Impact of hypothermia on emergence from isoflurane anesthesia in orexin neuron-ablated mice

1. Chiharu Kuroki, MD.

Title:

Affiliation: Departments of Physiology and Anesthesiology, Kagoshima University Graduate School of Medical and Dental Sciences

Email: chiharu@m2.kufm.kagoshima-u.ac.jp

Role: This author helped design the study, conduct the study, analyze the data, and write the manuscript

Conflicts: Chiharu Kuroki reported no conflicts of interest

Attestation: Chiharu Kuroki has seen the original study data, reviewed the analysis of the data, and approved the final manuscript

2. Yoshiko Takahashi, MD.

Title:

Affiliation: Departments of Physiology and Anesthesiology, Kagoshima University Graduate School of Medical and Dental Sciences

Email: yt445@m2.kufm.kagoshima-u.ac.jp

Role: This author helped design the study, conduct the study, and analyze the data

Conflicts: Yoshiko Takahashi reported no conflicts of interest

Attestation: Yoshiko Takahashi has seen the original study data, reviewed the analysis of the data, and approved the final manuscript

3. Youichirou Ootsuka, Ph.D.

Title: Associate Professor

Affiliation: Department of Physiology, Kagoshima University Graduate School of Medical and Dental Sciences

Email: yotsuka@m3.kufm.kagoshima-u.ac.jp

Role: This author helped design the study, analyze the data, and write the manuscript

Conflicts: Youichirou Ootsuka reported no conflicts of interest

Attestation: Youichirou Ootsuka has seen the original study data, reviewed the analysis of the data, and approved the final manuscript

4. Yuichi Kanmura, MD., Ph.D.

Title: Professor

Affiliation: Department of Anesthesiology, Kagoshima University Graduate School of Medical and Dental Sciences

Email: kanmura@m3.kufm.kagoshima-u.ac.jp

Role: This author helped analyze the data and write the manuscript

Conflicts: Yuichi Kanmura reported no conflicts of interest

Attestation: Yuichi Kanmura reviewed the analysis of the data and approved the final manuscript

5. Tomoyuki Kuwaki, Ph.D.

Title: Professor

Affiliation: Department of Physiology, Kagoshima University Graduate School of Medical and Dental Sciences

Email: kuwaki@m3.kufm.kagoshima-u.ac.jp

Role: This author helped design the study, analyze the data, and write the manuscript

Conflicts: Tomoyuki Kuwaki reported no conflicts of interest

Attestation: Tomoyuki Kuwaki has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files

Institution: Kagoshima University Graduate School of Medical and Dental Sciences

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Corresponding Author:

Tomoyuki Kuwaki, Ph.D.

Kagoshima University Graduate School of Medical and Dental Sciences

Sakuragaoka 8-35-1, Kagoshima 890-8544, Japan

Phone: +81-99-275-5227

FAX: +81-99-275-5231

Email: kuwaki@m3.kufm.kagoshima-u.ac.jp

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Abstract

BACKGROUND: Orexin neurons regulate the sleep/wake cycle and are proposed to influence general anesthesia. In animal experiments, orexin neurons have been shown to drive emergence from general anesthesia. In human studies, however, the role of orexin neurons remains controversial, owing at least in part to the fact that orexin neurons are multifunctional. Orexin neurons regulate not only the sleep/wake cycle, but also body temperature. We hypothesized that orexin neurons do not directly regulate emergence from anesthesia, but instead affect emergence indirectly through thermoregulation because anesthesia-induced hypothermia can greatly influence emergence time. To test our hypothesis, we used simultaneous measurement of body temperature and locomotor activity.

METHODS: We used male orexin neuron-ablated (ORX-AB) mice and their corresponding wild-type (WT) littermates to investigate the role of orexin neurons in emergence. Body temperature was recorded using an intraperitoneally implanted telemetric probe, and locomotor activity was measured using an infrared motion sensor. Induction of anesthesia and emergence from anesthesia were defined behaviorally as loss and return, respectively, of body movement. Mice received general anesthesia with 1.5% isoflurane in 100% oxygen for 30 min under three conditions: In the first experiment, the anesthesia chamber was warmed (32°C), ensuring a constant body temperature of animals during anesthesia. In the second experiment, the anesthesia chamber was maintained at room temperature (25°C), allowing body temperature to fluctuate. In the third experiment in WT mice, the anesthesia chamber was cooled (23°C) so that their body temperature would decrease to the comparable value to that obtained in the ORX-AB mice during room temperature condition.

