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Original Article

Hyperpolarization-Activated Cyclic Nucleotide-Gated Channel Blocker ZD7288 Prevents Sevoflurane-Induced Hyperactivity in A Novel Mice Behavioral Model

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ABSTRACT

Background: Besides effect of general anesthesia, sevoflurane also induces hyperactivity during induction and recovery, especially in young children. Lack of satisfied animal model impedes the investigation of causes of the hyperactivity as well as its prevention. Hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blocker might produce sedative effect. This study developed a novel mice model of hyperactive behaviors and further explored effects of HCN channel blocker on sevoflurane-induced hyperactivity.

Methods: C57BL/6 mice were used in the present study. Maximal speed, mean speed, total movement distance and resting percentage of mice were quantitatively measured by behavioral tracking software. Age-dependence of this model was also analyzed. HCN channel blocker ZD7288 at doses of 6.25 and 12.5 μg/kg were intraperitoneal injected to prevent sevoflurane-induced hyperactivity.

Results: In the behavioral model, sevoflurane could induce significant hyperactivity in mice under 1% sevoflurane inhalation and in recovery period, characterized as increased movement speed and total distance. The sevoflurane-induced hyperactivity was more significant in young mice than adult ($P<0.01$). Pre-administration of ZD7288 could significantly prevent sevoflurane-induced hyperactivity.

Conclusions: The mice behavioral model developed in this study could characterize sevoflurane-induced hyperactivity in induction and recovery period as well as age-dependence. In addition, by this animal model, HCN channel blocker ZD7288 could prevent sevoflurane-induced hyperactivity. Thus, HCN channel might be the underlying therapeutic target for sevoflurane-induced agitation in general anesthesia.

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Inhaled anesthetics have been widely used in clinic for more than 170 years; however, the mechanisms are not fully understood yet. Sevoflurane is one of the most commonly used inhaled anesthetic in clinic, particularly in pediatric anesthesia for its low respiratory irritation and short- lasting action. Unfortunately, like other general anesthetics, sevoflurane, could also cause some side effects. Exciting behavior is a common side effect during sevoflurane induction and recovery, including epileptiform electroencephalographic activity and seizure like movements (1-8). Some patients are also observed convulsions without epileptogenic EEG during sevoflurane induction (9). Convulsions percentage has been reported with an incidence of 5% during sevoflurane anesthesia (10). These hyper-excitatory side effects are of tremendous concern because potentially delayed neurologic and cognitive defects might be induced in neonates and infants (11).

Previous studies have reported the increased rates of emergence agitation after sevoflurane anesthesia in pediatric patients (12). Emergence agitation is defined as a dissociative state with altered cognitive perception, excitation, restless, agitation, hyperactivities and increased muscular tension during the early post-anesthetic period, which may be associated with physical injury as well as negative post-operative behaviors in children (12). The incidence of emergence agitation induced by sevoflurane greatly varies among studies and the highest incidence is up to 80% (12). Such events pose a risk for injury as well as decreased parental satisfaction, especially in the ambulatory and office-based setting.

There are many clinical studies about sevoflurane- induced emergence agitation, but the causes of sevoflurane-induced agitation are still unclear, especially the molecular causes. No satisfied animal model is one of the main reasons to impede the linkage between clinical observations and molecular mechanisms. Therefore, the therapy for agitation prevention and novel anti-agitation drug development are quite limited because of the poor understands. At this moment, the common therapy for emergence agitation is sedative drugs, such as midazolam, propofol and opioids in clinical setting (13- 16). However, combined with other general anesthetics in PACU could significantly increase general anesthetic risk and result in respiratory depression and prolonged recovery.

By previous studies, Hyperpolarization-activated cyclic nucleotide- gated (HCN) channel might be the target of general anesthetics. Intravenous general anesthetics including ketamine, inhaled anesthetics like isoflurane and sevoflurane have been found to interact with HCN channels (17, 18). Therefore, we hypothesized that HCN channel blocker could produce light sedative effect, which might attenuate sevoflurane-induced emergence agitation.

In this study, we developed a mice behavioral model to test its relevance to sevoflurane-induced exciting behaviors and further investigate prevention of HCN channel blocker ZD7288 on sevoflurane-induced exciting behaviors.

METHODS AND MATERIALS

Animals

The protocol of the present study was reviewed and approved by the committee of animal welfare, West China Hospital of Sichuan University (Chengdu, China). C57BL/6 mice were housed in standard conditions of a 12-hour light/dark cycle (50%-60% humidity and 22-24℃), with free access to food and water. For age-dependent behavioral test, the C57BL/6 mice at age about 2-3 weeks post-natal were used as young mice while the mice at age of 60-80 days were used (weighing 18-25 gram) as adult mice.

