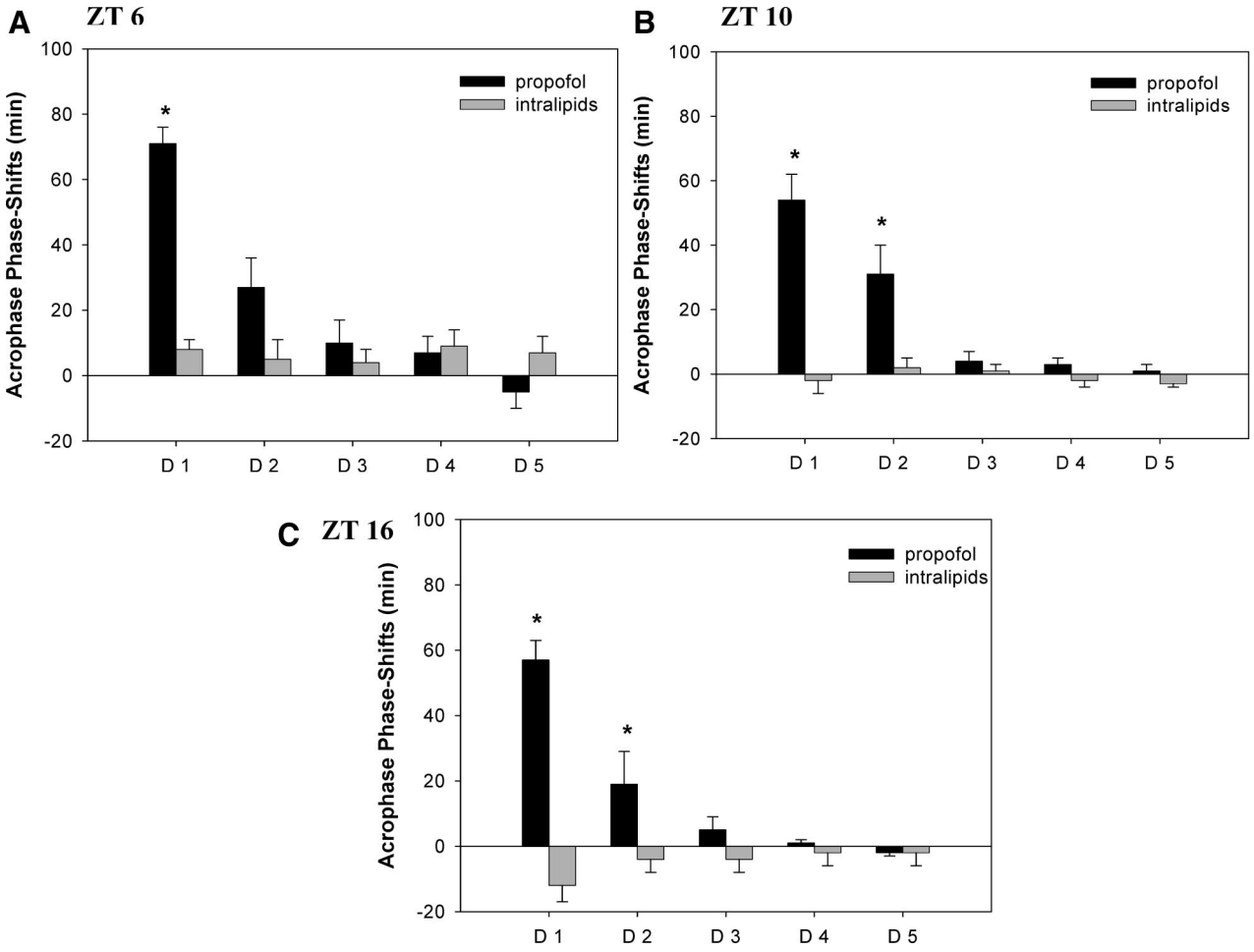

Fig. 2. Phase shift of rest–activity acrophase for the three Zeitgeber times (ZTs) of propofol administration (*A–C*) during the 5 days (D1, D2, D3, D4, D5) after propofol anesthesia or Intralipid administration. Positive values represent phase advances. Values are mean ± SEM. * Significant differences ($P < 0.05$) from mean values in preadministration period (the 5 days before propofol anesthesia).

with significant interaction (days × treatment: $F_{6,180} = 8.42$, $P = 0.001$).

Post hoc analysis for localization was performed on the acrophase phase shift from baseline calculated for each animal. For ZT6, the phase shift of the acrophase from baseline differed significantly between propofol and Intralipid on D1 (01:11 h ± 05 min *vs.* 00:08 h ± 03 min; $P < 10^{-4}$) (fig. 2A). For ZT10, the phase shift from baseline differed significantly between propofol and Intralipid on D1 (00:54 h ± 08 min *vs.* 00:02 h ± 03 min; $P < 10^{-4}$) and D2 (00:31 h ± 09 min *vs.* 00:02 h ± 03 min; $P = 0.001$) (fig. 2B). For ZT16, the observed shifts from baseline differed significantly between propofol and Intralipid on D1 (00:57 h ± 06 min *vs.* 00:12 h ± 05 min; $P < 10^{-4}$) and D2 (00:19 h ± 10 min *vs.* 00:04 h ± 04 min; $P = 0.05$) (fig. 2C).