RESULTS: In the warmed condition, there were no significant differences between the ORX-AB and control mice with respect to body temperature, locomotor activity, induction time, or emergence time. In the room temperature condition, however, anesthesia-induced hypothermia was greater and longer lasting in ORX-AB mice than that in WT mice. Emergence time in ORX-AB mice was significantly prolonged from the warmed condition (14.2±0.8 vs. 6.0±1.1 min) while that in WT mice was not different (7.4±0.8 vs. 4.9±0.2 min). When body temperature was decreased by cooling in WT mice, emergence time was prolonged to 12.4±1.3 min. Induction time did not differ among temperature conditions or genotypes.

CONCLUSIONS: The effect of orexin-deficiency to impair thermoregulation during general anesthesia is of sufficient magnitude that body temperature must be appropriately controlled when studying the role of orexin neurons in emergence from anesthesia.

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Introduction

Orexin neurons contribute to a wide range of physiological functions, including regulation of arousal, wakefulness, cardiovascular function, respiration, autonomic responses, the fight-or-flight response, and feeding.[1] The role of orexin neurons in regulating wakefulness is of particular interest for researchers and clinicians because degeneration of orexin neurons is implicated in the pathological dysregulation of wakefulness observed in narcolepsy,[2,3] and these neurons may also participate in the regulation of wakefulness during anesthesia. In animal experiments, orexin neurons have been proposed to drive emergence from a variety of anesthesias,[4-9] while orexin neurons exert a minimal influence on anesthesia induction. In human studies, the role of orexin neurons in induction and emergence from anesthesia remains controversial. Plasma orexin levels have been observed to increase after (but not before) emergence in patients undergoing general anesthesia.[10] Prolonged emergence time from general anesthesia in a narcolepsy patient has also been reported,[11] although information detailing the perioperative outcome and management for narcolepsy patients remains scarce. Theoretical complications for these patients include postoperative hypersomnia, prolonged emergence after general anesthesia,[11] apnea with sleep paralysis,[12] and interactions with medications.[13,14] However, the actual frequency of these complications has not been documented, and one study suggests that there narcoleptic patients undergoing anesthesia experience no increased risk for postoperative complications or difference in time for endotracheal extubation and in length of stay in the post anesthesia care unit and hospital.[15]

The thermoregulatory role of orexin neurons has become more widely appreciated in recent years.[16-19] We recently found that orexin neuron ablated (ORX-AB) mice (an animal model of human narcolepsy[20]) had no tolerance for cold exposure[21] and had attenuated stress-induced hyperthermia.[19] Because anesthesia-induced hypothermia greatly impacts

emergence time,[22] we hypothesized that orexin neurons may affect emergence time through their thermogenic actions. Delay of emergence from general anesthesia has been reported in ORX-AB mice.[4] However, the concomitant changes in body temperature have not been reported in previous studies. Therefore, the aim of the present study was to examine whether ablation of orexin neurons affected general anesthesia-induced hypothermia and induction and emergence time by using simultaneous measurement of body temperature and body movement.

Methods

Ethical approval

All experimental procedures were performed in accordance with the guiding principles for the care and use of animals in the field of physiological sciences published by the Physiological Society of Japan and were approved by the Institutional Animal Use Committee at Kagoshima University.