Chemicals

Hyperpolarization- activated cyclic nucleotide-gated (HCN) channel blocker ZD7288 (CAS: 133059-99-1) was purchased from Tocris Bioscience (Ellisville, MO) and prepared at concentration of 3 mg/mL with normal saline as stock. All the study solutions were prepared immediately before use. Sevoflurane (ULTANE) was purchased from AbbVie Inc., (North Chicago, IL, US).

Mice Agitation Behavioral Test

Movement traces of mice were continuously recorded by camera (Canon, Legria, HF R706) and analyzed with behavioral tracking software (Smart 2.5, Panlab, DL Naturgene Life Science, Inc., Beijing, China). The videos recorded were transformed into MPG format by video format

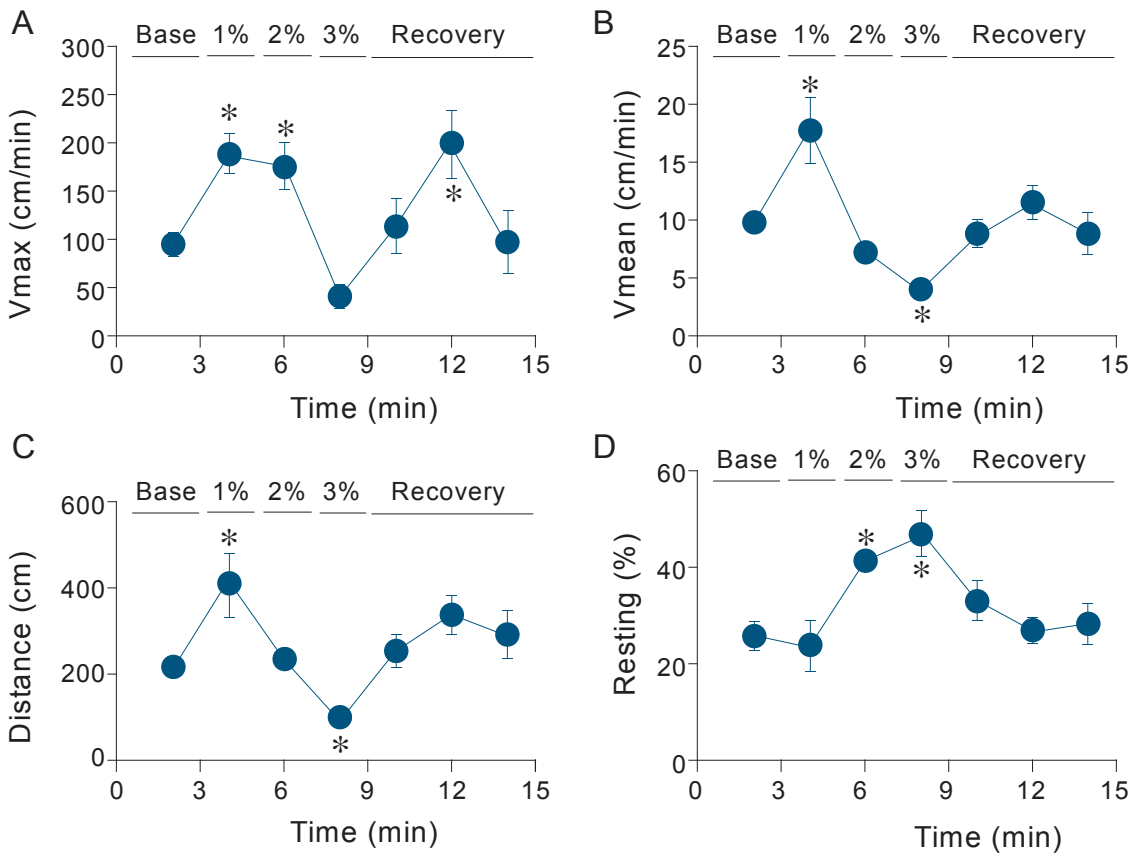

Figure 1. Behavioral Model Evaluation: Sevoflurane-Induced Hyperactivity in the Periods of Induction and Recovery.