Acrophase Body Temperature Rhythm

We observed a phase advance of body temperature acrophase during the 48 h after the administration of propofol anesthesia for all three Zeitgeber times, which was not detected after the Intralipid injection (fig. 3 and table 1). Analysis of variance on the baseline value of acrophase showed no significant effect of Zeitgeber time ($F_{2,30} = 2.73$, $P = 0.08$) or treatment ($F_{1,30} = 0.45$, $P = 0.5$). Multivariate repeated-measures analysis (between factors: Zeitgeber time and treatment [propofol *vs.* Intralipid]; within factor: days [baseline, D0, D1, D2, D3, D4, D5]) on body temperature rhythm acrophase showed significant effects of days ($F_{6,180} = 27.21$, $P < 10^{-4}$), treatment ($F_{1,30} = 14.43$, $P = 0.001$), and Zeitgeber time ($F_{2,30} = 8.66$, $P = 0.001$), with significant interactions (days × treatment: $F_{6,180} = 19.35$, $P < 10^{-4}$; Zeitgeber time × treatment: $F_{2,30} = 7.19$, $P = 0.003$).

Post hoc analysis for localization was performed on the acrophase phase shift from baseline calculated for each animal. For ZT6, the phase shift from baseline differed significantly between propofol and Intralipid on D1 (01:03 h ± 07 min *vs.* 00:10 ± 07 min), D2 (00:47 h ± 03 min *vs.* 00:06 h ± 03 min), D3 (00:19 h ± 03 min *vs.* 00:01 h ± 02 min; all $P < 10^{-4}$), and D4 (00:09 h ± 03 min *vs.* 00:01 h ± 02 min; $P = 0.03$) (fig. 3A). For ZT10,

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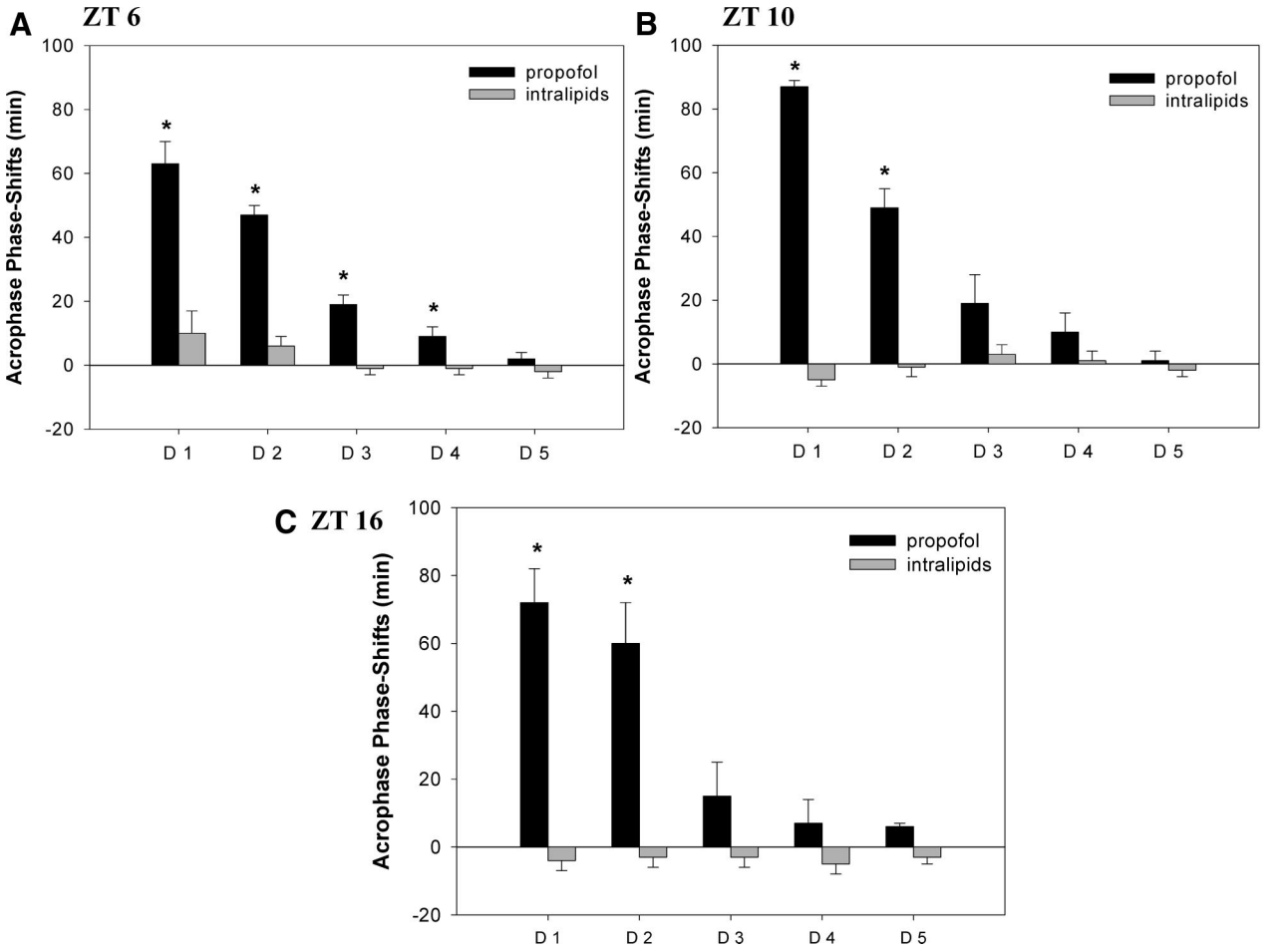

