

# Transient Receptor Potential A1 Activation Prolongs Isoflurane Induction Latency and Impairs Respiratory Function in Mice

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## ABSTRACT

**Background:** Isoflurane is a potent volatile anesthetic; however, it evokes airway irritation and neurogenic constriction through transient receptor potential (TRP) A1 channels and sensitizes TRPV1 channels, which colocalizes with TRPA1 in most of the vagal C-fibers innervating the airway. However, little is known about the precise effects of these two channels on the respiratory function during isoflurane anesthesia.

**Methods:** By using a rodent behavioral model and whole-body plethysmograph, the authors examined the response of *Trpa1*<sup>-/-</sup> and *Trpv1*<sup>-/-</sup> mice to isoflurane anesthesia and monitored their respiratory functions during anesthesia.

**Results:** This study showed that *Trpa1*<sup>-/-</sup> mice (n = 9), but not *Trpv1*<sup>-/-</sup> mice (n = 11), displayed a shortened induction latency compared with wild-type mice (n = 10) during isoflurane anesthesia (33 ± 2.0 s in wild-type and 33 ± 3.8 s in *Trpv1*<sup>-/-</sup> vs. 17 ± 1.8 in *Trpa1*<sup>-/-</sup> at 2.2 minimum alveolar concentrations). By contrast, their response to the nonpungent volatile anesthetic sevoflurane is indistinguishable from wild-type mice (24 ± 3.6 s in wild-type vs. 26 ± 1.0 s in *Trpa1*<sup>-/-</sup> at 2.4 minimum alveolar concentrations). The authors discovered that *Trpa1*<sup>-/-</sup> mice inhaled more anesthetic but maintained better respiratory function. Further respiration pattern analysis revealed that isoflurane triggered nociceptive reflexes and led to prolonged resting time between breaths during isoflurane induction as well as decreased dynamic pulmonary compliance, an indicator of airway constriction, throughout isoflurane anesthesia in wild-type and *Trpv1*<sup>-/-</sup> mice, but not in *Trpa1*<sup>-/-</sup> mice.

**Conclusion:** Activation of TRPA1 by isoflurane negatively affects anesthetic induction latency by altering respiratory patterns and impairing pulmonary compliance. (ANESTHESIOLOGY 2015; 122:768-75)

**I**NHALATION anesthetics play an essential role in clinical anesthesia.<sup>1,2</sup> Inhalation anesthesia is particularly useful for induction of anesthesia when intravenous administration is not available, especially for pediatric patients. Isoflurane is one of the most potent volatile anesthetics used in clinical settings. It is, however, also very irritating and may increase lung resistance at clinical concentrations.<sup>3,4</sup> Although isoflurane has been used as an anesthetic for decades, the precise pharmacological mechanism has not been conclusively determined. Even though modulation of  $\gamma$ -aminobutyric acid receptor function has been shown to be the principal mechanism of action for many anesthetic drugs, general anesthetics can act upon any one of several cellular systems including ion channels, second messenger pathways, and neurotransmitter receptors.<sup>5</sup> Recent studies have indeed revealed that isoflurane can directly activate the transient receptor potential (TRP) A1 ion channel to produce inflammation and neurogenic bronchoconstriction.<sup>6,7</sup>

### What We Already Know about This Topic

- The transient receptor potential (TRP) family of nonselective cation channels includes TRPA1 and TRPV1 members that respond to noxious chemical stimuli
- The role of TRP channels in the pulmonary effects of isoflurane and sevoflurane was studied using mutant mice deficient in TRPA1 or TRPV1

### What This Article Tells Us That Is New

- Mice not expressing TRPA1 had faster onset of isoflurane anesthesia than wild-type or TRPV1-deficient mice, whereas sevoflurane onset was independent of genotype
- Onset of the pungent anesthetic isoflurane is delayed due to activation of TRPA1 receptor-mediated nociceptive reflexes that reduce ventilation, pulmonary compliance, and anesthetic uptake

Transient receptor potential A1 is a ligand-gated, non-selective cation channel most well known for its integral role in neurogenic inflammation, pain, and detection of irritants including mustard oil, cannabinoids, acrolein,

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and toluene diisocyanate.<sup>8–15</sup> TRPA1 is highly expressed in the vagal fibers innervating the airway and lungs and plays a critical role in chemical detection in the airway and alters respiratory functions.<sup>16,17</sup> Exposure to aerosolized oxidants produces a dose-dependent end-expiratory pause (resting time between breaths) and decreases respiratory frequency in mice.<sup>17</sup> However, the effects of TRPA1 activation during anesthesia in clinical settings are not well understood. Few animal models have been developed and little is known regarding the effects of TRPA1 activation during inhaled general anesthesia, especially effects on anesthetic induction and the respiratory system. Furthermore, isoflurane has also been reported to sensitize TRPV1 channels,<sup>18</sup> which are coexpressed with TRPA1 in most of the C-fibers innervating the airway. To elucidate the relationship between activation of TRP channels and anesthesia induction, we tested the anesthetic effects of TRPA1 and TRPV1 deficiency on isoflurane-induced anesthesia and dynamic changes in respiratory function using mouse genetic knock-out models.

