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POST-SIGH SLEEP APNEAS IN MICE: SYSTEMATIC REVIEW AND DATA-DRIVEN DEFINITION

Short title: Post-Sigh Sleep Apnea in Mice

Stefano Bastianini[1], Sara Alvente[1], Chiara Berteotti[1], Marcello Bosi[2], Viviana Lo Martire[1], Alessandro Silvani[1], Alice Valli[1], Giovanna Zoccoli[1]*.

[1]PRISM Lab, Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy.

[2]ASL of Romagna, Department Thoracic Diseases, Pulmonary Operative Unit, Morgagni-Pierantoni Hospital, Forlì, Italy.

***Corresponding author:**

Prof. Giovanna Zoccoli, MD, PhD

Dipartimento di Scienze Biomediche e Neuromotorie, Alma Mater Studiorum - Università di Bologna.

Piazza di Porta San Donato 2, 40126 Bologna, Italy

Phone: +390512091726 Fax: +390512091737

E-mail: giovanna.zoccoli@unibo.it

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Study design: AS, MB, SB, GZ. Surgery, recordings and sleep scoring: VLM, SB, SA, CB, AV. Data analysis and statistics: AS, SB. Manuscript draft: SB. All authors reviewed the manuscript for important intellectual content.

ABSTRACT

Sleep apneas can be categorized as post-sigh (prevailing in non-rapid-eye-movement sleep) or spontaneous (prevailing in rapid-eye-movement sleep) according to whether or not they are preceded by an augmented breaths (sigh). Notably, the occurrence of these apnea subtypes changes differently in hypoxic/hypercapnic environments and in some genetic diseases, highlighting the importance of an objective discrimination. We aim to: a) systematically review the literature comparing the criteria used in categorizing mouse sleep apneas; b) provide data-driven criteria for this categorization, with the final goal of reducing experimental variability in future studies.

Twenty-two wild-type mice, instrumented with electroencephalographic/electromyographic electrodes, were placed inside a whole-body plethysmographic chamber to quantify sleep apneas and sighs. Wake-sleep states were scored on 4-s epochs based on electroencephalographic/electromyographic signals.

Literature revision showed that highly different criteria were used for post-sigh apnea definition, the intervals for apnea occurrence after sigh ranging from 1 breath up to 20 seconds. In our data, the apnea occurrence rate during non-rapid-eye-movement sleep was significantly higher than that calculated before the sigh only in the 1st and 2nd 4-s epochs following a sigh.

These data suggest that, in mice, apneas should be categorized as post-sigh only if they start within 8 s from a sigh; the choice of shorter or longer time windows might underestimate or slightly overestimate their occurrence rate, respectively.

KEYWORDS

Breathing pattern, plethysmography

INTRODUCTION

The study of the pathogenesis of common sleep-related breathing disorders (Fleury Curado et al., 2018), such as sleep apneas, and associated comorbidities is a topic of ongoing interest which can be accelerated using genetically-modified mouse models (Davis and O'Donnell, 2013). The mouse breathing pattern can be assessed throughout the wake-sleep cycle by combining whole-body plethysmography (WBP) with electroencephalographic (EEG) and electromyographic (EMG) recordings, but an accurate discrimination of the wake-sleep states may be also obtained by relying solely on visual inspection of WBP signal (Bastianini et al., 2017). Apneas and augmented breaths (sighs) have been documented as physiological phenomena both in humans and mice and are considered pathological when increased in number (Davis and O'Donnell, 2013).

The mechanisms underlying apneas and, particularly, those underlying sighs are still incompletely understood. Sighs may be the results of an inspiratory-augmenting reflex elicited by activation of lung and chest wall receptors in response to reduced lung compliance or by stimulation of peripheral chemoreceptors in response to hypoxia or hypercapnia (Qureshi et al., 2009, Nakamura et al., 2003). Consistently, carotid body denervation and sectioning of the vagus nerve abolish spontaneous sighing (Qureshi et al., 2009). Moreover, other inputs from peripheral chemoreceptors, laryngeal irritation or noxious stimuli, may interact with vagal activation in producing augmented breaths. It has been hypothesized that these inputs increase inspiratory flow and deepen inspiration, thereby increasing the discharge of lung inflation receptors which stimulate inspiration and thus set up a “positive feedback” (Glogowska et al., 1972). Consistent evidence indicates that sighs play an important role in restoring lung volume, preventing atelectasis, reducing hypercapnia and hypoxia, and changing the properties of the neuro-respiratory control system (with an improvement of long-range stability, short-range memory and respiratory variability) (Qureshi et al., 2009, Vlemincx et al., 2010, Baldwin et al., 2004). It has been proposed that sighs could play a role in the regulation of breathing by resetting the autonomic tonus in conditions of autonomic imbalance, such as it appears to occur in

future sudden infant death syndrome (SIDS) victims (Franco et al., 2003). Finally, sighs are associated with cardiovascular changes (peripheral vasoconstriction (Chalacheva and Khoo, 2015) and tachycardia followed by bradycardia (Ramirez et al., 2013)).