Fig. 3. Phase shift of body temperature acrophase for the three Zeitgeber times (ZTs) of propofol administration (*A–C*) during the 5 days (D1, D2, D3, D4, D5) after propofol anesthesia or Intralipid administration. Positive values represent phase advances. Values are mean ± SEM. * Significant differences ($P < 0.05$) from mean values in preadministration period (the 5 days before propofol anesthesia).

the phase shift from baseline differed significantly between propofol and Intralipid on D1 (01:27 h ± 02 min *vs.* 00:05 h ± 02 min; $P = 0.001$) and D2 (00:49 h ± 06 min *vs.* 00:01 h ± 03 min; $P < 10^{-4}$) (fig. 3B). For ZT16, the phase shift from baseline differed significantly between propofol and Intralipid on D1 (01:12 h ± 10 min *vs.* 00:04 h ± 03 min; $P < 10^{-4}$) and D2 (01:00 h ± 12 min *vs.* 00:03 h ± 03 min; $P < 10^{-4}$) (fig. 3C).

Amplitude of Rest–Activity Rhythm

We observed a decreased in the amplitude of the circadian rest–activity rhythm after propofol anesthesia, which was not detected after the Intralipid administration (fig. 4 and table 1). A significant decrease of the amplitude during the 48 h after propofol anesthesia was only detected when propofol was administered at ZT10. Indeed, for ZT6 and ZT10, only a tendency of decreased amplitude was observed, which was not significant. Analysis of variance on baseline value showed no significant effect of treatment ($F_{1,30} = 0.45$, $P = 0.506$) or Zeitgeber time ($F_{2,30} = 2.73$, $P = 0.08$). Multivariate repeated-measures analysis (between factors: Zeitgeber time and treatment [propofol *vs.* Intralipid]; within factor: days [baseline, D0, D1, D2, D3, D4, D5]) on the amplitude of rest–activity rhythm showed a significant effect of days ($F_{6,180} = 8.59$, $P < 10^{-4}$) and treatment ($F_{1,30} = 5.74$, $P = 0.023$) but no significative effect of Zeitgeber time ($F_{2,30} = 2.55$, $P = 0.09$), with significant interaction (days × treatment: $F_{6,180} = 4.56$, $P < 10^{-4}$). *Post hoc* analysis for localization was performed on the amplitude value. For ZT6, propofol anesthesia had no significant effect on the amplitude of rest–activity rhythm on D1–D5 (fig. 4A). For ZT10, propofol anesthesia significantly decreased the amplitude of rest–activity rhythm on D1 (27%; $P = 0.004$) and D2 (8%; $P = 0.013$) (fig. 4B). For ZT16, propofol anesthesia had no significant effect on the amplitude of rest–activity rhythm (fig. 4C).

Amplitude of Body Temperature Rhythm

We observed a decrease in the amplitude of body temperature circadian rhythm during the 48 h after propofol anesthesia, which was not detected after Intralipid administration (fig. 5 and table 1).

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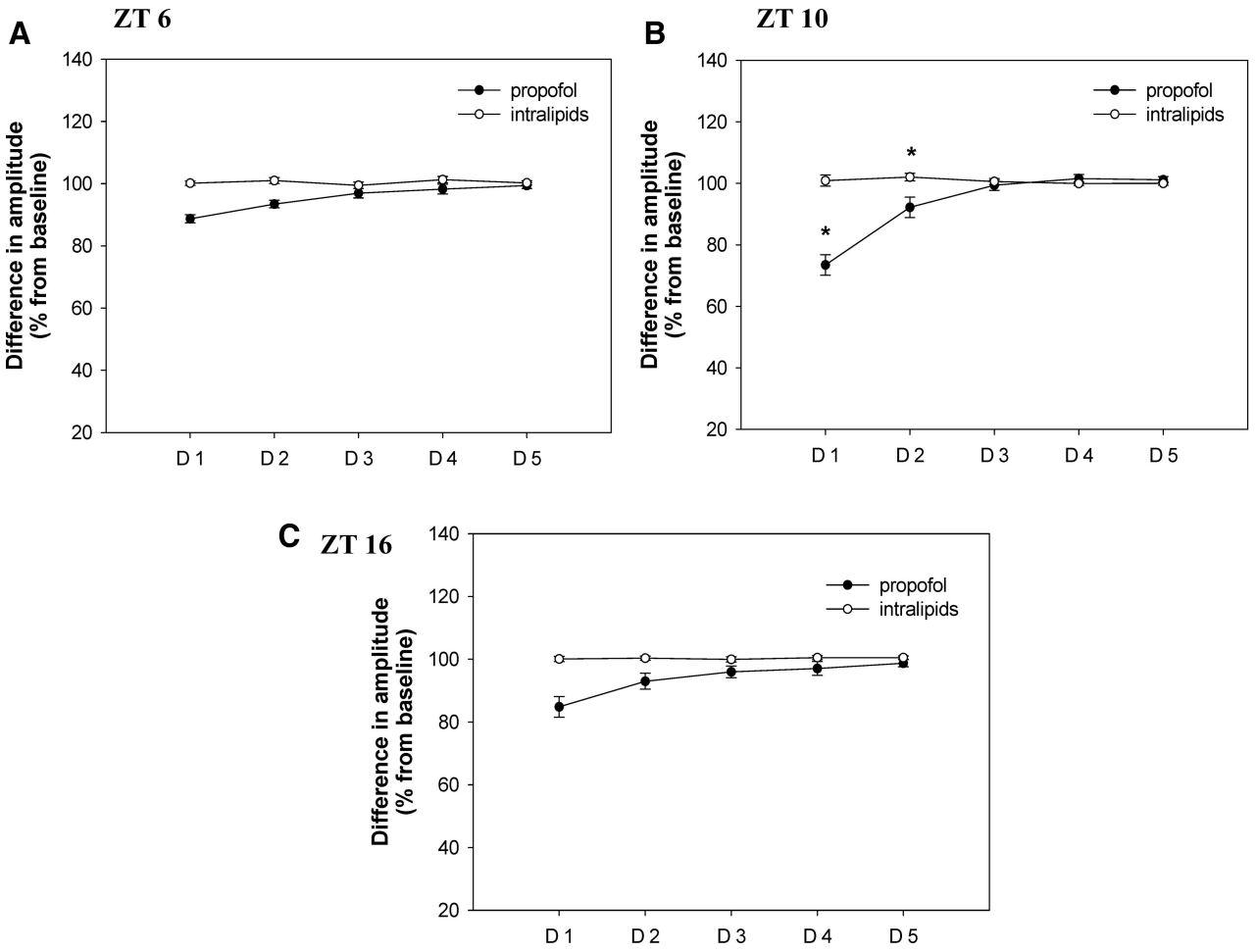