A. Maximal speed of mice increased in the time period of 1% sevoflurane, 2% sevoflurane and recovery; B. Average speed of mice increased in the time period of 1% sevoflurane and recovery; C. Total move distance of mice significantly increased in the time period of 1% sevoflurane; D. Resting percentage decreased with induction. The mice almost anesthetized in 3% sevoflurane. *P<0.05 vs. baseline. Vmax, maximal speed; Vmean, average speed; Resting %, resting percentage.

software to facilitate the identification and analysis of Smart 2.5 software. By the software, maximal speed (Vmax), mean speed (Vmean), total movement distance (Distance) and resting time percentage (Resting %) of the mice tracking could be quantitatively determined. Briefly, mice were put into a transparent plastic cylinder (35 cm in length and 15 cm in diameter) with flow of air at about 3 L/min. Because the mice were black color, white background was placed under the plastic cylinder for better contrast ratio. For Smart 2.5 software, the focus of tracking was adjusted at body of mice. Every single mouse was put into the transparent plastic cylinder (with an electric blanket insulation at 37℃) for total duration of 15 min. Under normal air, first 3 min was considered as baseline tracks. Then, sevoflurane was introduced at concentrations of 1%, 2% and 3%, respectively, monitoring with a gas detector (Datex- Ohmeda, Louisville, CO, US) connected to the gas outlet. Every concentration of sevoflurane was maintained for 2 min. At last, mouse was allowed to recovery under normal air for 6 min. Sample size was 8-10 mice for each group and all the mice were used only once. For ZD7288 prevention study, ZD7288 at doses of 6.25 and 12.5 μg/kg were intraperitoneal injected respectively 10 min before behavioral tests.

STATISTICAL ANALYSIS

SPSS version 16.0 (SPSS Inc.) was used for all

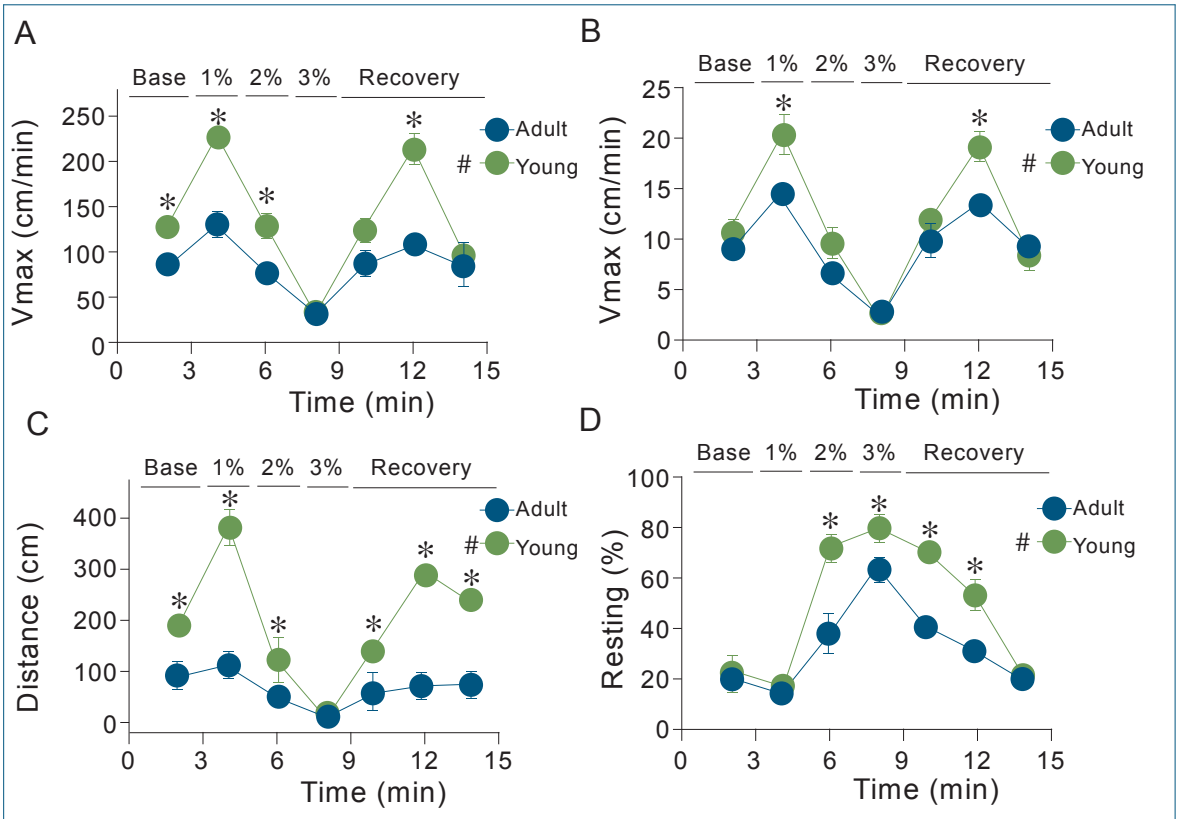

Figure 2. Sevoflurane Induced Hyperactivities in the Mice Model Was in an Age-Dependent Manner. Young Mice Were at 2-3 Weeks Post-Natal.