In mice, sighs occur almost exclusively during non-rapid-eye-movement sleep (NREMS) as opposed to rapid-eye-movement sleep (REMS) (Nakamura et al., 2003, Lo Martire et al., 2017). On the contrary, the relationship between sigh occurrence and sleep states is still not conclusive in humans. Indeed some works reported that sighs occurred in humans more frequently during NREMS than during REMS (Qureshi et al., 2009, McNamara et al., 2002), whereas others reported the opposite result (Perez-Padilla et al., 1983). Moreover, sighs are much more frequent in infants than in adults (Qureshi et al., 2009). Both in mice (Nakamura et al., 2003) and humans (particularly in children) (Ramirez et al., 2013, Qureshi et al., 2009), sighs during NREMS are frequently followed by apneas which may represent an inhibitory response evoked by the activation of pulmonary stretch receptors (Davis and O'Donnell, 2013, Saito et al., 2002) or by the decreased CO_2 levels sensed by chemoreceptors. Thus, sleep apneas might be categorized as post-sigh if they occur within an arbitrary time window after an augmented breath or as spontaneous if they do not. However, the definition of this time window varies widely among studies, ranging from “1 breath” (Samarasinghe et al., 2015) up to “within 20 s” (Yamauchi et al., 2008), increasing inter-study variability and limiting experimental reproducibility.

With the present study, we aimed to: a) systematically review the criteria previously used for the definition of post-sigh sleep apneas in mice; b) provide data-driven criteria to be used for this purpose in future experiments.

METHODS

The study protocol was approved by the Committees on the Ethics of Animal Experiments of the University of Bologna and of the Italian Ministry of Health (Authorizations 291/2013-B issued November 25, 2013, and 245/2015-PR issued May 10, 2015). All the experimental procedures were conducted in conformity with the institutional guidelines in compliance with the national (Legislative Decree n. 26, March 4, 2014) and international law and policies (EEC Council Directive 2010/63/EU), and in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Systematic Review of the literature

The present review follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria (Moher et al., 2009). We conducted a search in PUBMED (last update: July 3, 2018) using the keywords "mice whole-body plethysmography" and retrieved and screened all relevant publications to systematically review the criteria used to discriminate between post-sigh and spontaneous apneas in mice. For inclusion in this systematic review, studies were required to meet the following criteria:

1) Results were presented in English as a full original research paper published in a peer-reviewed journal.

2) Studies were performed on mice using a WBP chamber

3) A clear and objective definition of apneas was provided

4) A clear and objective definition of sigh was provided

5) A definition, even not stringent, of post-sigh apneas was included

The process for selecting studies is displayed in Fig. 1.

A total of 435 articles (12 of which did not appear in the initial PUBMED search but were cited by other articles) were screened but only 44 full-text articles were then included in the present report because they explicitly indicate the methodological definition of apnea. Finally, among these 44

articles, only 11 also showed the methodological definition of sigh and the criteria for distinguishing post-sigh from spontaneous apneas and, thus, they were included in Table 1. From the included studies, the following data were extracted: cutoff values for the discrimination of apneas and sighs, and criteria used to discriminate post-sigh from spontaneous apneas.

Experimental evaluation of sleep apnea in mice

We analyzed WBP data recorded in our laboratory to provide a data-driven definition of post-sigh apneas. In particular, 22 adult male wild-type mice with a C57BL/6 genetic background, maintained with a 12:12 hour light-dark cycle and free access to food and water, underwent surgery at 18-24 weeks of age for the implantation of electrodes to record EEG and neck EMG signals (Bastianini et al., 2014). After at least 7 days of recovery, each mouse was placed inside a WBP chamber (PLY4223, Buxco, Wilmington, NC, USA) flushed with air at 1.5 L/h for the first 8 hours of the light period. The WBP chamber was modified to reduce the internal volume to 0.97 L and to accommodate a rotating electrical swivel (SL6C/SB, Plastics One, Roanoke, VA, USA) to simultaneously record the respiratory (WBP chamber pressure), EEG and EMG signals. All these signals were digitalized and stored at 128 Hz together with chamber humidity and temperature which were both stored at 4 Hz. Eighteen of these mice were part of a previously published dataset (Bastianini et al., 2015, Silvani et al., 2014), and four more mice were added in the present study using the same protocol as the others. Wake-sleep states were scored on 4-s epochs based on EEG and EMG signals as previously described (Bastianini et al., 2014). Briefly, wakefulness was scored when the EMG tone was high and the EEG was at low voltage with possible δ (0.5-4 Hz) and θ (6-9 Hz) frequency components. NREMS was scored when the EMG tone was lower than in wakefulness and the EEG was at high voltage with prominent δ frequency components. REMS was scored when the EMG indicated muscle atonia with occasional muscle twitches and the EEG was at low voltage with predominant θ frequency components. Each epoch was scored based on the EEG/EMG features that were present for most of its duration (i.e., > 2 s). Epochs with signals borderline between two different states were scored as

"undetermined" and excluded from subsequent analyses. Since sleep sighs are commonly associated with brief EEG desynchronization, we prevented any bias in their evaluation by scoring wake-sleep states while blinded to WBP signal.