## Materials and Methods

### Animals

*C57BL/6J* wild-type (Stock#: 000664) *Trpv1*<sup>-/-</sup> (Stock#: 003770) mice were purchased from Jackson Laboratory (Bar Harbor, ME). *Trpa1*<sup>-/-</sup> mutants were generously gifted by Dr. Zhou-Feng Chen, Ph.D., of Washington University Pain Center, St. Louis, Missouri. All behavior tests used naive, congenic 2-month-old male mice that have been backcrossed to the *C57BL/6* background for at least 10 generations. For all the experiments, researchers were blinded to mouse genotype throughout experimentation and analysis, and animal testing sequence was randomly assigned as previously described.<sup>19</sup> After data analysis, behavioral results of tested mice were grouped based on their genotypes. All experiments were performed under protocols approved by the Animal Care and Use Committee of Washington University in St. Louis School of Medicine.

### Anesthesia Induction Latency

Age-matched 8 to 10-week-old male mice were anesthetized under either 1.5 to 2.5% isoflurane (Butler Schein, USA) or 3 to 5% sevoflurane (Butler Schein), delivered by 1 l/min oxygen (Airgas, USA), in a 1 l Plexiglass chamber (Lyon Electric Company, Inc., Chula Vista, CA), which was prefilled with anesthetics for 150 s. Anesthesia induction latency was defined as the time elapsed between entering the chamber and loss of movement. Mice were removed from the chamber after 150 s and laid on their right flank. Anesthesia recovery latency was defined as the time elapsed between removal from the chamber and voluntary, coordinated roll back onto its belly.

### Whole-body Plethysmograph

Respiration data were collected using a Buxco plethysmograph system operated in accordance with the manufacturer's

protocol in flow mode. In brief, age-matched 8 to 10-week-old male mice were placed inside the unrestrained whole-body plethysmograph (Buxco, USA). Delivered *via* oxygen, 3% isoflurane or 5% sevoflurane (both from Butler Schein) was infused into the plethysmograph chamber through the aerosol inlet port at a rate of 2 l/min. Gas and anesthetic circulation was maintained *via* a constant 1 l/min suction using a small rodent bias flow supply (Buxco) connected to the bias outlet located on the bottom of the plethysmograph. All respiration signals were collected continuously *via* a transducer and amplified (Buxco). Collected signals were analyzed in real time using BioSystem XA software (Buxco) and transformed into respiratory parameters using the Epstein algorithm. Tested animals were allowed to roam freely and habituate inside the chamber, with oxygen and bias flow for 2 min before anesthesia. Isoflurane and sevoflurane were delivered by anesthetic vaporizers (both from Ohmeda, USA) with oxygen supply outside the plethysmograph chamber and administered for 180 and 150 s, respectively.

### Statistical Analysis

Sample sizes were chosen based on recently published articles that are relevant to our study.<sup>20</sup> Anesthesia induction and recovery latencies were scored using videotapes by observers blinded to mouse genotypes. All plethysmography measurements, except accumulated ventilation (AV), were collected and processed in real time by BioSystem XA software. No data point or test animal was excluded from our analysis. AV was calculated using the formula  $AV_t = AV_{(t-1)} + MV_t (60^{-1})$  (reference),<sup>21,22</sup> where  $t$  represents seconds after the start of data recording, which occurs 20 s before infusion of anesthetics, and  $MV_t$  represents instantaneous minute ventilation. Please note that plethysmograph data are recorded once every second and  $MV_t$  is extrapolated from the data captured during the immediately preceding second and not the sum of ventilation from the previous minute. Graphs were generated, and statistical significances were determined using GraphPad Prism V (GraphPad, USA). All data are presented as mean  $\pm$  SEM. Statistical comparisons for AV were made using a repeated-measures two-way ANOVA that compared genotypes against AV and time. Bonferroni *post hoc* tests were performed to compare time-matched ventilation data between genotypes. For comparison between groups in all other tests, a one-way ANOVA at each time point followed by a Tukey–Kramer *post hoc* test was used as previously described.<sup>23</sup> Differences were considered significant if  $P$  value was 0.05 or less.

