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Isoflurane-Induced Changes in Righting Response and Breathing are Modulated by RGS Proteins

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Abstract

Background—Recent evidence suggests that G protein coupled receptors, especially those linked to $G\alpha_i$, contribute to the mechanisms of anesthetic action. Regulator of G protein signaling (RGS) proteins bind to activated $G\alpha_i$ and inhibit its signal transduction. Genomic knock-in mice with an RGS-insensitive $G\alpha_{i2}$ G184S ($G\alpha_{i2}$ GS) allele exhibit enhanced $G\alpha_{i2}$ signaling and provide a novel approach for investigating the role of $G\alpha_{i2}$ signaling and RGS proteins in general anesthesia.

Methods—Homozygous $G\alpha_{i2}$ GS/GS and wild type (WT) mice were anesthetized with isoflurane and time (s) to loss and resumption of righting response was quantified. During recovery from isoflurane anesthesia breathing was quantified in a plethysmography chamber for both lines of mice.

Results—$G\alpha_{i2}$ GS/GS mice required significantly less time for loss of righting and significantly more time for resumption of righting than WT mice. During recovery from isoflurane anesthesia, $G\alpha_{i2}$ GS/GS mice exhibited significantly greater respiratory depression. Poincaré analyses show that GS/GS mice have diminished respiratory variability compared to WT mice.

Conclusion—Modulation of $G\alpha_{i2}$ signaling by RGS proteins alters loss and resumption of wakefulness, and state-dependent changes in breathing.

Introduction

Volatile anesthetics are thought to produce a loss of consciousness by numerous mechanisms that include activating inhibitory ionotropic gamma-aminobutyric acid type A ($GABA_A$) receptors, inhibiting excitatory ionotropic glutamate receptors, and direct effects on ion channels such as the two-pore potassium channels (1–5). Less is known about the contribution of metabotropic receptor signaling to the generation of anesthetic states (6–8).

There is evidence that G protein coupled receptors (GPCRs) are altered by anesthetics (8–11), and GPCRs may contribute to anesthesia by mimicking signaling that occurs during normal states of sleep and wakefulness (6,7,12–14). GPCRs activate heterotrimeric G proteins by inducing the binding of GTP, and activated G proteins are subsequently turned off by hydrolysis of GTP to GDP to restore the inactive state (15). There are four families of G proteins, referred to as G_s, G_i, G_q, and G_{12}. Receptors that activate G_i lead to inhibition of

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neural activity by multiple mechanisms (15,16). G_i linked pathways in the brain are known to modulate states of behavioral arousal. For example, M2 and M4 muscarinic cholinergic receptors contribute to the regulation of wakefulness (17) and ketamine inhibits muscarinic receptors (18) as well as acetylcholine release (19). Volatile anesthetics act on GPCRs (6,20), including cholinergic (21) and hypocretinergic (12) receptors that promote cortical and behavioral arousal.

Regulators of G protein signaling (RGS) proteins inhibit G protein signaling by speeding the rate of GTP hydrolysis (22–24), which allows RGS proteins to tightly control G protein activity and subsequent downstream signaling. Knock-in mice containing an RGS-insensitive $G\alpha_{i2}$ G184S allele recently have been described (25,26). These mice lose the negative regulatory activity of RGS proteins such that receptor-stimulated $G\alpha_{i2}$ proteins remain active for a longer period of time. As a result, the $G\alpha_{i2}$ RGS-insensitive knock-in mice (either homozygous $G\alpha_{i2}$ GS/GS or heterozygous $G\alpha_{i2}$ GS/+) exhibit enhanced $G\alpha_{i2}$ signaling compared to wild type (WT) mice (25,26). Some (25,26) but not all (27) physiological responses are enhanced by the $G\alpha_{i2}$ G184S mutation, while other physiological responses are selectively enhanced by a similar $G\alpha_o$ G184S mutation (27). The prolonged inhibitory signaling makes the $G\alpha_{i2}$ GS/GS mice a novel tool for examining the role of $G\alpha_{i2}$ signaling and RGS proteins in anesthetic action.