Animals

Six 5- to 8-month-old male ORX-AB mice[20] (original mating pairs were a kind gift from Professor Takashi Sakurai at Kanazawa University, Japan) and 12 wild-type (WT) littermates weighing 31 to 38 g were used in this study. In ORX-AB mice, almost all of orexin neurons are ablated by 4 months of age through expression of the neurotoxin ataxin-3, under the control of the human orexin promoter.[20] ORX-AB mice were maintained as heterozygotes and crossed with C57BL/6 mice (CLEA Japan, Inc., Tokyo, Japan) to obtain ORX-AB mice and WT mice. To maximize genetic homogeneity, we backcrossed the ORX-AB mice with C57BL/6 mice for more than 10 generations. The genotype of ORX-AB mice was identified by PCR of DNA extracted from the tail.[20] Mice were housed in a room maintained at 22 to 25°C, with lights on at 0700 and off at 1900. Mice had food and tap water available *ad libitum* during housing and all experimental procedures.

Physiological recording

Body temperature was recorded using intraperitoneally implanted telemetric probes (TA10TA-F10 or TA11TA-F10, Data Science International, MN, USA). For implantation of the telemetric probe, mice were anesthetized with 2-3% isoflurane. After surgery, mice were given penicillin G and acetaminophen. Locomotor activity was recorded with passive infrared type motion sensor (AMN 1111, Panasonic Co., Osaka, Japan) attached on the ceiling of the

anesthesia chamber.

Body temperature and locomotor activity signals were continuously monitored and digitized by PowerLab (ADInstruments, Castle Hill, Australia) and were stored and analyzed using LabChart software (ADInstruments). Body temperature and locomotor activity data were averaged and integrated for every 1s, respectively. They were further averaged across 5-min segments of time for analysis of time-related changes in them.

Anesthesia regimen and assessment of induction and emergence

After a 1-week period of recovery from surgery, mice were acclimated to an anesthesia chamber. Mice were gently placed in the anesthesia chamber (a 600 ml container with in- and out-flow gas tubing and water supply) that was continuously flushed with 100% oxygen (300 ml/min) for 3 h. This procedure was repeated on every other day for 1 week (3 times in total).

All experiments were performed in a quiet, air-conditioned (25 ± 1°C) room from 1900 to 2100, just after lights off, when the mice's locomotor activity, wakefulness, and orexin neuron activity are maximal.[23] Mice were gently placed in the anesthesia chamber continuously flushed with 100% oxygen for 2 h prior to the anesthesia regimen.

Mice received general anesthesia with 1.5% isoflurane (Escain, Mylan Inc., Tokyo, Japan) in 100% oxygen for 30 min using a vaporizer (VIP Sigma, IMI CO. LTD., Saitama, Japan). Anesthetic gas concentration was checked with a gas monitor prior to experimentation (AS/3, Datex-Ohmeda Co. Helsinki, Finland). We set isoflurane inspiration concentration at 1.5% for both ORX-AB mice and WT mice because the minimum alveolar concentration (MAC) of WT mice (C57BL/6 strain) is 1.30 ± 0.11%[24] and the MAC of ORX-AB mice is equivalent to that of WT mice.[4]

In the first series, mice received anesthesia in a warm (32±1°C) chamber (heated using a water bath) so that their body temperature would not change during anesthesia. In the second

series, mice received anesthesia in room atmosphere (25±1°C), allowing their body temperature to fluctuate. After the anesthesia regimen and physiological recording, mice were returned to their home cage allowed to rest for at least 3 days before another experiment. The order of experiments (warm or room temperature condition) was randomized. In the third series using a different set of the WT mice (n=6), the animals received anesthesia in a cooled (23±1°C) chamber, so that their body temperature would decrease to the comparable value (33°C, see results section) to that obtained in the ORX-AB mice during room temperature condition.

Anesthesia induction and emergence times were defined as lag time to loss and return, respectively, of body movement signals over successive 3 time bins (3 sec). Sensitivity of the motion sensor was set so that muscle twitching on a background of quiet immobility (an indicator of rapid-eye-movement sleep) would not be detected.

Statistics

The effect of anesthesia with isoflurane on body temperature and locomotor activity was assessed by Friedman test. When *P*-value was less than 0.05, within-subjects effects over time was analyzed by Dunn's post hoc test. Possible body temperature difference at each time point was determined by 2-way ANOVA with genotype as the main factor and time as a repeated measure and Scheffe's post hoc test. The time to induction and emergence were analyzed by 1 factor ANOVA followed by Scheffe's post hoc test. Data are presented as mean ± SEM. Differences were considered significant at $P < 0.05$.