## Results

### *Trpa1*<sup>-/-</sup> Mice Exhibit Decreased Induction Latency during Isoflurane Anesthesia

Pungent volatile anesthetics, including isoflurane and desflurane, have been shown to robustly activate TRPA1 channels and sensitize TRPV1 channels.<sup>6,18</sup> To test whether TRPA1-deficient

or TRPV1-deficient mice exhibit any differences in their response to pungent anesthetics, we used controlled concentrations of isoflurane to anesthetize *Trpa1*<sup>-/-</sup> (n = 9), *Trpv1*<sup>-/-</sup> (n = 11), and wild-type (n = 10) mice. *Trpa1*<sup>-/-</sup> mutants showed a significantly faster induction of anesthesia than wild-type and *Trpv1*<sup>-/-</sup> mice at high concentrations of isoflurane,  $33 \pm 2.0$  s (mean  $\pm$  SEM) in wild-type and  $34 \pm 3.8$  s in *Trpv1*<sup>-/-</sup> versus  $17 \pm 1.8$  s in *Trpa1*<sup>-/-</sup> at 2.5% isoflurane (fig. 1A). By contrast, there was no significant difference in anesthesia recovery latency for isoflurane between these groups,  $67 \pm 6.0$  s in wild-type and  $60 \pm 4.5$  s in *Trpv1*<sup>-/-</sup> versus  $71 \pm 8.1$  s in *Trpa1*<sup>-/-</sup> at 2.5% isoflurane (fig. 1B). As a control, sevoflurane, a nonpungent anesthetic agent, elicited similar induction and recovery latencies in *Trpa1*<sup>-/-</sup> mutants and wild-type controls (fig. 1, C and D).

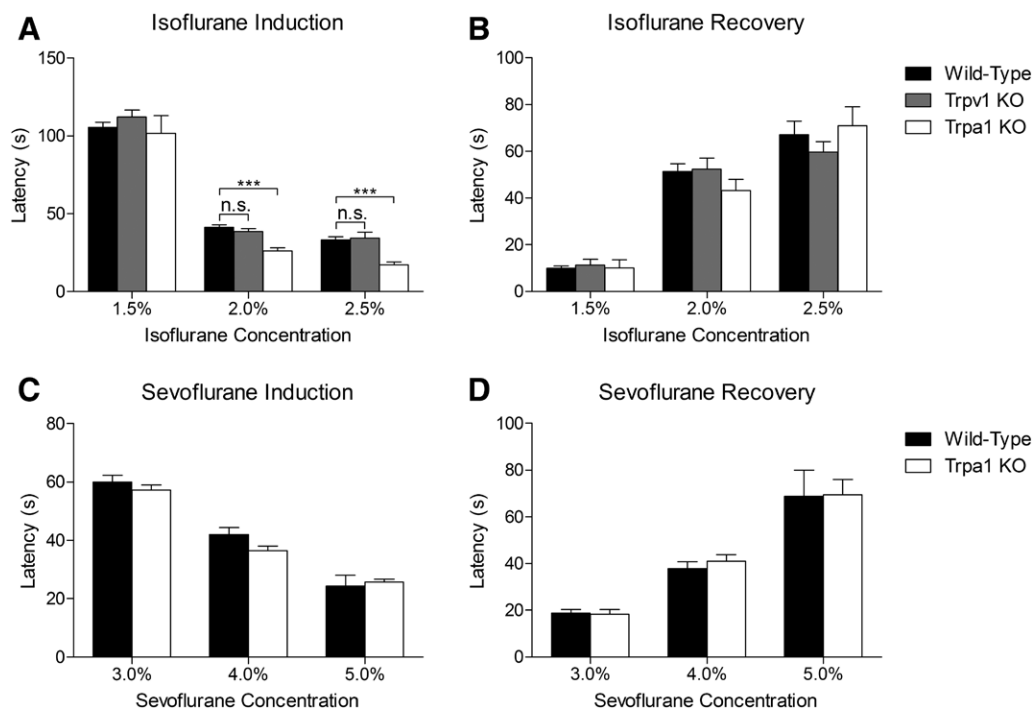
### Isoflurane Impairs Respiration Patterns via TRPA1

We hypothesized that the faster induction of isoflurane anesthesia in *Trpa1*<sup>-/-</sup> mutants is due to increased drug uptake. Isoflurane likely activated TRPA1 receptors along the respiratory tract and elicited pungent sensations in wild-type mice and induced avoidance behavior (e.g., breath holding and/or airway constriction) in these mice. By contrast, TRPA1-deficient mutants did not detect isoflurane pungency during anesthesia and continued to breathe normally. To test this hypothesis, we recorded respiration signals of unintubated, spontaneously breathing animals during anesthesia using a