This study tested the hypothesis that compared to WT mice, increased $G\alpha_{i2}$ signaling in $G\alpha_{i2}$ GS/GS mice alters the loss of consciousness caused by isoflurane anesthesia and breathing. The results provide an initial phenotyping of the effects of isoflurane in $G\alpha_{i2}$ GS/GS mice and suggest that RGS proteins contribute to isoflurane anesthesia. Portions of these data have been presented as abstracts (28,29).

Methods

Animals

The University of Michigan Committee on Use and Care of Animals reviewed and approved all procedures. Experiments were conducted in accordance with the National Institutes of Health Policy on Humane Care and Use of Laboratory Animals (NIH publication 80-23). Adult male homozygous $G\alpha_{i2}$ GS/GS knock-in (n=16) and WT littermate control mice (n=10) derived from the C57BL/6J strain were obtained from an internal colony. The development of the knock-in mice and the genotyping protocol have been described in detail (25). Mice were housed in a temperature and humidity controlled environment with *ad libitum* access to food and water, and were kept on a 24h light cycle to minimize circadian influences. For data collection, experimenters were blinded to mouse genotype.

Quantifying loss of righting response

$G\alpha_{i2}$ GS/GS and WT mice were used to quantify the time to become immobile after a single exposure to isoflurane (Hospira Inc, Lake Forest, IL). Mice (n=8 per genotype) were conditioned to handling and to being placed in an acrylic chamber for one week prior to experiments. On the experiment day, mice were placed in the acrylic chamber (25 cm × 11 cm × 13 cm) and administered 5% isoflurane in 100% oxygen at a flow rate of 1 L/min. Loss of righting response was operationally defined as the isoflurane-induced loss of a weight-bearing posture and the inability to resume a normal weight-bearing posture after being placed in dorsal recumbency. The time (s) from onset of isoflurane administration until the loss of righting response was recorded manually. Each mouse was used for one trial.

Quantifying resumption of righting response

$G\alpha_{i2}$ GS/GS and WT (n=5 for each group) were used to quantify time for resumption of righting response after isoflurane anesthesia. Mice were conditioned to handling as described above. On the day of the experiment, mice were placed in the acrylic chamber and administered 3% isoflurane in 100% oxygen until they exhibited loss of righting. Mice were then transferred to a Kopf Model 923-B mouse anesthesia mask (David Kopf Instruments Tujunga, CA) and anesthesia was maintained with 1.3% isoflurane in 100% oxygen (30). Administered isoflurane concentration was monitored continuously using a Datex-Ohmeda Cardiocap 5 (Madison, WI), and body temperature was held at 37°C throughout the entire experiment using a water-filled heating pad (Gaymar Industries, Orchard Park, NY). Isoflurane delivery was discontinued 30 min later and mice were placed in dorsal recumbency under a heating lamp. The time (s) until mice resumed a normal, upright posture was recorded. Each mouse was used for 6 experiments with one week between experiments.

Quantifying breathing during recovery from isoflurane anesthesia

$G\alpha_{i2}$ GS/GS and WT littermate controls were used to quantify isoflurane-induced alterations in breathing. Mice were conditioned to handling as described above and to being placed in a PLY 3211 Buxco plethysmography chamber (Wilmington, NC). The same anesthesia protocol used for the resumption of righting response experiments was followed. After 30 min of isoflurane anesthesia, mice were placed in the plethysmography chamber for 1 h and exposed to room air while breathing was recorded. Temperature in the plethysmography chambers was maintained between 26–28°C. The plethysmography system allowed for the quantification of respiratory rate, tidal volume, minute ventilation, inspiratory time, expiratory time, total respiratory cycle time, inspiratory flow, and inspiratory effort. All respiratory measurements containing volume units were expressed as per g body weight to normalize for weight differences between mice. Each mouse was used for 4 trials with one week between experiments. The number of mice used in the present experiments is comparable to previous studies with statistical power adequate for phenotyping strain-specific aspects of ventilatory control (31).