A. Increase of maximal speed was significantly higher in young mice; B. Increase of mean speed was significantly higher in young mice; C. Increase of total move distance was significantly longer in young mice; D. Resting percentage of mice was significantly higher in young mice under 2% and 3% sevoflurane, indicating young mice might be prone to be anesthetized. *P<0.05 vs. adult mice by t-test. #P<0.05 vs. adult mice by repeated measured ANOVA. Vmax, maximal speed; Vmean, average speed; Resting %, resting percentage.

the statistical analysis, except where noted. Sample size was 8-10 mice for each group based on the preliminary test (significance between 1% sevoflurane-induced hyperactivity and baseline). All data were expressed as mean SD unless otherwise specified. Vmax, Vmean, Distance and Resting % of mice under sevoflurane (1%, 2% and 3%, respectively) or in recovery period were compared to their baselines by one-way ANOVA with post hoc of Bonferroni test. For comparisons between adult and young mice, t-test was applied at each condition while repeated measured ANOVA was used for the whole durations. To determine effects of ZD7288, area under curves (AUC) of Vmax, Vmean, Distance and Resting % were calculated by WinNonlin (version 5.2) software (Pharsight, Mountain View, CA), and compared by Kruskal-Wallis test followed by post hoc test of Dunn test (software Prism 5.0). For other discontinued data or skewed data (abnormal distribution), Mann-Whitney U test was applied. For all the cases, $P < 0.05$ was considered as statistically significant.

RESULTS

Sevoflurane Induced Hyperactivities Both in Induction and Recovery

Adult mice were used in this part. Compared to baselines, sevoflurane could significant increase Vmax (Figure 1A), Vmean (Figure 1B) and Distance (Figure 1C) of adult mice under concentration of 1% sevoflurane ($P<0.05$). All the mice were nearly anesthetized in the condition of 3% sevoflurane, characterized by decreased Vmean ($P<0.05$), shorter distance ($P<0.05$) and in-

creased Resting % (Figure 1D, P<0.05). During recovery, Vmax of mice were also significant increased (P<0.05) before returned to baseline (Figure 1A).

Sevoflurane-Induced Hyperactivities Were Age-Dependent

As shown in Figure 2A, Vmax of young mice was significant higher than adult mice during the whole 15 min (P<0.001), and significant increase were found at the time periods of baseline, 1% sevoflurane, 2% sevoflurane and recovery (P<0.05). As show in Figure 2B, Vmean of young mice was significant higher than adult mice during the whole 15 min (P=0.001), and significant increase were found at the time periods of 1% sevoflurane and recovery (P<0.05). As show in Figure 2C, Distance of young mice was significant longer than adult mice during the whole 15 min (P<0.001), and significant increase were found at the time periods of baseline, 1% sevoflurane and recovery (P<0.05). For Resting %, as show in Figure 2D, young mice was significant higher than adult mice during the whole 15 min (P<0.001), and significant increase were found at the time periods of 2% sevoflurane and recovery (P<0.05), indicating young mice were easier to be anesthetized.

Because there might be differences of baselines between adult and young mice, the ratio between 1% sevoflurane and baseline was calculated. As shown in Figure 3, increased ratio of Vmax was larger in young mice, although no significance (P=0.067). Baselines of Vmean were similar between young and adult mice. Thus, increased ratio of Vmean was significantly larger in young mice than adult (Figure 3, P=0.041). Increased ratio of Distance was significantly longer in young mice than adult (Figure 3, P=0.015). No age-dependence was found in the increased ratio of Resting % between young and adult mice (Figure 3, P=0.526).

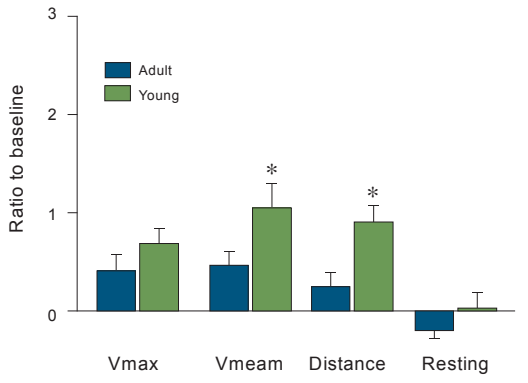

Figure 3. Sevoflurane-Induced Hyperactivity Was Revealed as the Ratio between the Period of 1% Sevoflurane and Their Baselines.
Because baseline activities between young and adult mice might be different; therefore, the ratio between periods of 1% sevoflurane and baseline could be better way to characterize the age-dependence of sevoflurane-induced hyperactivity. *P<0.05 vs. adult mice. Vmax, maximal speed; Vmean, average speed; Resting %, resting percentage.