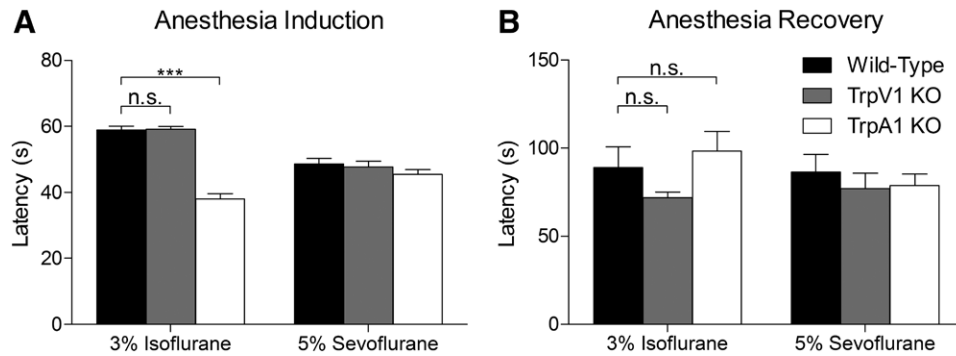
rodent whole-body plethysmograph. To compensate for the decreased drug delivery efficiency in the plethysmograph apparatus, isoflurane concentration was increased to 3%. Isoflurane induction latencies were  $59 \pm 1.2$  s and  $38 \pm 1.6$  s for wild-type and *Trpa1*<sup>-/-</sup> mice, respectively. By contrast, 5% sevoflurane induction latencies were similar in wild-type and *Trpa1*<sup>-/-</sup> mice ( $49 \pm 1.7$  s and  $46 \pm 1.4$  s, respectively; fig. 2A). No significant differences were found between wild-type and *Trpa1*<sup>-/-</sup> mice during anesthesia recovery (fig. 2B).

Respiration signals were collected throughout the duration of anesthesia, which was subdivided into five phases for separate analysis: induction, maintenance, dyspnea, early recovery, and late recovery. Induction was defined as the period between onset of anesthesia and loss of righting reflex. Maintenance was defined as the period between the loss of righting reflex and onset of dyspnea characterized by infrequent, labored, deep breaths. Early recovery was defined as the period after recovery from dyspnea and when the animal regains the righting reflex. Late recovery was defined as the period after early recovery and when the animals regained the ability to produce coordinated movement.

Under 3% isoflurane, respiratory rate was more quickly depressed during the induction phase in wild-type and *Trpv1*<sup>-/-</sup> mice than in *Trpa1*<sup>-/-</sup> mice ( $250 \pm 26$  breaths/min in wild-type,  $250 \pm 11$  in *Trpv1*<sup>-/-</sup> vs.  $360 \pm 13$  in *Trpa1*<sup>-/-</sup>) and recovered more slowly in wild-type and *Trpv1*<sup>-/-</sup> mice



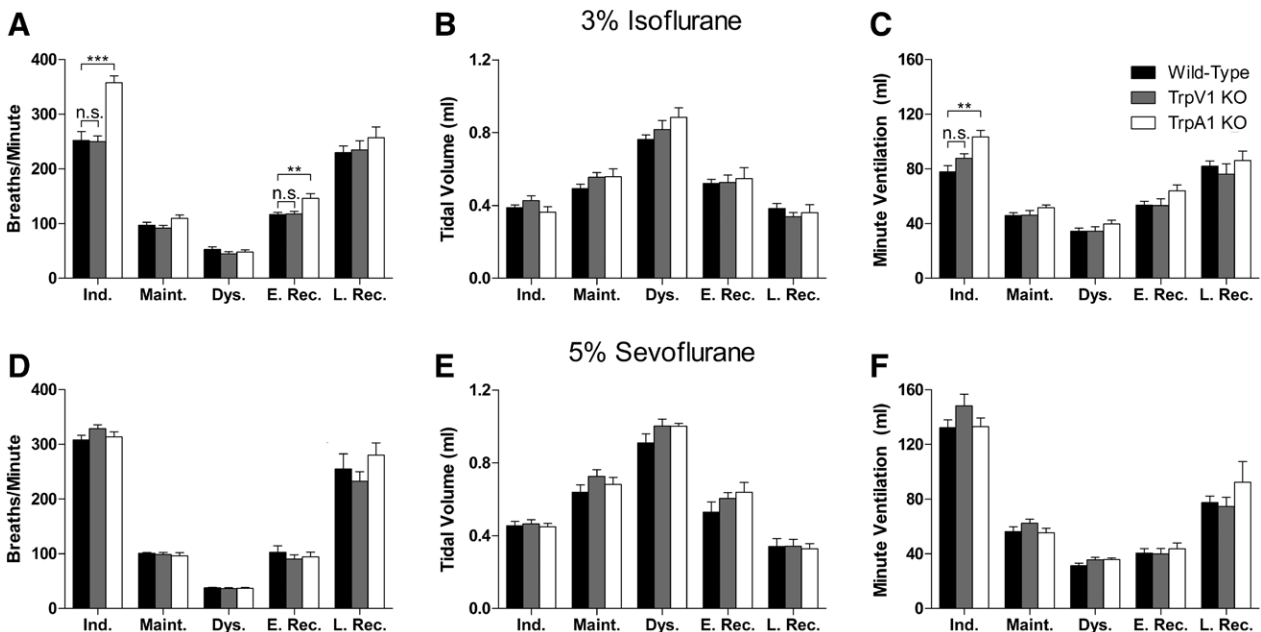
**Fig. 1.** *Trpa1*<sup>-/-</sup> mice exhibit decreased induction latency during isoflurane anesthesia. (A) *Trpa1*<sup>-/-</sup> mice (n = 9) showed significantly shortened induction latencies during anesthesia with 2.0 and 2.5% isoflurane, relative to wild-type controls (n = 10). In contrast, *Trpv1*<sup>-/-</sup> mice (n = 11) were not significantly different from wild-type controls. (B) There is no significant difference in anesthesia recovery latency between any groups at all tested concentrations of isoflurane. As a control, sevoflurane (C) induction and (D) recovery latencies were similar in wild-type and *Trpa1*<sup>-/-</sup> mice at tested concentrations. Statistical significances were calculated using one-way ANOVA followed by a Tukey–Kramer *post hoc* test. \*\*\**P* ≤ 0.001. KO = knock-out; n.s. = no significance.