Data Analysis

Descriptive statistics, Student's t-test, and Wilcoxon Rank Sum test were used to determine significant differences between genotypes. Respiratory rate during recovery from isoflurane anesthesia was also plotted using Poincaré analyses in which the mean respiratory rate for 5 min segments was plotted against the mean respiratory rate for the subsequent 5 min segment. As described in detail elsewhere (32,33), Poincaré analysis provides unique insights into the temporal organization of periodic phenomena. Data are reported as mean ± standard error of the mean (SEM). A p value less than 0.05 was interpreted to indicate a statistically significant difference.

Results

Figure 1A shows that $G\alpha_{i2}$ GS/GS mice exhibited a significant decrease in time to loss of righting response compared to WT mice. To rule out changes in kinetics of isoflurane distribution, recovery from anesthesia also was assessed. Figure 1B illustrates that time required for resumption of righting after cessation of isoflurane was significantly increased in $G\alpha_{i2}$ GS/GS mice compared to WT mice.

All measures of breathing were analyzed and expressed as per g body weight. Phenotyping of baseline ventilatory behavior was provided by pre-anesthesia measures of respiratory rate, inspiratory flow, and inspiratory time (Figs. 2A, B, and C; left histograms). The GS/GS mice

revealed a significantly slower rate of breathing than WT before isoflurane anesthesia (Fig. 2A). In contrast to baseline, all respiratory measures were significantly different between WT and GS/GS mice after isoflurane anesthesia (Figs. 2A, B, and C; right histograms). Table 1 summarizes the percent change in breathing caused by isoflurane anesthesia. The greatest change in breathing was the increased duration of inspiration in GS/GS mice (Fig. 2C, Post-Isoflurane).

The Poincaré plots illustrate and quantify the variability in respiratory rate characteristic of WT (Fig. 3A) and $G\alpha_{i2}$ GS/GS (Fig. 3B) mice. For one h following cessation of isoflurane delivery, WT mice exhibited a wider range of respiratory rate variability than $G\alpha_{i2}$ GS/GS mice. The diminished variability in rate of breathing by the $G\alpha_{i2}$ GS/GS mice can be visualized by contrasting the distribution of points in Figs. 3A and 3B. Poincaré analyses quantify the differences in variability in two ways. The first standard deviation (SD_1) represents breath-to-breath (short-term) variability with respect to the line of identity ($y = x$). The second standard deviation (SD_2) represents the amount by which the data points varied from a line orthogonal to and intersecting the line of identity at the mean. SD_2 reflects the overall (long-term) variability in respiratory cycle length throughout the one h post-isoflurane recovery period. $G\alpha_{i2}$ GS/GS mice exhibited significantly less breath-to-breath variation than WT mice.

Discussion

The results show decreased time to loss of righting response with exposure to isoflurane and increased time for resumption of righting following cessation of isoflurane in GS/GS knock-in mice (Fig. 1). These findings support the interpretation that RGS proteins can modulate, in part via $G\alpha_{i2}$ signaling, both the loss and the resumption of wakefulness. The Fig. 1 data could be due to a direct action of isoflurane on a GPCRs (10–12) or to the effect of isoflurane on signaling molecules downstream of the primary site of anesthetic action (6,7). This new finding concerning RGS proteins is consistent with recent data regarding the role of GPCRs in arousal state control. For example, the hypothalamic peptide hypocretin/orexin promotes wakefulness via two G protein coupled receptors (34). Wake-active hypocretin neurons are inhibited by isoflurane, and genetic deletion of hypocretin/orexin delays the resumption of righting following isoflurane anesthesia (12). Adenosine promotes sleep via four GPCRs and recovery from isoflurane anesthesia can be significantly delayed or prolonged by cortical delivery of an adenosine A_1 receptor agonist or antagonist, respectively (11).

Arousal state-dependent alterations in breathing can be modulated by $G\alpha_i$ coupled-receptors, including muscarinic M2 and M4, adenosine A1, and adrenergic α_2 (7,21,35–38). $G\alpha_{i2}$ GS/GS mice exhibited greater isoflurane-induced respiratory depression compared to WT littermate controls (Figs. 2 and 3). The significantly decreased inspiratory flow and increased inspiratory time in the $G\alpha_{i2}$ GS/GS mice show that enhanced $G\alpha_{i2}$ signaling also contributes to respiratory rate depression during recovery from isoflurane anesthesia.