HCN Channel Blocker ZD7288 Could Prevent Sevoflurane-Induced Hyperactivity

As shown in Figure 4, ZD7288 at dose of 12.5 μg/kg could significantly prevent sevoflurane-induced increase of Vmax (P=0.002), Vmean (P=0.001) and Distance (P=0.003) during the whole 15 min observation, and significant decrease was found at the time periods of both induction and recovery (P<0.05 vs. control). For the low dose of 6.25 μg/kg, ZD7288 could significantly decrease Vmax, Vmean and Distance at the time periods of recovery (P<0.05 vs. control). As show in Figure 4D, ZD7288 at both doses of 12.5 and 6.25 μg/kg could significantly increase Resting % of mice since baseline and during the whole 15 min observation (P<0.001). As shown in Figure 5, AUC of Vmax, Vmean and Distance were significantly decreased by ZD7288 while Resting % was increased. These results indicated that ZD7288 at both low (6.25 μg/kg) and high (12.5 μg/kg) doses could produce light sedation, while ZD7288 at high dose (12.5 μg/kg) could completely prevent sevoflurane-induced hyperactivity.

DISCUSSION

In this study, a novel mice behavioral model was developed to evaluate sevoflurane induced exciting behaviors. By this mice model, sevoflurane was found to induce hyperactivity in both induction and recovery period, characterized by increased maximal speed, mean speed and total move distance in given duration. The hyperactivities of mice are similar to sevoflurane-induced

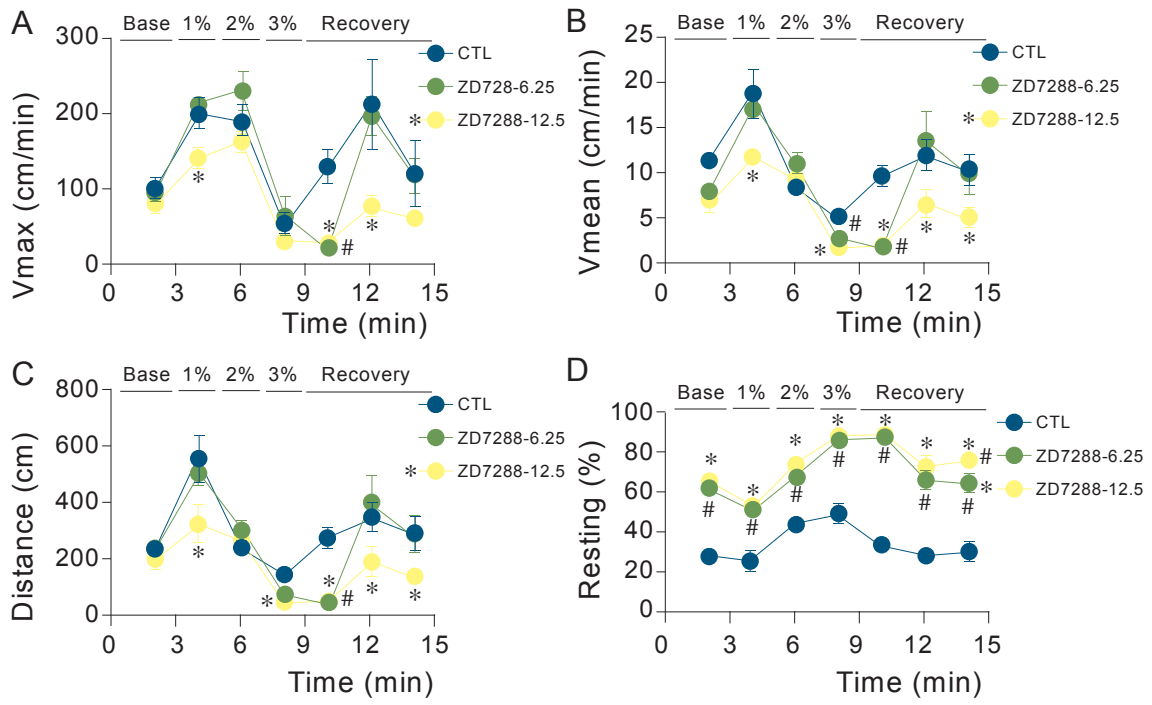

Figure 4. HCN Channel Blocker ZD7288 could Prevent Sevoflurane-Induced Hyperactivity. ZD7288 at both Low (6.25 μg/kg) and High (12.5 μg/kg) Doses Were Used.