**Fig. 2.** *Trpa1*<sup>-/-</sup> mice exhibit decreased induction latency during isoflurane anesthesia in plethysmograph tests. (A) Compared with wild-type controls, *Trpa1*<sup>-/-</sup> mice showed significantly shortened induction latencies during anesthesia with 3% isoflurane delivered into the plethysmograph chamber. In contrast, *Trpv1*<sup>-/-</sup> mice were not significantly different from wild-type controls. (B) There is also no significant difference in anesthesia recovery latency between any groups at tested concentration of isoflurane. As a control, 5% sevoflurane (A) induction and (B) recovery latencies were similar in wild-type, *Trpa1*<sup>-/-</sup>, and *Trpv1*<sup>-/-</sup> mice. Statistical significances were calculated using one-way ANOVA followed by a Tukey–Kramer *post hoc* test. \*\*\* *P* ≤ 0.001. KO = knock-out; n.s. = no significance.

than in *Trpa1*<sup>-/-</sup> mice (120 ± 4.3 breaths/min in wild-type, 120 ± 4.9 in *Trpv1*<sup>-/-</sup> vs. 150 ± 8.2 in *Trpa1*<sup>-/-</sup> during early recovery phase; fig. 3A). However, tidal volume was comparable between all three genotypes throughout all phases of anesthesia (fig. 3B). Minute ventilation was therefore higher in *Trpa1*<sup>-/-</sup> mice during the induction period (78 ± 4.5 ml/min in wild-type, 88 ± 3.2 in *Trpv1*<sup>-/-</sup> vs. 100 ± 4.8 in *Trpa1*<sup>-/-</sup>; fig. 3C). In contrast, these noted differences are not present under 5% sevoflurane (fig. 3, D–F).

Furthermore, based on the changes in the slope of the curve (AV vs. time), the rate of ventilation in wild-types decreased sharply after administration of isoflurane, whereas *Trpa1*<sup>-/-</sup> mice remained at the same level within the first minute of anesthesia (fig. 4A). Consequently, AV of *Trpa1*<sup>-/-</sup> mice was 18.8% more than that in wild-type controls within the first minute of anesthesia (120 ± 3.4 ml vs. 100 ± 4.8 ml). The rate of ventilation of wild-types also continued to lag behind *Trpa1*<sup>-/-</sup> mice until the recovery phases, well after