Poincaré analyses provide a novel tool for quantifying and visualizing breath-to-breath variability in respiratory rate (39). GPCRs amplify transmembrane signaling in the time domain and previous studies demonstrate that RGS proteins alter cardiovascular control (25,26). In view of the relevance of cardio-respiratory control for anesthesiology, a logical extension of these previous findings was for the present study to quantify the extent to which RGS proteins might alter respiratory rhythm generation. A waxing and waning of respiratory rate is one characteristic of disordered breathing associated with the loss of wakefulness (40). Poincaré analyses can uniquely phenotype the temporal organization of ventilatory behavior (31,41). Furthermore, variability in breathing pattern has been shown to be

clinically useful as a weaning predictor for post-anesthesia patients (32), for diagnosis of sleep apnea (42), and for elucidating which components of the central respiratory rhythm generator are most altered by opioids (43).

The present Poincaré analyses show (Fig. 3) that compared to WT mice, the $G\alpha_{i2}$ GS/GS mice have a less variable respiratory rate during recovery from isoflurane anesthesia. A highly variable rate of breathing (Fig. 3A) observed among WT mice is characteristic of a ventilatory control system that can rapidly respond to rapidly changing metabolic and environmental demands. In the GS/GS mice (Fig. 3B) a clustering of points along the line of identity indicates that long duration breaths were followed by similarly timed breaths. In contrast to WT mice that have normal RGS proteins, the $G\alpha_{i2}$ GS/GS mice have prolonged inhibitory signaling (24). Thus, the phenotype of ventilatory timing revealed by Poincaré analysis fits with differences in RGS protein dynamics between WT and GS/GS mice. Compared to WT control mice, isoflurane caused a rapid loss of consciousness and a delayed resumption of wakefulness in the GS/GS mice (Fig. 1). The isoflurane effects on breathing may also be due to the loss of a wakefulness stimulus on breathing (44).

Respiratory depression and decreased breathing rate variability may also be a consequence of the enhanced $G\alpha_{i2}$ signaling outside the brain. For example, a decrease in upper airway and respiratory pump muscle activity due to lowered Ca^{2+} levels in the respiratory accessory and pump muscle cells (45,46) may also contribute to decreased variability in breathing. Some anesthetics inhibit upper airway muscles (47) and decrease diaphragm contractility (48). The greater respiratory rate depression in the $G\alpha_{i2}$ GS/GS mice may reflect a synergistic effect of enhanced $G\alpha_{i2}$ signaling on central and peripheral control mechanisms.

A general limitation of the approach used in this study is that transgenic animals can develop compensatory modulation by other genes and gene products (49,50). Any study of genetically modified animals has the potential to be confounded by compensatory changes that occur during development. Indeed, $G\alpha_{i2}$ knock-out mice show that $G\alpha_{i3}$ is upregulated (51). While such secondary changes could cause or contribute to the observed changes in anesthetic responses, the evidence for Gi-coupled receptor signaling in arousal state control suggests that the simple model of enhanced $G\alpha_{i2}$ signaling as the mechanism is reasonable. Further study with other $G_{i/o}$ protein mutants (either RGS-insensitive or knockout) would provide additional support for this interpretation. Future studies designed to identify the receptor and signaling pathways involved in the presently observed changes in arousal state and respiratory effects of isoflurane can provide additional important information.

The present study is also limited by lack of data regarding relevant isoflurane minimum alveolar concentration (MAC). It is not yet known how MAC values in these knock-in $G\alpha_{i2}$ GS/GS mice compare to MAC of the WT mice. The present study was undertaken in part to determine if phenotype differences justify the effort and cost of creating enough GS/GS mice and heterozygote mice for a study designed to quantify MAC (30). The present finding of statistically significant differences, even with the relatively small number of WT and GS/GS mice studied, supports the interpretation of a robust effect of RGS proteins on breathing as well as loss and recovery of righting. These results encourage development of a transgenic colony large enough to enable studies of receptor systems and neural networks through which RGS proteins modulate anesthesia and breathing phenotypes.