Fig. 4. Cosinor parameter of the amplitude of rest–activity rhythm during the 5 days after anesthesia (D1, D2, D3, D4, D5) for the three Zeitgeber times (ZTs) of propofol anesthesia or Intralipid administration (*A–C*). Baseline represents values of rest–activity amplitude 5 days before propofol administration taken as 100%. *Error bars* represent SEM. * Significant differences ($P < 0.05$) from mean values in preadministration period.

Analysis of variance on baseline value showed no significant effect of Zeitgeber time ($F_{2,30} = 0.977$, $P = 0.388$) or treatment ($F_{1,30} = 4.173$, $P = 0.05$). Multivariate repeated-measure analysis (between factors: Zeitgeber time and treatment [propofol *vs.* Intralipid]; within factor: days [baseline, D0, D1, D2, D3, D4, D5]) on the amplitude of body temperature rhythm showed a significant effect of days ($F_{6,174} = 65.70$, $P < 10^{-4}$) but no significant effect of treatment ($F_{1,29} = 1.01$, $P = 0.32$) or Zeitgeber time ($F_{2,29} = 0.60$, $P = 0.55$), with significant interaction (days × treatment: $F_{6,174} = 48.51$, $P < 10^{-4}$).

Post hoc analysis for localization was performed on the amplitude value. For ZT6, propofol anesthesia significantly decreased body temperature rhythm amplitude on D1 (36%; $P = 0.003$) and D2 (19%; $P = 0.006$) (fig. 5A). For ZT10, propofol anesthesia significantly decreased body temperature rhythm amplitude on D1 (57%; $P < 10^{-4}$) and D2 (29%; $P = 0.01$) (fig. 5B). For ZT16, propofol anesthesia significantly decreased body temperature rhythm amplitude on D1 (42%; $P = 0.04$) (fig. 5C).

MESOR of Rest–Activity Rhythm

We observed a decrease in the MESOR of the rest–activity rhythms during the 24 h after propofol anesthesia, which was not detected after the administration of Intralipid (fig. 6 and table 1).

Analysis of variance on baseline value showed no significant effect of Zeitgeber time ($F_{2,30} = 1.228$, $P = 0.307$) or treatment ($F_{1,30} = 0.049$, $P = 0.827$). Multivariate repeated-measure analysis (between factors: Zeitgeber time and treatment [propofol *vs.* Intralipid]; within factor: days [baseline, D0, D1, D2, D3, D4, D5]) on the MESOR of rest–activity rhythm showed a significant effect of days ($F_{6,180} = 32.03$, $P < 10^{-4}$) but no significant effect of treatment ($F_{1,30} = 0.82$, $P = 0.372$) or Zeitgeber time ($F_{2,30} = 0.94$, $P = 0.40$), with significant interaction (days × treatment: $F_{6,180} = 30.16$, $P < 10^{-4}$).

For ZT6, propofol anesthesia had no significant effect on the MESOR of the rest–activity rhythm during the days after the anesthesia (fig. 6A). For ZT10, propofol anesthesia significantly decreased the MESOR of the