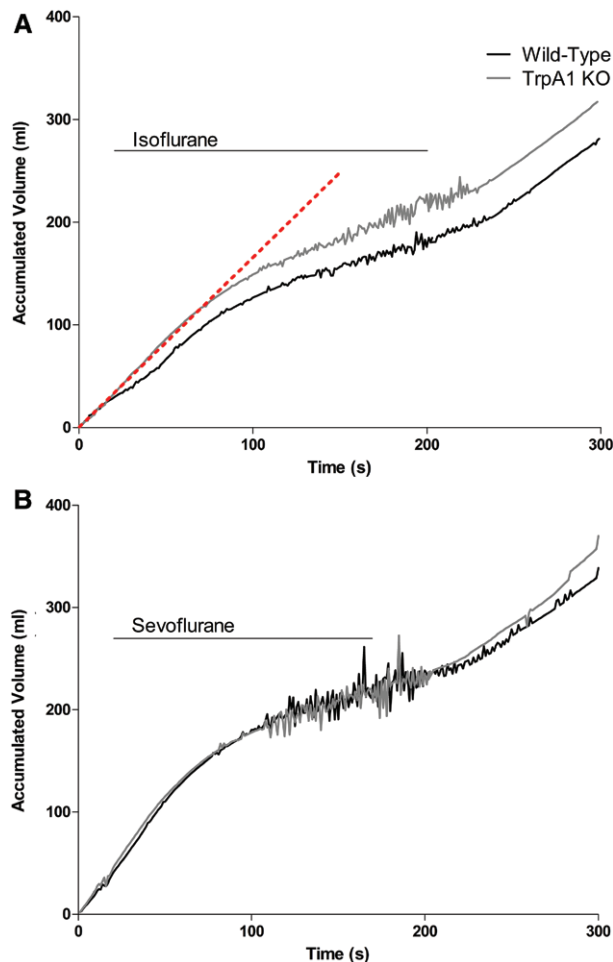


**Fig. 3.** *Trpa1*<sup>-/-</sup> mice maintain higher respiratory functions during isoflurane anesthesia. (A) The respiratory rate of wild-type, *Trpa1*<sup>-/-</sup>, and *Trpv1*<sup>-/-</sup> mice during various phases of isoflurane anesthesia. *Trpa1*<sup>-/-</sup> mice maintained significantly higher respiratory rate than wild-type mice during the induction phase. Respiratory rate also recovered more quickly in *Trpa1*<sup>-/-</sup> mice than in wild-type mice during the early recovery phase. (B) Tidal volume was similar across all genotypes during various phases of isoflurane anesthesia. (C) *Trpa1*<sup>-/-</sup> mice maintained significantly higher minute ventilation than wild-type mice during the induction phase of isoflurane anesthesia. *Trpv1*<sup>-/-</sup> mice were not significantly different from wild-type controls in any measurement (A–C). (D–F) Respiratory functions were affected similarly across all genotypes during all the phases of sevoflurane anesthesia. Statistical significances were calculated using one-way ANOVA followed by a Tukey–Kramer *post hoc* test. \*\**P* ≤ 0.01, \*\*\**P* ≤ 0.001. Dys. = dyspnea; E. Rec. = early recovery; Ind. = induction; KO = knock-out; L. Rec. = late recovery; Maint. = maintenance; n.s. = no significance.



the anesthetic was turned off (fig. 4A). However, when we applied sevoflurane, a nonpungent anesthetic, the breathing patterns of wild-type and *Trpa1*<sup>-/-</sup> mice were nearly identical throughout anesthesia (fig. 4B).

We speculate that the decreased ventilation during the induction phase in wild-type mice is the result of activating nocifensive airway reflexes by isoflurane. Further analysis revealed that the breathing cycle was prolonged in wild-type mice; and the end-expiration pause was increased dramatically in wild-type mice during this period (fig. 5, A and B). The prolonged duration of each breath and resting time between



**Fig. 4.** Accumulated ventilation of wild-type and *Trpa1*<sup>-/-</sup> mice. *Trpa1*<sup>-/-</sup> mice inhaled more isoflurane during anesthesia. (A) Ventilation rate (slope) was similar in *Trpa1*<sup>-/-</sup> and wild-type mice before isoflurane administration but dropped quickly in wild-type mice upon exposure to isoflurane. The red dotted line represents extrapolated baseline ventilation in the absence of isoflurane. Ventilation rate in wild-type mice continued to lag behind *Trpa1*<sup>-/-</sup> mice until recovery phases. (B) Ventilation was similar in *Trpa1*<sup>-/-</sup> and wild-type mice during sevoflurane anesthesia. Traces represent the averages of nine wild-type and six *Trpa1*<sup>-/-</sup> mice. Statistical significances were calculated using two-way ANOVA,  $P = 0.0061$  and  $P = 0.5590$  for isoflurane and sevoflurane, respectively. Bonferroni *post hoc* tests indicate that  $P \leq 0.05$  throughout the induction and maintenance phases during isoflurane anesthesia. KO = knock-out.

breaths sharply reduced the breathing frequency. No significant difference was observed between wild-type and *Trpa1*<sup>-/-</sup> mice during sevoflurane anesthesia (fig. 5, C and D).