A. Maximal speed of mice was significantly decreased by higher dose of ZD7288 in induction while both doses decreased Vmax in recovery; B. Mean speed of mice was significantly decreased by higher dose of ZD7288 in induction while both doses decreased Vmean in recovery; C. Total move distance of mice was significantly decreased by higher dose of ZD7288 in induction while both doses decreased it in recovery; D. Both doses of ZD7288 could increase resting percentage of mice. *P<0.05 vs. control. CTL, control; Vmax, maximal speed; Vmean, average speed; Resting %, resting percentage.

exciting behaviors in clinical practice. In clinic, sevoflurane- induced emergence agitation is more prevalence in preschool children. In the present study, we found that the hyperactive behaviors of this animal model were also age-dependent: maximal move speed, mean speed and total move distance of were significantly higher in young mice than adult mice. Of note, although baseline activity is also higher in young mice, the ratio between sevoflurane induction and baseline is larger in young mice, which indicating sevoflurane induced hyperactivity is age-dependent with the same direction in clinic. Thus, the mice model might be useful for further study about inhaled anesthetics induced exciting behavior. Base on this model, we demonstrated pre-administration of Hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blocker ZD7288 could significantly attenuate sevoflurane-induced exciting behavior. This result might provide molecular target for exciting behavior therapy. Behavioral test of mice is notorious for great deviation within group and not easy for repetition. In this study, deviation within group is quite acceptable even when sample size is only 8-10 mice. By analyze movement tracking of mice under sevoflurane induction and/or recovery could mimic clinical agitation like behavior.

Exciting behavior introduces great risk in general anesthesia induction and recovery and possibly has long-term impact on brain development, especially in infant. There are already a lot of clinical studies about sevoflurane-induced agitation; however, the causes of this unexpected hyper-excitability are quite unclear. No satisfied animal model is one of the main reasons to impede the linkage between clinical observations and molecular mechanisms. Thus, the therapy to sevoflurane-induced exciting behavior is still unsatisfied. Sedative agents like propofol and midazolam are commonly used to prevent sevoflu-