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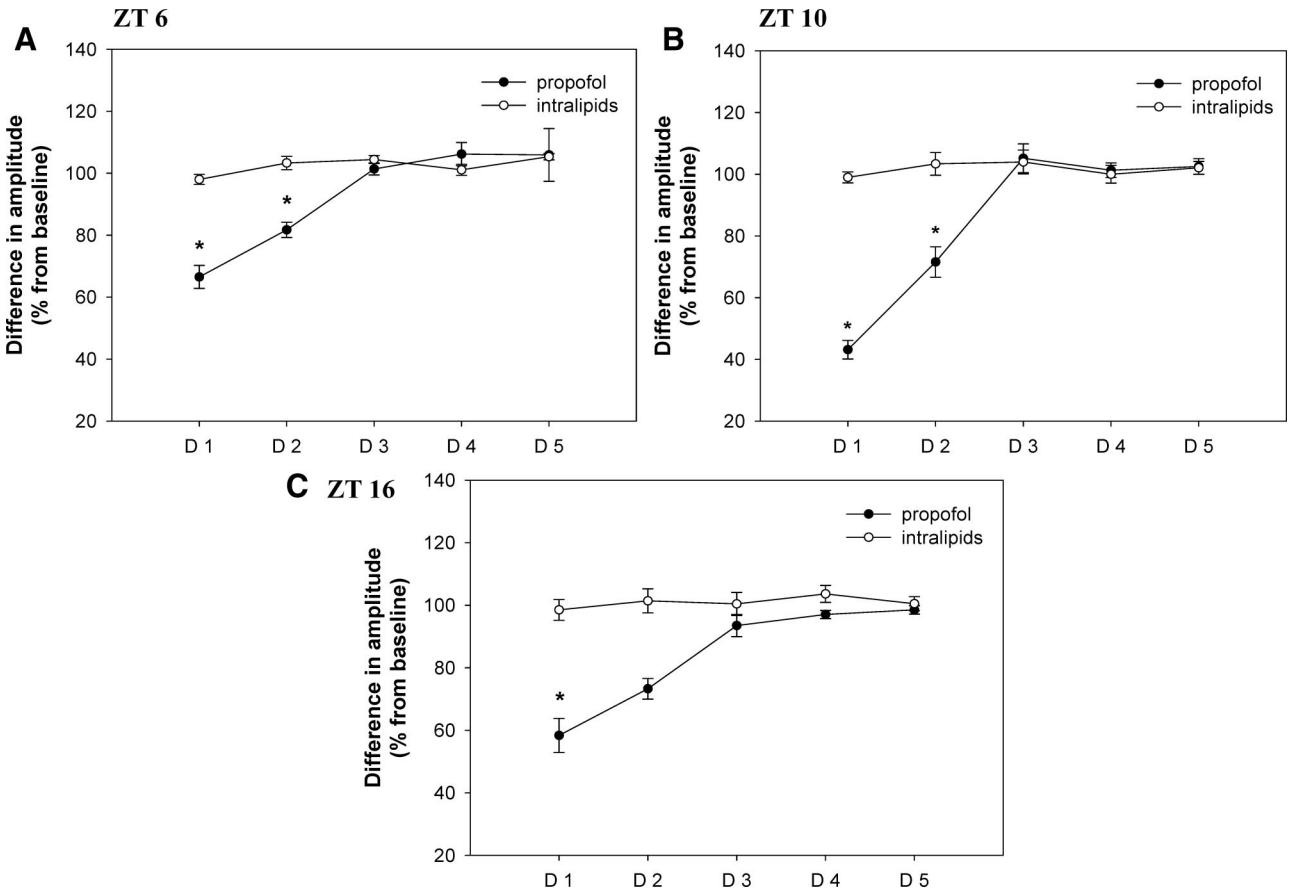

Fig. 5. Cosinor parameter of the amplitude of body temperature rhythm during the 5 days after anesthesia (D1, D2, D3, D4, D5) for the three Zeitgeber times (ZTs) of propofol anesthesia or Intralipid administration (*A–C*). Baseline represents values of rest–activity amplitude 5 days before propofol administration taken as 100%. *Error bars* represent SEM. * Significant differences ($P < 0.05$) from mean values in preadministration period.

rest–activity rhythm on D1 (19%; $P = 0.048$) (fig. 6B). For ZT16, propofol anesthesia had no significant effect on rest–activity rhythm MESOR during the days after anesthesia (all $P > 0.05$) (fig. 6C).

MESOR of Body Temperature Rhythm

We did not observe any modification of the MESOR of body temperature rhythm after the administration of propofol anesthesia (figs. 7A–C and table 1), except on the day of injection (D0, table 1). Excluding the day of anesthesia (D0), multivariate repeated-measures analysis (between factors: Zeitgeber time and treatment [propofol *vs.* Intralipid]; within factor: days [baseline, D1, D2, D3, D4, D5]) on the MESOR of body activity rhythm showed a significant effect of days ($F_{5,150} = 10.736$, $P < 10^{-4}$) but no significant effect of treatment ($F_{1,30} = 3.60$, $P = 0.067$) or Zeitgeber time ($F_{2,30} = 2.625$, $P = 0.089$).

Table 2 illustrates and recapitulates all the significant changes induced by propofol administration on the three cosinor parameters during the postanesthesia days described in the Results section. It recaps the days during which the cosinor parameters were significantly disturbed by propofol anesthesia.

Discussion

The current study shows that general propofol anesthesia decreases the amplitude and induces a 1-h phase advance of both rest–activity and body temperature circadian rhythms during the 48 h after general anesthesia. These results demonstrate the disturbing effects of general propofol anesthesia on circadian rhythms in rats under usual alternation of light and darkness.