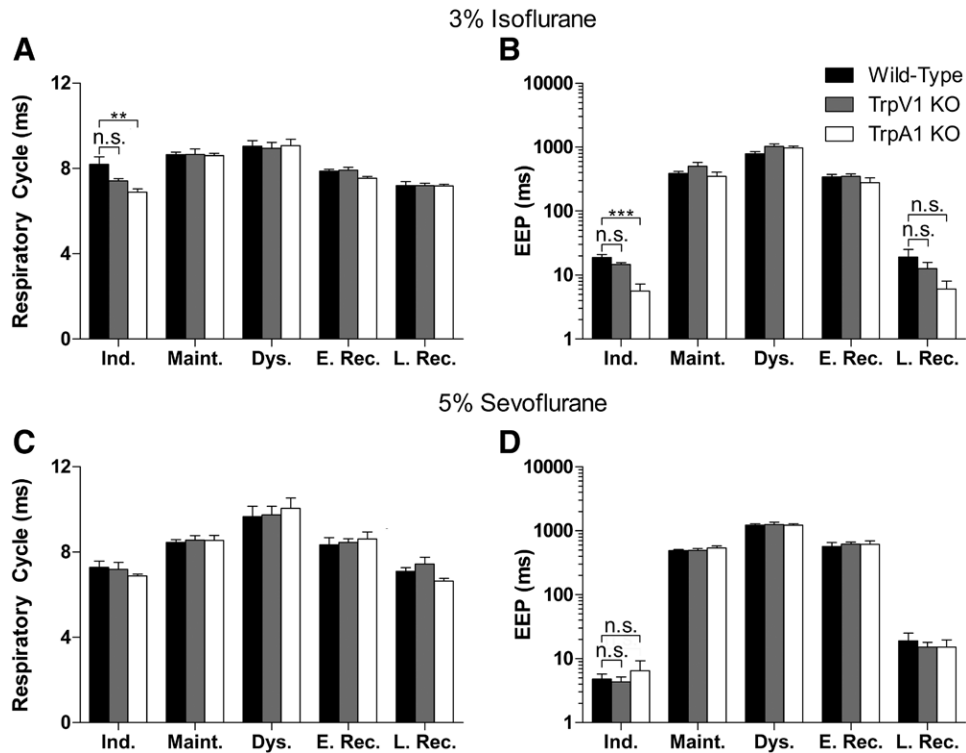
### TRPA1<sup>-/-</sup> Mice Better Maintain Respiratory Functions during Isoflurane Anesthesia

We observed a more prominent loss of dynamic airway compliance ( $C_{dyn}$ ), an indicator of airway resistance and turbulence along the airway, in wild-type mice than in *Trpa1*<sup>-/-</sup> mice during isoflurane anesthesia.  $C_{dyn}$  was computed automatically from raw tidal volume and inspiration pressure data by our plethysmograph system.<sup>24</sup> Decreased compliance most often results from stiff lungs when airway and chest wall lose elasticity, air turbulence, or airways narrowing. Patients with low pulmonary compliance may suffer from dyspnea when breathing spontaneously and are at an increased risk of lung injury when delivered by pressure control ventilation. Interestingly, except during dyspnea and late recovery, airway compliance was substantially less compromised in *Trpa1*<sup>-/-</sup> than in wild-type mice (fig. 6A). As a control, sevoflurane reduced the dynamic airway compliance equally in all mice during the whole duration of anesthesia (fig. 6B).

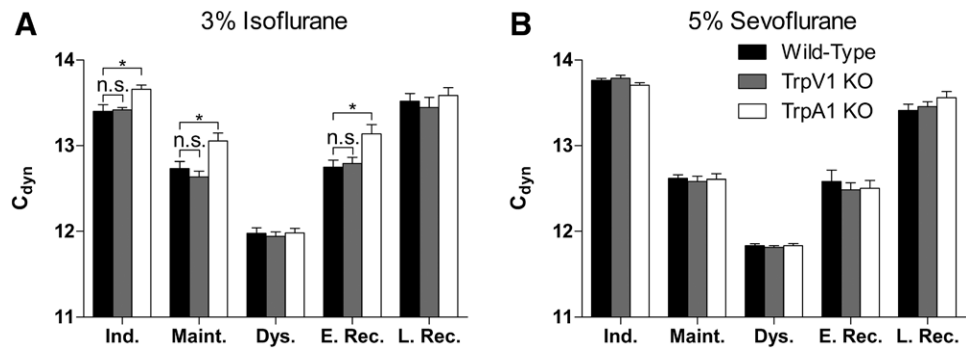
### Discussion

We discovered that TRPA1 activation adversely affects isoflurane induction latency by lowering respiration rate, reducing ventilation, prolonging end-expiration pause, and impairing dynamic airway compliance. Several factors can affect the uptake of an anesthetic from its administration from a vaporizer and its deposition in the brain.<sup>5</sup> The inhaled gas mixture depends mainly on the fresh gas flow rate, the volume of the breathing system, and absorption in the breathing circuit. Lower fresh gas flow rates, larger breathing systems, and greater circuit absorptions all decrease the inhaled gas concentration. Clinically, these attributes translate into longer induction and recovery latencies. Previous studies have also reported that C-fibers innervating the airway trigger nocifensive reflexes and breaking response (increased end-expiration pause) when activated by cinnamaldehyde.<sup>16</sup> Also, respiratory depression evoked by zinc exposure in the airway was absent in *Trpa1*<sup>-/-</sup> mice,<sup>25</sup> as well as the hypoxic ventilator response is attenuated when using the TRPA1 antagonist.<sup>20</sup> Our behavioral observations are consistent with these reports and further reveal how TRPA1-mediated nocifensive reflexes to isoflurane compromises anesthetic effectiveness.