Results

Time-related changes in body temperature and locomotor activity

In the warmed condition, body temperature did not change during or after anesthesia (Fig. 1A). Body temperature before anesthesia was 37.4 [95% confidence interval; 36.5 to 38.3] °C in WT mice (n = 6) and 37.6 [37.0 to 38.2] °C in ORX-AB mice (n = 6), and these values were maintained within ± 0.5°C throughout the experiment. There was no significant difference in body temperature between ORX-AB mice and WT mice at any time point. Although the locomotor activity reappeared after anesthesia, it did not reach the control value (Fig. 1B), presumably because locomotor activity is lower in hot environments. Locomotor activity in ORX-AB mice was significantly lower at 15 min and significantly greater at 30 min after anesthesia than that observed in WT mice.

By contrast, isoflurane induced a marked hypothermia in WT mice in the room temperature condition (Fig. 1C). Body temperature was significantly different between the control period (37.0 [36.5 to 37.5] ºC) and the time period from the 20 min under anesthesia to the 20 min after anesthesia, with its nadir at 34.1 [33.3 to 34.8] °C (fall of - 3.1 [-2.3 to -3.9] °C, n = 6). Although there was a delay (20 min) in the recovery of body temperature after anesthesia, locomotor activity soon reappeared when anesthesia was discontinued (Fig. 1D).

In ORX-AB mice in the room temperature condition, body temperature at the control period (37.3 [36.4 to 38.2] °C) was not different from that in the WT mice. However, body temperature continued to decrease even after the anesthesia was discontinued (Fig. 1C). Consequently, body temperature in the ORX-AB mice was significantly different from that at the control period from 20 min under anesthesia to 25 min after anesthesia, with its nadir of 33.1 [32.3 to 34.0] °C (fall of - 4.2 [-3.6 to -4.9] °C, n = 6). There was a significant difference in body temperature between ORX-AB and WT mice at 15 to 30 min after the anesthesia was

discontinued. In ORX-AB mice, recovery of locomotor activity was also delayed and attenuated during the emergence period (Fig. 1D).

In an additional experiment, how much the decreased body temperature would contribute to the delayed arousal was examined in the WT mice. For this purpose, WT mice (n=6) were anesthetized in a cooled (23°C) chamber and their body temperature was decreased to 32.9 [32.6 to 33.1] °C (Fig. 1C). As was the case in ORX-AB mice in the room temperature condition, recovery of body temperature was delayed and attenuated during the emergence period (Fig. 1C).

Time to induction and emergence from anesthesia

Because locomotor activity changes much more rapidly than body temperature, we next quantified the time to induction of anesthesia and time to emergence from anesthesia with a higher time resolution to give a more detailed analysis of induction and emergence. There was no difference in the induction time among 5 groups (genotype and temperature conditions) of the animals ($F_{4,25}$=1.251; p=0.316; statistical power was 0.10, Fig. 2A). As to the emergence time, on the other hand, ANOVA revealed significant difference among the animals ($F_{4,25}$=19.557; p=0.0001; statistical power was 1.00, Fig. 2B). At the warmed condition, emergence time in ORX-AB mice (6.0±1.1 min) was seemingly larger than that in WT mice (4.9±0.2 min) but the difference was not significant (p=0.958, Scheffe's procedure). At the room temperature condition, emergence time in ORX-AB mice (14.2±0.8 min) was significantly longer than that in WT mice (7.4±0.8 min, p=0.0008). This was because temperature-dependent prolongation of the emergence time was evident in the ORX-AB mice (p=0.0001) but not in the WT mice (p=0.475). In a cooled condition, prolongation of the emergence time also became evident in the WT mice (p=0.0002 vs. warmed condition and p=0.018 vs. room temperature condition).