Our data show that propofol induces a significant shift of the acrophase of both rest–activity and core body temperature (60–80 min) on the day after anesthesia (D1) when compared with reference days (figs. 2 and 3). The 1-h phase advance of rest–activity and body temperature rhythm represents approximately half of the maximal resetting effect of a light pulse in rodents in constant darkness.[13] Moreover, rats were only anesthetized during 30 min, and therefore, the observed phase advance of the two rhythms is twice greater than the duration of propofol anesthesia. Rest–activity and body temperature rhythms are often used as circadian phase markers in mammals because of their coupling with the endogenous circadian profile. However, the core body temperature rhythm is less influenced by external fac-

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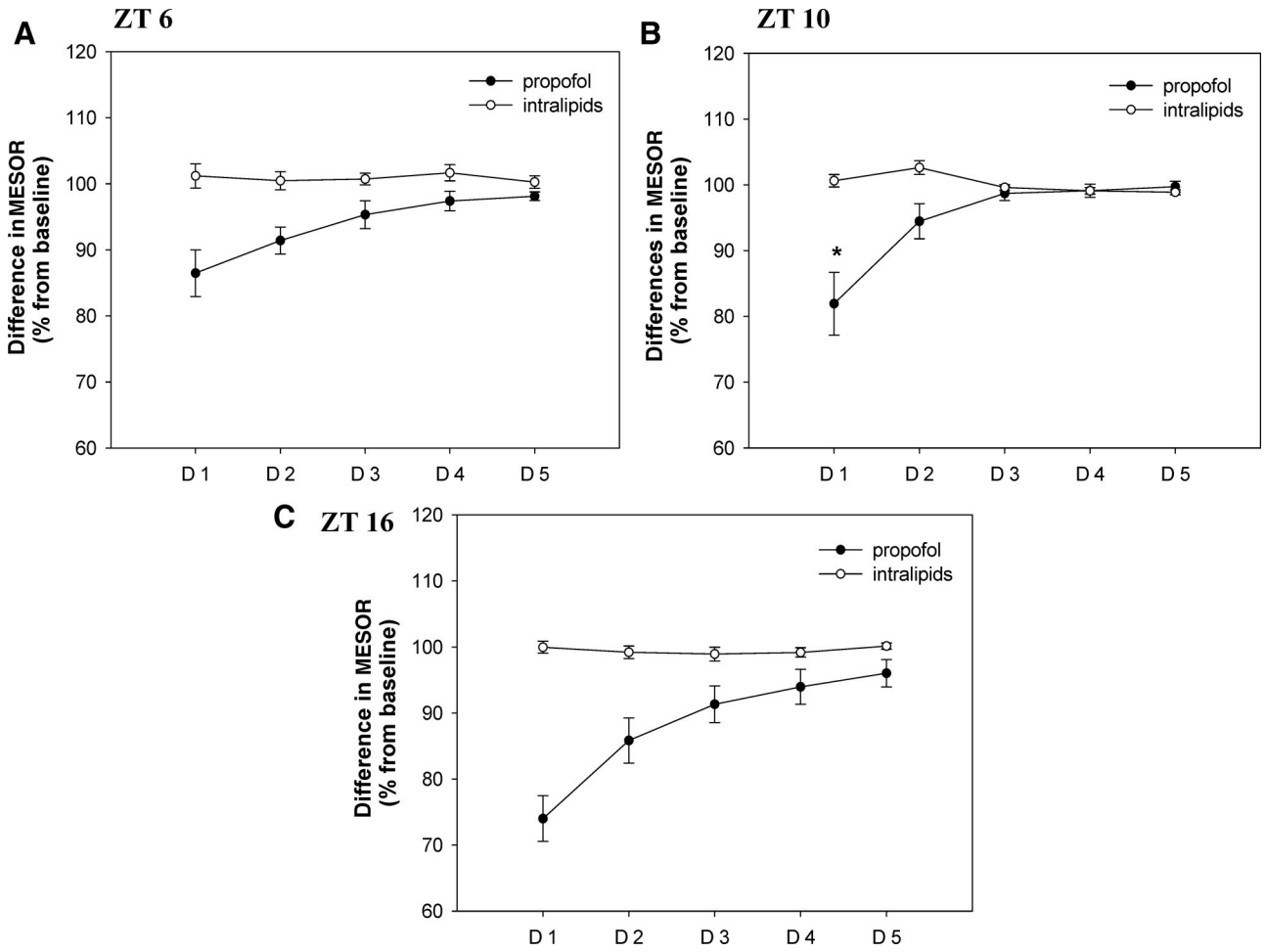

Fig. 6. Cosinor parameter of the midline estimating statistic of rhythm (MESOR) of rest–activity on the 5 days after anesthesia (D1, D2, D3, D4, D5) for the three Zeitgeber times (ZT) of propofolanesthesia or Intralipid administration (*A–C*). Baseline represents values of rest–activity MESOR 5 days before propofol administration taken as 100%. *Error bars* represent SEM. * Significant differences ($P < 0.05$) from mean values in preadministration period.

tors and the sleep-wake cycle than rest–activity rhythm and thus seems to be a more robust parameter to reflect the endogenous circadian profile.[14] In the current study, we showed that both rest–activity and core body temperature circadian markers were disturbed by general anesthesia, which is indicative of a significant dysregulation of endogenous circadian organization. Few studies have focused on the effects of general anesthesia on rest–activity and body temperature rhythms. In humans (healthy volunteers), it has been shown that isoflurane (subanesthetic dose) does not disturb circadian parameters of the MESOR and acrophase of body temperature rhythm, but decreases the amplitude on the day of anesthesia.[15] In rats, it has been shown that ketamine anesthesia disturbs the circadian parameters (MESOR, amplitude, acrophase) of body temperature and general locomotor activity rhythms on the day of anesthesia[16] but returns to basal values on the day after anesthesia (except for locomotor activity MESOR, which is still disturbed 3 days after anesthesia). One previous study has also shown that propofol induces a 1-h phase advance of rest–activity rhythm when free-running rats are anesthetized during the rest period.[6] We would have thought that this 1-h phase advance obtained in constant darkness would not be repeated in conditions of light–dark alternation. Indeed, light could diminish the impact of propofol on the circadian time structure by creating a masking effect. Light is the most powerful synchronizer, and exposure to light is currently used to faster resynchronize disturbed circadian rhythms.[17–19] The presence of light during our experiment could have induced a masking effect that could decrease propofol effects on the circadian time structure. Indeed, a measured circadian rhythm is a mixture of an endogenous component (that reflects the body clock and can be estimated in constant darkness conditions) and a nonendogenous component (*e.g.*, light, social synchronizers).[14] The nonendogenous component can thus mask the endogenous effects of an external factor such as propofol on the circadian time structure. We have shown that the effects of propofol anesthesia on the circadian time structure, which could have been decreased by exposure to light,