In conclusion, compared to WT controls, homozygous knock-in $G\alpha_{i2}$ GS/GS mice with enhanced $G\alpha_{i2}$ signaling exhibited shorter time for loss of righting caused by isoflurane and increased time for recovery of righting after isoflurane anesthesia. $G\alpha_{i2}$ GS/GS mice also revealed decreased breathing rate and inspiratory flow, increased inspiratory time, and a less variable respiratory rate during recovery from isoflurane anesthesia. The results are

consistent with the interpretation that $G\alpha_{i2}$ signaling contributes to the loss of consciousness and respiratory depression caused by isoflurane. Finally, these data suggest a link between RGS proteins and isoflurane modulation of wakefulness and breathing, and encourage future studies to determine whether RGS proteins contribute to the mechanisms of anesthetic action.

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References

1. Hemmings HC, Akabas MH, Goldstein PA, Trudell JR, Orser BA, Harrison NL. Emerging molecular mechanisms of general anesthetic action. Trends Pharmacol Sci. 2005; 26:503–510. [PubMed: 16126282]
2. Mashour GA, Forman SA, Campagna JA. Mechanisms of general anesthesia: from molecules to mind. Best Pract Res Clin Anaesthesiol. 2005; 19:349–364. [PubMed: 16013686]
3. Orser BA. Extrasynaptic $GABA_A$ receptors are critical targets for sedative-hypnotic drugs. J Clin Sleep Med. 2006; 2:S12–S18. [PubMed: 17557502]
4. Vanini G, Watson CJ, Lydic R, Baghdoyan HA. Gamma-aminobutyric acid-mediated neurotransmission in the pontine reticular formation modulates hypnosis, immobility, and breathing during isoflurane anesthesia. Anesthesiology. 2008; 109:978–988. [PubMed: 19034094]
5. Zecharia AY, Nelson LE, Gent TC, Schumacher M, Jurd R, Rudolph U, Brickley SG, Franks NP. The involvement of hypothalamic sleep pathways in general anesthesia: testing the hypothesis using the GABA-A receptor β3 N265M knock-in mouse. J Neurosci. 2009; 29:2177–2187. [PubMed: 19228970]
6. Franks NP. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. Nat Rev Neurosci. 2008; 9:370–386. [PubMed: 18425091]
7. Lydic R, Baghdoyan HA. Sleep, anesthesiology, and the neurobiology of arousal state control. Anesthesiology. 2005; 103:1268–1295. [PubMed: 16306742]
8. Rudolph U, Antkowiak B. Molecular and neuronal substrates for general anaesthetics. Nat Rev Neurosci. 2004; 5:709–720. [PubMed: 15322529]
9. Hollmann MW, Strumper D, Herroeder S, Durieux ME. Receptors, G proteins, and their interactions. Anesthesiology. 2005; 103:1066–1078. [PubMed: 16249682]
10. Ishizawa Y, Pidikiti R, Liebman PA, Eckenhoff RG. G protein-coupled receptors as direct targets of inhaled anesthetics. Mol Pharmacol. 2002; 61:945–952. [PubMed: 11961111]
11. Van Dort CJ, Baghdoyan HA, Lydic R. Adenosine A1 and A2A receptors in mouse prefrontal cortex modulate acetylcholine release and behavioral arousal. J Neurosci. 2009; 29:871–881. [PubMed: 19158311]
12. Kelz MB, Sun Y, Chen J, Cheng Meng Q, Moore JT, Veasey SC, Dixon S, Thornton M, Funato H, Yanagisawa M. An essential role for orexins in emergence from general anesthesia. Proc Nat Acad Sci USA. 2008; 105:1309–1314. [PubMed: 18195361]
13. Clement EA, Richard A, Thwaites M, Ailon J, Peters S, Dickson CT. Cyclic and sleep-lke spontaneous alternations of brain state under urethane anaesthesia. PLoS ONE. 2008; 3
14. Van Dort, CJ.; Baghdoyan, HA.; Lydic, R. Neurochemical modulators of sleep and anesthetic states. In: Mashour, GA., editor. Int Anesthesiol Clin. Philadelphia: Lippencott Williams & Wilkins; 2008. p. 75-104.
15. Wettschureck N, Offermanns S. Mammalian G proteins and their cell type specific functions. Physiol Rev. 2005; 85:1159–1204. [PubMed: 16183910]