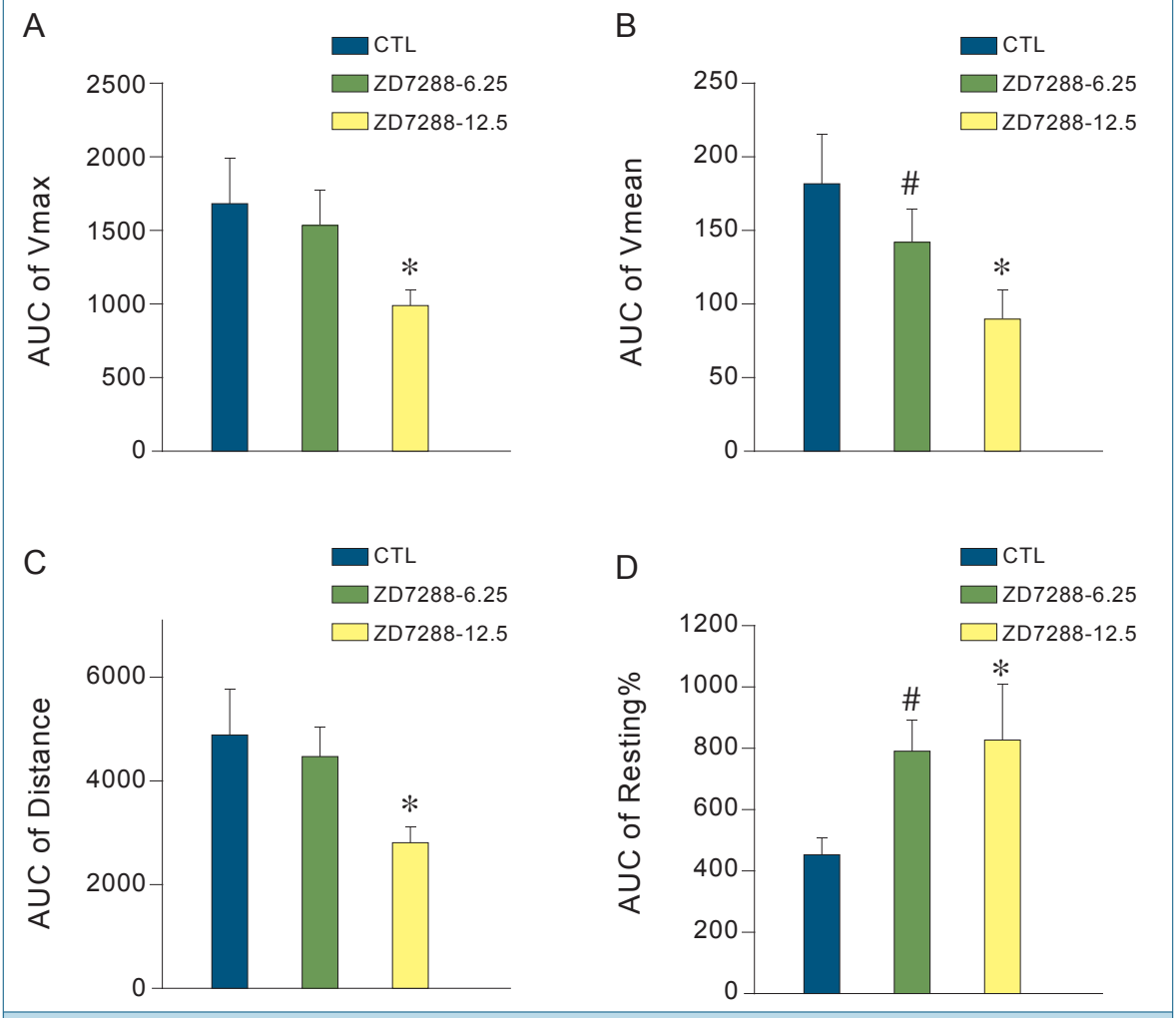

Figure 5. AUC of Vmax, Vmean and Distance Were Significantly Decreased by ZD7288 while Resting % Was Increased.

These results indicated that ZD7288 at both low (6.25 μg/kg) and high (12.5 μg/kg) doses could produce light sedation, while ZD7288 at high dose (12.5 μg/kg) could completely prevent sevoflurane-induced hyperactivity. *P< 0.05, 12.5 μg/kg group vs. control. #P<0.05, 6.25 μg/kg group vs. control. CTL, control; AUC, area under curve; Vmax, maximal speed; Vmean, average speed; Resting %, resting percentage.

rane-induced hyperactivity. However, combined with other general anesthetics in sevoflurane induction or recovery would significantly increase general anesthetic rick and prolong emergence time, which is even worse than exciting behaviors alone. The animal behavioral model developed in this study is technically easy and reliable. We choose the mice at age of 2-3 weeks as young group because this is the youngest age of mice with the ability of free movement that could be involved in behavioral test.

HCN channel has been identified as an important molecular target for general anesthetics. Previous studies demonstrate HCN channel deletion significantly affects potency of general anesthetic including ketamine, isoflurane and sevoflurane (17- 19). For isoflurane and sevoflurane, HCN channel might be involved in their hypnotic and amnesic effects. 18 Based on these finding, HCN channel blocker might decrease excitability of neurons in central nervous system by hyperpolarize neural membrane potential. Of note, in this study, HCN channel blocker ZD7288 at dose of 12.5 μg/kg did not produce significant sedative effect in mice before sevoflurane induction (data not shown) but significantly prevent sevoflurane-

induced hyperactivity. These results indicate that HCN channel blocker might prevent sevoflurane-induced agitation at very low dose that without significant effect on its general anesthetic effect. Thus, the risk of ZD7288 in agitation therapy might be quite acceptable. In this study, the mice received ZD7288 12.5 μg/kg recovered from sevoflurane anesthesia rapidly. Based on this study, development of selective HCN channel blocker might have great potential in prevention and therapy of sevoflurane induced exciting behaviors, while diminishes other side effects. At the same time, the recovery time and quality from sevoflurane anesthesia may be only slightly affected as long as the dose of HCN channel blocker is appropriate.

There are still some limitations in this study. The brain development of pre-adult mice is definitely different to human. Because of requirement of movement ability, mice at younger age like 7 days post-natal could not be involved; however, the mice with younger age might be even more hyperactive in sevoflurane induction and recovery. At the same time, our model could only analyze movement tracking and other important distinguishing feature of exciting phenomenon such as increased muscular tension, could be monitored at the same time. Combination of other remote in vivo physiological measurement system might be available. Furthermore, we did not investigate expression of HCN channel. It will even better to quantify HCN expression in the brain region after sevoflurane intervention and after ZD7288 intraperitoneal administration.