DISCUSSION

Anesthesia induced hypothermia was exaggerated in ORX-AB mice

In this study, anesthesia-induced hypothermia was greater and longer lasting in ORX-AB mice than that in WT mice when the animals were not warmed (Fig. 1C). This result suggests that orexin neurons are important for maintaining body temperature during general anesthesia with isoflurane. Orexin neurons have been shown to contribute to the regulation of body temperature through their axonal projections to the raphe pallidus (RPa),[1,17] which activates brown adipose tissue (BAT) via sympathetic innervation. Consistent with this mechanism, intraventricular administration[16] or nanoinjection of orexin-A into the RPa[17] increased the sympathetic firing rate and raised core temperature. Orexin neurons are activated from cold exposure, and ORX-AB mice exhibit extremely low tolerance for cold temperatures.[21] Taken together, the lack of thermogenic action by orexin neurons in ORX-AB mice seems to account for the exaggerated hypothermia observed in this experiment.

Emergence time in ORX-AB mice was prolonged at room temperature

In this study, emergence time from anesthesia in ORX-AB mice was significantly prolonged in room temperature experiment (Fig. 2B), but not in the warmed experiment. This result was surprising to us because orexin neurons have been proposed to drive emergence from anesthesia. However, previous reports proposing orexin's role in emergence time did not report simultaneous recording of the body temperature during anesthesia.[4-9] The present study is, to our knowledge, the first report to demonstrate the importance of simultaneous recording of body temperature during assessment of emergence time. Our notion is further supported by a prolonged emergence time at the cooled condition in WT mice (Fig. 2B).

It is worth noting that the definitions of induction and emergence differ across studies. While we used motion sensor data, EEG[4-6] and measurement of righting reflex[4,5,7-9] are alternate

methods to assessing induction and emergence. However, we expect our results to be robust to different methods of measurement because these variables are well correlated with one another.[25] We were also concerned about possible baseline difference in locomotor activity that would affect induction/emergence time because ORX-AB mice is less active than the WT mice.[20] However, baseline locomotor activity was not different between the genotypes during both warmed (Fig. 1B) and the room temperature (Fig. 1D) conditions and thus our interpretation seems valid. Acclimatization to the chamber in our protocol seemed effective because less locomotor activity in orexin deficient mice would become apparent under stressful condition such as food restriction[26] or forced running.[27]

Perspectives and conclusion

Thermoregulation during anesthesia may differ between humans and rodents. Rodents rely more heavily on BAT activity for thermoregulation than do humans.[28] Despite these differences, our work lends important insights into the management of body temperature during anesthesia, particularly for narcolepsy patients. In this patient population, anesthesia induced hypothermia causes not only prolonged emergence time but also other adverse effects, including impaired coagulation, wound infection, and myocardial events.[22] Our results suggest that (at least in some conditions) differences between control and orexin-ablated subjects can be minimized by ensuring appropriate artificial thermoregulation.

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Figure Legends

Figure 1. Time-related changes of body temperature and locomotor activity during isoflurane-anesthesia in orexin neuron-ablated (AB) and wild-type (WT) mice.

Body temperature (A) and Locomotor activity (B) during the warmed condition (32°C) of WT mice (WT32) and ORX-AB mice (AB32). Body temperature (C) and Locomotor activity (D) during the room temperature condition (25°C) of WT mice (WT25) and ORX-AB mice (AB25) and cooled condition (23°C) of WT mice (WT23). Data are presented as mean ± SEM of 6 animals (each group), * $P < 0.05$ between AB25 and WT25. † $P < 0.05$ between WT23 and WT25. #1 $P < 0.05$ vs. baseline in WT32 or WT25. #2 $P < 0.05$ vs. baseline in AB32 or AB25. #3 $P < 0.05$ vs. baseline in WT23.

Figure 2. Time to induction and emergence from isoflurane-anesthesia.

Time to induction (A) and time of emergence from 1.5% isoflurane-anesthesia (B) was evaluated under the warmed condition (32°C), room temperature condition (25°C), and the cooled condition (23°C) in orexin neuron-ablated (AB) mice and wild-type (WT) mice (n=6 each). Number following genotype (WT or AB) designates temperature condition. Data are presented as mean ± SEM. *P* values over horizontal lines were obtained with Scheffe's *post hoc* test.