16. Tas PW, Eisemann C, Roewer N. Indirect activation of adenosine A1 receptors in cultured rat hippocampal neurons by volatile anaesthetics. Eur J Anaesthesiol. 2005; 22:694–702. [PubMed: 16163917]

17. Baghdoyan HA, Lydic R. M2 muscarinic receptor subtype in the feline medial pontine reticular formation modulates the amount of rapid eye movement sleep. Sleep. 1999; 22:835–847. [PubMed: 10566902]

18. Durieux ME. Inhibition by ketamine of muscarinic acetylcholine receptor function. Anesth Analg. 1995; 81:57–62. [PubMed: 7598283]

19. Lydic R, Baghdoyan HA. Ketamine and MK-801 decrease ACh release in the pontine reticular formation, slow breathing, and disrupt sleep. Sleep. 2002; 25:617–622. [PubMed: 12224840]

20. Nakayama T, Penheiter AR, Penheiter SG, Chini EN, Thompson M, Warner DO, Jones KA. Differential effects of volatile anesthetics on M3 muscarinic receptor coupling to the G alpha q heterotrimeric G protein. Anesthesiology. 2006; 105:313–324. [PubMed: 16871065]

21. Dong HL, Fukuda S, Murata E, Higuchi T. Excitatory and inhibitory actions of isoflurane on the cholinergic ascending arousal system of the rat. Anesthesiology. 2006; 104:122–133. [PubMed: 16394698]

22. Hepler JR. RGS protein and G protein interactions: a little help from their friends. Mol Pharmacol. 2003; 64:547–549. [PubMed: 12920189]

23. Neubig RR. Regulators of G protein signaling (RGS proteins): novel central nervous system drug targets. J Pept Res. 2002; 60:312–316. [PubMed: 12464108]

24. Neubig RR, Siderovski DP. Regulators of G-protein signalling as new central nervous system drug targets. Nat Rev Drug Discov. 2002; 1:187–197. [PubMed: 12120503]

25. Huang X, Fu Y, Charbeneau RA, Saunders TL, Taylor DK, Hankenson KD, Russell MW, D'Alecy LG, Neubig RR. Pleiotropic phenotype of a genomic knock-in of an RGS-insensitive G184S Gnαi2 allele. Mol Cell Biol. 2006; 26:6870–6879. [PubMed: 16943428]

26. Fu Y, Huang X, Zhong H, Mortensen RM, D'Alecy LG, Neubig RR. Endogenous RGS proteins and Gα subtypes differentially control muscarinic and adenosine-mediated chronotropic effects. Circ Res. 2006; 98:659–666. [PubMed: 16456099]

27. Goldenstein BL, Nelson BW, Xu K, Luger EJ, Pribula JA, Wald JM, O'Shea LA, Weinshenker D, Charbeneau RA, Huang X, Neubig RR, Doze VA. Regulator of G protein signaling protein suppression of $G\alpha_o$ protein-mediated α_{2A} adrenergic receptor inhibition of mouse hippocampal CA3 epileptiform activity. Mol Pharm. 2009; 75:1222–1230.

28. Icaza EE, Fu Y, Huang X, Neubig RR, Baghdoyan HA, Lydic R. Role of an inhibitory G protein ($G\alpha_{i2}$) and regulators of G protein signaling (RGS proteins) in isoflurane anesthesia. Society for Neuroscience Abstract Viewer/Itinerary Planner Online. 2006 Program No. 795.3.

29. Icaza EE, Huang X, Neubig RR, Baghdoyan HA, Lydic R. Genomic knock-in mice with enhanced $G\alpha_{i2}$ signaling exhibit altered breathing during recovery from isoflurane anesthesia compared to wild type mice. Sleep. 2007; 30 Abstr Suppl:0024.