In summary, the mice behavioral model developed in this study could well reveal sevoflurane-induced hyperactivity in the period of induction and recovery. This hyperactivity is age-dependence, which as the main characterization of sevoflurane induced exciting behavior. This animal behavioral model might be useful for further mechanism study about sevoflurane-induced exciting behavior. In addition, by this animal model, HCN channel blocker ZD7288 could prevent sevoflurane-induced exciting behavior and HCN channel might be an underlying therapeutic target for exciting behavior.

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No potential conflict of interest relevant to this article was reported.

References

1. Zeiler SR, Kaplan PW. Propofol withdrawal seizures (or not). *Seizure* 2008;17:665-7.
2. Welborn LG, Hannallah RS, Norden JM, Ruttimann UE, Callan CM. Comparison of emergence and recovery characteristics of sevoflurane, desflurane, and halothane in pediatric ambulatory patients. *Anesth Analg* 1996;83:917-20.
3. Veyckemans F. Excitation phenomena during sevoflurane anaesthesia in children. *Curr Opin Anaesthesiol* 2001;14:339-43.
4. Constant I, Seeman R, Murat I. Sevoflurane and epileptiform EEG changes. *Paediatr Anaesth* 2005;15:266-74.
5. Mohanram A, Kumar V, Iqbal Z, Markan S, Pagel PS. Repetitive generalized seizure-like activity during emergence from sevoflurane anesthesia. *Can J Anaesth* 2007;54:657-61.
6. Griffeth BT, Mehra A. Etomidate and unpredicted seizures during electroconvulsive therapy. *J ECT* 2007;23:177-8.
7. Harrison JL. Postoperative seizures after isoflurane anesthesia. *Anesth Analg* 1986;65:1235-6.
8. McManus KF. Convulsion after propofol/enflurane. *Anaesth Intensive Care* 1992;20:245.
9. Pilge S, Jordan D, Kochs EF, Schneider G. Sevoflurane-induced epileptiform electroencephalographic activity and generalized tonic-clonic seizures in a volunteer study. *Anesthesiology* 2013;119:447.
10. Vakkuri A, Yli-Hankala A, Särkelä M, Lindgren L, Mennander S, Korttila K, et al. Sevoflurane mask induction of anaesthesia is associated with epileptiform EEG in children. *Acta Anaesthesiol Scand* 2001;45:805-11.
11. Tekgul H, Gauvreau K, Soul J, Murphy L, Robertson R, Stewart J, et al. The current etiologic profile and neurodevelopmental outcome of seizures in term newborn infants. *Pediatrics* 2006;117:1270-80.
12. Dahmani S, Mantz J, Veyckemans F. Case scenario: severe emergence agitation after myringotomy in a 3-yr-old child. *Anesthesiology* 2012;117:399-406.
13. Cho EJ, Yoon SZ, Cho JE, Lee HW. Comparison of the effects of 0.03 and 0.05 mg/kg midazolam with placebo on prevention of emergence agitation in children having strabismus surgery. *Anesthesiology* 2014;120:1354-61.
14. Jildenstål PK, Rawal N, Hallén JL, Berggren L, Jakobsson JG. Routines for reducing the occurrence of emergence agitation during awakening in children, a national survey. *Springerplus* 2014;3:572.
15. Tan Y, Shi Y, Ding H, Kong X, Zhou H, Tian J. μ-Opioid agonists for preventing emergence agitation under sevoflurane anesthesia in children: a meta-analysis of randomized controlled trials. *Paediatr Anaesth* 2016;26:139-50.
16. Jiang S, Liu J, Li M, Ji W, Liang J. The efficacy of propofol on emergence agitation--a meta-analysis of randomized controlled trials. *Acta Anaesthesiol Scand* 2015;59:1232-45.
17. Zhou C, Douglas JE, Kumar NN, Shu S, Bayliss DA, Chen X. Forebrain HCN1 channels contribute to hypnotic actions of ketamine. *Anesthesiology* 2013;118:785-95.
18. Zhou C, Liang P, Liu J, Ke B, Wang X, Li F, et al. HCN1 channels contribute to the effects of amnesia and hypnosis but not immobility of volatile anesthetics. *Anesth Analg* 2015;121:661-6.
19. Chen X, Shu S, Kennedy DP, Willcox SC, Bayliss DA. Subunit-specific effects of isoflurane on neuronal Ih in HCN1 knockout mice. *J Neurophysiol* 2009;101:129-40.