Even though changes in pulmonary compliance are frequently reported during administration of high dose of isoflurane,<sup>4,26</sup> the precise mechanisms responsible for these changes have not been determined. Our study showed that during isoflurane anesthesia, *Trpa1*<sup>-/-</sup> mice maintained higher compliance than wild-type mice during the induction and maintenance phases and recovered more quickly during the recovery phases (fig. 6A). We provide the first evidence that the pungency of volatile anesthetics can directly compromise airway compliance *via* TRPA1 channels. Indeed,



**Fig. 5.** Isoflurane negatively affects the respiration pattern during anesthesia via TRPA1. (A) Compared with *Trpa1*<sup>-/-</sup> mice, wild-type mice exhibited significantly slowed breathing and (B) prolonged end-expiratory pause (EEP, braking time) during isoflurane induction phase. (C and D) Respiratory cycle and EEP were affected similarly across all genotypes during all the phases of sevoflurane anesthesia. *Trpv1*<sup>-/-</sup> mice were not significantly different from wild-type controls in any measurement. Statistical significances were calculated using one-way ANOVA followed by a Tukey–Kramer *post hoc* test. \*\**P* ≤ 0.01; \*\*\**P* ≤ 0.001. Dys. = dyspnea; E. Rec. = early recovery; Ind. = induction; KO = knock-out; L. Rec. = late recovery; Maint. = maintenance; n.s. = no significance.



**Fig. 6.** *Trpa1*<sup>-/-</sup> mice maintain higher dynamic pulmonary compliance during isoflurane anesthesia. (A) *Trpa1*<sup>-/-</sup> mice maintained significantly higher *C<sub>dyn</sub>* than wild-type mice during isoflurane induction and maintenance phases. *C<sub>dyn</sub>* also recovered more quickly in *Trpa1*<sup>-/-</sup> mice than wild-type mice after removal of drugs in the early recovery phase. *Trpv1*<sup>-/-</sup> mice were not significantly different from wild-type controls in any measurement. (B) Pulmonary compliance was affected similarly across all genotypes during all the phases of sevoflurane anesthesia. Statistical significances were calculated using one-way ANOVA followed by a Tukey–Kramer *post hoc* test. \**P* ≤ 0.05. *C<sub>dyn</sub>* = dynamic pulmonary compliance; Dys. = dyspnea; E. Rec. = early recovery; Ind. = induction; KO = knock-out; L. Rec. = late recovery; Maint. = maintenance; n.s. = no significance.

desflurane, another pungent anesthetic that activates TRPA1 channels in airway, was reported to increase lung resistance concomitant with a decrease in dynamic airway compliance in guinea pigs when compared with sevoflurane.<sup>27,28</sup>

During deep anesthesia, which induced dyspnea, we did not observe a difference in pulmonary compliance between wild-type and *Trpa1*<sup>-/-</sup> mice. This could be due

to the fact that mice used in our study were not intubated during anesthesia, and upper airway obstruction that typically occurs during dyspnea may have confounded phenotypic differences between *Trpa1*<sup>-/-</sup> and wild-type mice in this phase.

As recovery from anesthesia depends on clearance of the anesthetic from brain tissue, anesthetics can be

eliminated by biotransformation, transcutaneous loss, and exhalation. The speed of recovery also depends on the duration of anesthetic administration. Because tissues keep absorbing anesthetic and lowering availability to the brain until the alveolar partial pressure falls below tissue partial pressure, prolonged anesthesia ends this drug redistribution effect when the anesthetic reaches its equilibrium concentrations in peripheral tissues. However, we only administered anesthesia for a brief period to our mice. The anesthetic agents that we used are unlikely to have reached equilibrium in all tissues in such a short duration. Therefore, the peripheral tissue absorption of drug probably contributed significantly to recovery latency. Furthermore, the respiratory rate, tidal volume, and minute volume during recovery are similar in both *Trpa1*<sup>-/-</sup> and wild-type mice (fig. 3), suggesting that the anesthetics were exhaled at the same rate. These two major factors contributed predominately to our observations and may have obscured any small phenotypic differences between mutant and control mice.

In conclusion, our study examined the physiological effects of isoflurane pungency on its uptake and the dynamic changes to the respiratory function using mouse genetic knock-out models. Based on our results, isoflurane activation of TRPA1 during anesthesia induction has a profound effect on the speed of anesthesia onset. Furthermore, we found that TRPA1 activation by isoflurane elicits strong nociceptive reflexes that reduce ventilation and airway compliance at clinically relevant concentrations. Together, these findings provide novel evidence that TRPA1 activation during isoflurane anesthesia impairs respiratory function and prolongs the induction latency of anesthesia.

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## Competing Interests

The authors declare no competing interests.

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