30. Sonner JM, Gong D, Li J, Eger EI 2nd, Laster MJ. Mouse strain modestly influences minimum alveolar anesthetic concentration and convulsivity of inhaled compounds. Anesth Analg. 1999; 89:1030–1034. [PubMed: 10512285]

31. Yamauchi M, Dostal J, Kimura H, Strohl KP. Effects of buspirone on posthypoxic ventilatory behavior in C57BL/6J and A/J mouse strains. J Appl Physiol. 2008; 105:518–526. [PubMed: 18511527]

32. Bien M-Y, Shu-Shya H, Benjamin I-TK, Yu-Ting L, Jia-Horng W, Yu RK. Breathing pattern variability: a weaning predictor in postoperative patients recovering from systemic inflammatory response syndrome. Intensive Care Med. 2004; 30:241–247. [PubMed: 14647889]

33. Woo MA, Stevenson WG, Moser DK, Trelease RB, Harper RM. Patterns of beat-to-beat heart rate variability in advanced heart failure. Am Heart J. 1992; 123:704–710. [PubMed: 1539521]

34. Nishino, S.; Sakurai, T. The Orexin/Hypocretin System. In: Lydic, R.; Baghdoyan, HA., editors. Contemporary Clinical Neuroscience. Humana Press; 2006. p. 1-397.

35. Evers, AS.; Crowder, CM.; Balser, JR. General anesthetics. In: Brunton, LL.; Lazo, JS.; Parker, KL., editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. Eleventh. New York: McGraw-Hill; 2006. p. 341-368.

36. Barros RC, Branco LG, Carnio EC. Respiratory and body temperature modulation by adenosine A1 receptors in the anteroventral preoptic region during normoxia and hypoxia. Respir Physiol Neurobiol. 2006; 153:115–125. [PubMed: 16352472]
37. Bischoff, P.; Schneider, G.; Kochs, E. Anesthetics drug pharmacodynamics. In: Schuttler, J.; Schwilden, H., editors. Handbook of Experimental Pharmacology. Berlin: Springer; 2008. p. 379-408.
38. Joseph V, Pequignot JM, Van Reeth O. Neurochemical perspectives on the control of breathing during sleep. Respir Physiol Neurobiol. 2002; 130:253–263. [PubMed: 12093622]
39. Chun HH, Spiegel ET, Solomon IC. Burst-to-burst variability in respiratory timing, inspiratory-phase spectral activity, and inspiratory neural network complexity in urethane-anesthetized C57BL/6 mice in vivo. Adv Exp Med & Biol. 2008; 605:408–412.
40. Strohl KP. Periodic breathing and genetics. Respir Physiol Neurobiol. 2003; 135:179–185. [PubMed: 12809618]
41. Gonsenhauser I, Wilson CG, Han F, Strohl KP, Dick T. Strain differences in murine ventilatory behavior persists after urethane anesthesia. J Appl Physiol. 2004; 97:888–894. [PubMed: 15333626]
42. Morillo DS, Rojas JL, Crespo LF, Leon A, Gross N. Poincaré analysis of an overnight arterial oxygen saturation signal applied to the diagnosis of sleep apnea hypopnea syndrome. Physiol Meas. 2009; 30:405–420. [PubMed: 19332895]
43. Mitsis GD, Governo RJM, Rogers R, Pattinson KTS. The effect of remifentanil on respiratory variability, evaluated with dynamic modeling. J Appl Physiol. 2009; 106:1038–1049. [PubMed: 19196914]
44. Fink BR. Influence of cerebral activity in wakefulness on regulation of breathing. J Appl Physiol. 1961; 16:15–20. [PubMed: 13699604]
45. Chen X, Yamakage M, Namiki A. Inhibitory effects of volatile anesthetics on K+ and Cl− channel currents in porcine tracheal and bronchial smooth muscle. Anesthesiology. 2002; 96:458–466. [PubMed: 11818782]
46. Ikeda SR, Jeong SW. Use of RGS-insensitive Gα subunits to study endogenous RGS protein action on G-protein modulation of N-type calcium channels in sympathetic neurons. Methods Enzymol. 2004; 389:170–189. [PubMed: 15313566]
47. Akhtar S, Brull SJ. Effect of isoflurane on endothelin-1 mediated airway smooth muscle contraction. Pulm Pharmacol Ther. 1998; 11:227–230. [PubMed: 9918761]
48. Fauroux B, Cordingley J, Hart N, Clement A, Moxham J, Lofaso F, M I P. Depression of diaphragm contractility by nitrous oxide in humans. Anesth Analg. 2002; 94:340–345. [PubMed: 11812695]
49. Robert C. Knockout mice: advantages and limitations for biological modeling. Definitions, principles and examples. Ann Dermatog Venereol. 1998; 125:946–947.
50. Crawley, J. Behavioral Phenotyping of Transgenic and Knockout Mice. New York: Wiley-Liss; 2000. What's Wrong with my Mouse?.
51. Han SB, Moratz C, Huang NN, Kelsall B, Cho H, Shi CS, Schwartz O, Kehrl JH. Rgs1 and Gnai2 regulate the entrance of B lymphocytes into lymph nodes and B cell motility within lymph node follicles. Immunity. 2005; 22:343–354. [PubMed: 15780991]