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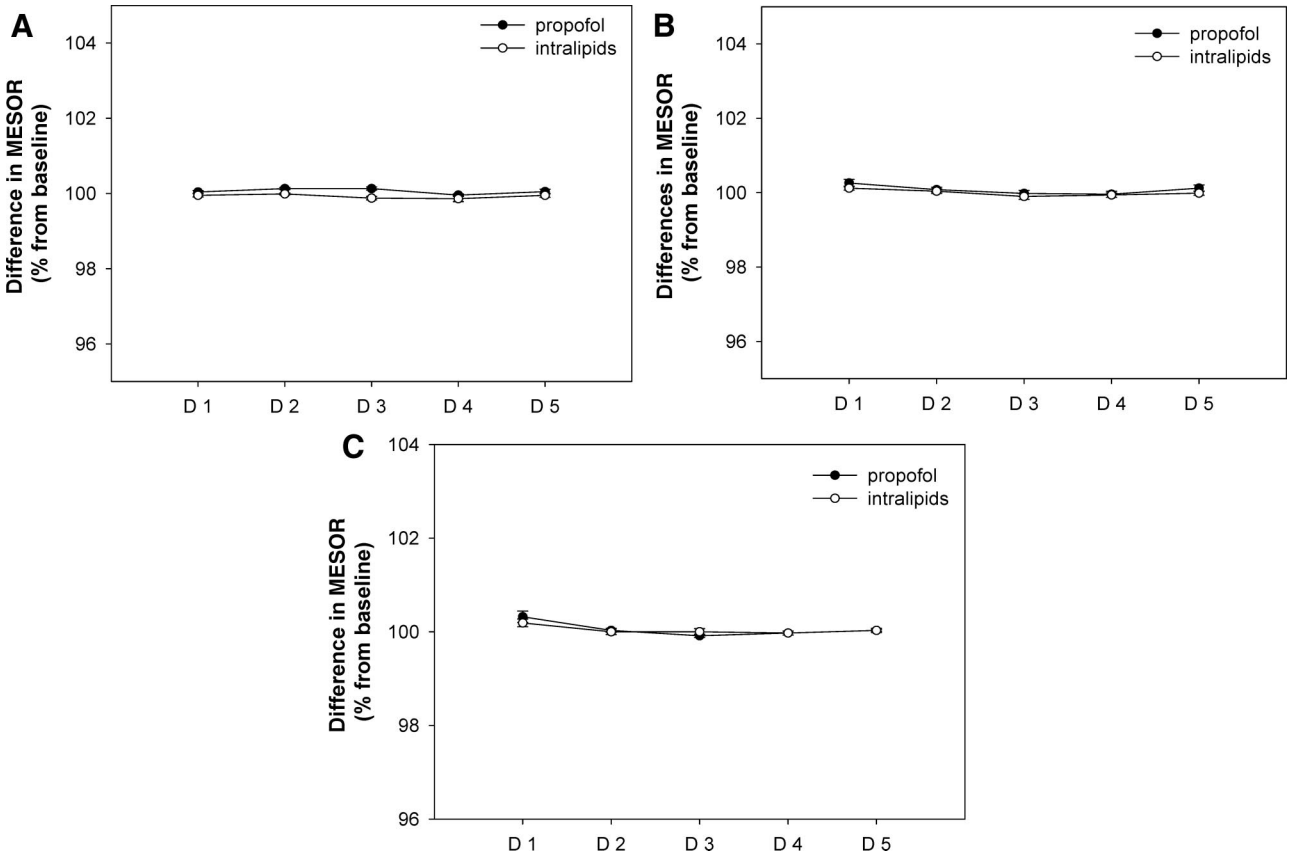

Fig. 7. Cosinor parameter of the midline estimating statistic of rhythm (MESOR) of body temperature on the 5 days after anesthesia (D1, D2, D3, D4, D5) for the three Zeitgeber times of propofol administration (*A–C*). Baseline represents values of rest–activity MESOR 5 days before propofol administration taken as 100%. *Error bars* represent SEM. * Significant differences ($P < 0.05$) from mean values in preadministration period.

persist for at least 2 days. Therefore, the fact that, even in the presence of light, our two circadian rhythms studied are still phase shifted demonstrates the important effect of propofol on the circadian structure. We therefore consider that a 1-h phase advance of the circadian time structure in normal light–dark conditions induced by a single dose of propofol is important. According to the literature, hypothermia can induce disturbing effects on the circadian time structure. Indeed, changes in temperature can phase shift circadian neuronal activity rhythms of suprachiasmatic nuclei maintained *in vitro*.[20,21] General anesthesia can be associated with transient hypothermia, but we did not observe significant difference in the MESOR of body temperature when comparing Intralipid and anesthetic injections during the 5 days after drug administration (fig. 7). Indeed, we observed that intraperitoneal injections of propofol or Intralipid were both associated with a transient decrease in the MESOR of core body temperature on the day of injection, which then returns to baseline values the day after injection (D1). Moreover, we observed a positive correlation between the shifts in circadian rhythms of rest–activity and body temperature in rats injected with propofol or Intralipid on day 1. Therefore, the observed changes in body core temperature during the days after general anesthesia could not be biased by anesthesia-induced possible hypothermia.