Figure1
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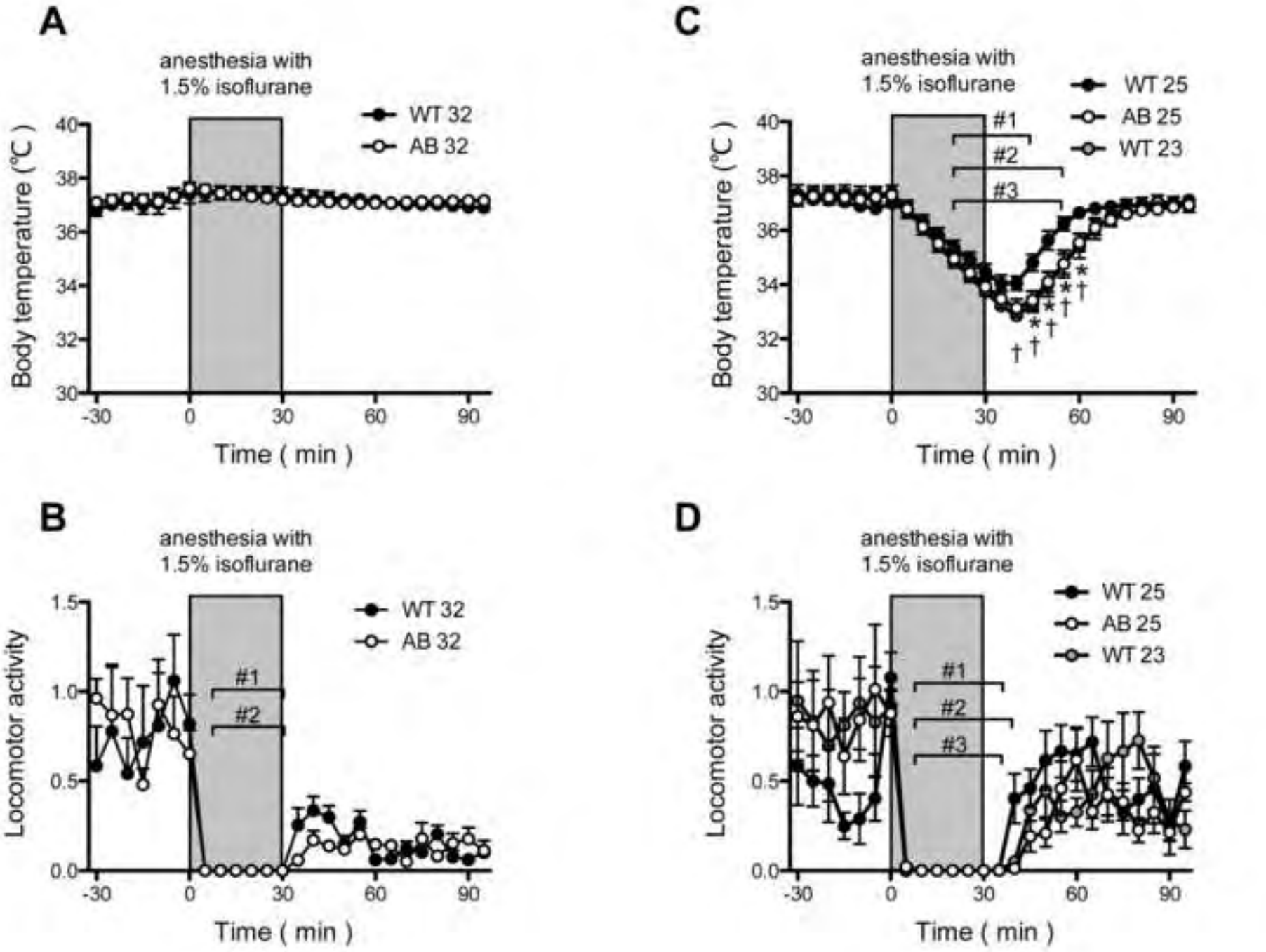

Figure2
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