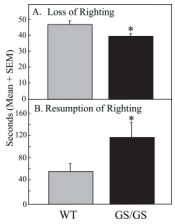

Figure 1.

$G\alpha_{i2}$ GS/GS mice show a more rapid loss of righting caused by isoflurane than WT controls. **A.** Time for loss of righting response was significantly shorter (*, p = 0.01) in $G\alpha_{i2}$ GS/GS mice compared to WT control mice. **B.** Time to resumption of righting response in $G\alpha_{i2}$ GS/GS mice was significantly longer (*, p = 0.02) compared to WT control.

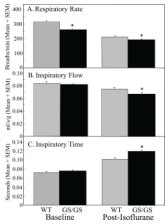

Figure 2.

$G\alpha_{i2}$ GS/GS mice exhibited greater respiratory depression during the initial 20 min post-isoflurane anesthesia recovery period compared to WT mice. Each bar summarizes data from 3 mice randomly chosen from the two genotypes. The left pair of histograms in frames A, B, and C plots baseline measures prior to isoflurane exposure. **A.** At baseline, respiratory rate in $G\alpha_{i2}$ GS/GS mice was significantly less than in WT mice. **B.** Inspiratory flow and **C.** inspiratory time were not different between $G\alpha_{i2}$ GS/GS and WT mice during baseline recordings. The right pair of histograms illustrates breathing measures in $G\alpha_{i2}$ GS/GS and WT mice after isoflurane. **A.** After isoflurane, respiratory rate in $G\alpha_{i2}$ GS/GS mice was significantly (*, $p = 0.01$) lower than WT mice. **B.** Inspiratory flow was significantly (*, $p = 0.008$) decreased compared to WT mice by isoflurane. **C.** Inspiratory time was significantly (*, $p < 0.0001$) increased in $G\alpha_{i2}$ GS/GS mice compared to WT mice during recovery from isoflurane anesthesia.

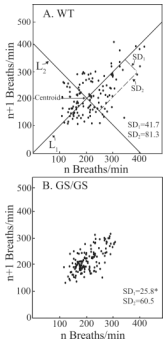

Figure 3.

Poincaré plots illustrating the range of respiratory rates in WT (**A**) and $G\alpha_{i2}$ GS/GS (**B**) mice during the 1 h recovery period following cessation of isoflurane delivery. Each point represents the mean respiratory rate (n = 3 mice per genotype) of a 5 min segment plotted against the next 5 min segment. The plot was analyzed by determining two standard deviation values: SD_1 and SD_2 for each data point. SD_1 is defined as the dispersion of points perpendicular to the line-of-identity (L_1). SD_1 is used to analyze short-term variation. For these analyses SD_1 corresponds to breath-to-breath variation of the respiratory cycle. The centroid is the point representing the overall mean respiratory rate. SD_2 is defined as the dispersion of points along the line-of-identity passing through the centroid. SD_2 is also referred to as long-term variation and for these data SD_2 characterizes variability in respiratory rate due to genotype. Breath-to-breath respiratory rate of Gαi2 GS/GS mice was significantly (*, $p < 0.001$) less variable (smaller SD_1) than WT mice.

Table 1

Percent Change in Breathing Caused by Isoflurane

	Respiratory Rate	Inspiratory Flow	Inspiratory Time
WT	−32	−11	39
GS/GS	−26	−18	58