Table 2. Days during Which Cosinor Parameters Were Significantly Disturbed by Propofol Anesthesia

	ZT6	ZT10	ZT16
Acrophase			
Rest–activity	D1	D1, D2	D1, D2
Body temperature	D1, D2, D3, D4	D1, D2	D1, D2
Amplitude			
Rest–activity	—	D1, D2	—
Body temperature	D1, D2	D1, D2	D1
MESOR			
Rest–activity	—	D1	—
Body temperature	—	—	—

This tables recapitulates the postoperative days (D1, D2, D3, D4) within the cosinor parameters studied for rest–activity and body temperature rhythms (acrophase, amplitude, midline estimating statistic of rhythm [MESOR]) significantly disturbed by propofol anesthesia administered at three different Zeitgeber times (ZTs).

At first glance, general propofol anesthesia seems to affect the body temperature circadian rhythm more than the rest–activity circadian rhythm. Indeed, the magnitude of phase shift and the decrease in amplitude seem more pronounced with regard to body temperature than to rest–

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activity. Changes in phase shift and amplitude were also more prolonged for body temperature than for rest–activity rhythms. Rats were submitted to light synchronization during the days after anesthesia, as well as during the reference period; therefore, it might be that light is more efficient in resynchronizing the rest–activity rhythm than the body temperature rhythm, which is less susceptible to external photic synchronizers than the rest–activity rhythm.

Differences in the reentrainment of rest–activity and body temperature rhythms have already been observed.[22] These differences seem to be linked to differential projections from the neurons of the suprachiasmatic nucleus to paraventricular zona, with the dorsal paraventricular zona being involved in the control of body temperature and the ventral paraventricular zona being involved in the control of rest–activity rhythm.[23]

Alterations of the acrophases, MESORs, and amplitudes of rest–activity and body temperature rhythms were different on the days after anesthesia according to the Zeitgeber time of injection. Indeed, as shown in the table 2, when propofol is administered at ZT6 and ZT10, circadian parameters of rest–activity and body temperature rhythms (acrophase, amplitude, and MESOR) are more disturbed than after propofol administration at ZT16. These results could be explained by the chronopharmacology of propofol, because the duration of propofol anesthesia exhibits a significant circadian rhythm. Indeed, chronopharmacologic studies in animals showed that the longest duration of general anesthetics (ketamine, halothane, pentobarbital, propofol) and their maximum hypnotic effect occur during the rest period.[6,24–26] Chronopharmacology of general anesthetics could be linked to circadian variations in the postsynaptic γ-aminobutyric acid receptor, whose peak activity and maximal receptor-binding affinity occur during the rest period.[27,28] Propofol, like the majority of general anesthetics, acts on γ-aminobutyric acid type A receptors,[29] and therefore, circadian variations in the activity of the γ-aminobutyric acid receptor could explain the circadian variability observed in the duration of propofol anesthesia. Our results show also that the maximal impact of propofol anesthesia on circadian rest–activity and body temperature rhythms occurs during the rest period. In our study, an alternative explanation for the observed differences between ZT6, ZT10, and ZT16 groups is the powerful effect of light. Indeed, for the ZT16 time of injection, rats were injected 4 h after the beginning of their activity period and thus were exposed to light 8 h after injection. This fast reexposure to light, which was not observed for the two other injection times, could thus interfere with the effects of propofol anesthesia and therefore minimize the observed effects of propofol in animals.

From our results, we cannot demonstrate the cellular mechanisms by which general anesthesia has affected the circadian temporal structure. This is currently under examination.

However, our study demonstrates for the first time that propofol administered at an anesthetic dose impacts the circadian rhythms of rest–activity and body temperature in rats submitted to light–dark alternation. The persistence of disturbing effects of propofol anesthesia on the circadian time structure under naturalistic light conditions in rats may be of particular interest to understand general anesthesia effects on the circadian time structure in humans. Indeed, in humans, general anesthesia can induce several clinical symptoms on the postanesthesia days (*e.g.*, fatigue, sleep disorders, drowsiness).[30] Studying propofol anesthesia effects on the circadian time structure in humans is of particular clinical relevance because propofol is one of the most used anesthetics in humans, particularly in ambulatory surgery. Therefore, even if the effects of propofol observed in rats persist for only a few days after anesthesia, it is of particular importance in ambulatory procedures, where patients return home on the day of anesthesia and go back to work and social activities on the day after anesthesia. Indeed, it was previously shown that in ambulatory practice of anesthesia for medical procedures, more than 30% of patients reported fatigue, drowsiness, decrease in vigilance, and mood alterations.[31] Regarding our results, it is thus possible that disturbing effects on circadian rhythms induced by propofol could be, at least in part, responsible for fatigue symptoms and the decrease in vigilance observed during the postanesthesia days.

The authors thank Mounir Chennaoui, Ph.D., and Danièle Gomez, Ph.D. (Researchers, Institut de Médecine Aérospatiale du Service de Santé des Armées, Brétigny-sur-Orge, France), for their technical assistance.

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