Individual breaths were identified automatically from the upward (+) WBP pressure deflection peak. Errors in breath detection as well as WBP pressure artefacts (due to mouse movements) were manually excluded from the analyses. Quantitative analysis of breathing was restricted to stable sleep episodes ≥ 12 s because of the frequent occurrence of movement artefacts during wakefulness. Instantaneous total breath duration (T_{TOT}) and tidal volume (V_T) per gram body weight were obtained as previously reported (Bastianini et al., 2017). By analogy with the criterion used for sleep apnea in humans (cessation of breathing for > 10 s, which corresponds to 2 or 3 times basal respiratory period; Berry et al., 2012) and in other mouse studies (Nakamura et al., 2003, Boukari et al., 2016), apneas were automatically-detected for each mouse and sleep state as a cessation of WBP pressure signals with T_{TOT} > 3 times (3x) the average T_{TOT} for that mouse and sleep state (Fig. 2). Moreover, as in previous mouse studies (Nakamura et al., 2003, Boukari et al., 2016), sigh discrimination was based on breath VT, and sighs were automatically-detected as breaths with V_T > 3 times (3x) the average V_T for each sleep state. For the sake of the robustness of these computations, the average values of T_{TOT} and V_T for each mouse and sleep state were computed after exclusion of the breaths with T_{TOT} and/or V_T that deviated more than 3 standard deviations from the respective mean value in the whole recording (Bastianini et al., 2015). Each automatically-detected event was then visually checked and confirmed before proceeding with subsequent analyses. In particular, we visually confirmed breathing cessation during apnea episodes and we excluded movement artifacts as cause of augmented WBP signal. In additional analyses, both apneas and sighs were also calculated as breaths with T_{TOT} or V_T > 2 times (2x) the average T_{TOT} or V_T for each sleep state. We decided to apply the 2x threshold in additional analyses because we aimed at comparing the breathing pattern obtained with the criteria applied in our previous works (3x) (Bastianini et al., 2017, Bastianini et al., 2015, Lo

Martire et al., 2017, Silvani et al., 2014) with those obtained using a criterion (2x) less restrictive but widely used (Table 1).

Because sighs are nearly absent during REMS (Lo Martire et al., 2017, Nakamura et al., 2003), we confined to NREMS periods the analysis of the maximum time interval following a sigh within which an apnea should fall to be categorized as a post-sigh apnea. Thus, we compared the apnea occurrence rate in the 8 s preceding each NREMS sigh (used as reference value) with the apnea occurrence rate calculated in the following 32 s (split into 4 or 8-s bins). The time interval elapsed between a sigh and a subsequent apnea was computed as the interval between the upward (+) WBP pressure deflection peak of the sigh and the upward WBP pressure deflection peak of the last breath before the apnea (Fig. 2). Thus, for example, for each mouse the apnea occurrence rate in the first 4-s bin after a sigh was computed on the number of instances in which this time interval was < 4 s. We reasoned that apneas in a given time bin should be considered related to the preceding sigh only if their occurrence rate in that specific bin resulted significantly higher than the occurrence rate of apneas before the sigh. We arbitrarily chose as a baseline a pre-sigh baseline window because this is an easily identifiable and reproducible time window that we can compare with post-sigh periods. The pre-sigh baseline window was a-priori set at 8 s (instead of 32 s as the post-sigh window) in order to include in the subsequent analyses as many episodes as possible, taking into account that most of NREMS episodes during the light period in mice last less than 2 minutes (Silvani et al., 2014). Ancillary analyses with the Kaplan-Meier estimator (Fig. S1) confirmed that extending the pre-sigh time window from 8 to 32 s (i.e. same length of the analyzed post-sigh window) would have importantly decreased the number of analyzable NREMS episodes containing sighs (by 29.4% using the 2x cutoff value and by 20.5% using the 3x cutoff value for sigh discrimination) and, consequently, the statistical power of our approach.

Non-parametric statistical analyses were performed using SPSS software (Chicago, USA). Friedman and Wilcoxon tests were used to evaluate apnea distribution across and between time bins and to

compare event occurrence rate using the 2x or the 3x cutoff value. Results are shown as median (range) with significance at $P < 0.05$.

RESULTS

The results of our PUBMED search are shown in Table 1. In the 11 articles that reported the definition of post-sigh sleep apnea, highly different criteria were applied. In particular, apneas were categorized as post-sigh if they occurred in a time interval ranging from "1 breath" (Samarasinghe et al., 2015) up to "within 20 s from a sigh" (Yamauchi et al., 2008) and, in 5 articles, only a generic "up to several normal breaths" was indicated as the time interval elapsed between an apnea and the preceding sigh. In the same table, it can also be noted that the 2x cutoff value was used by all groups, except ours, to define apneas (i.e. we applied a more restrictive 3x cutoff value) whereas the cutoff value for sigh definition ranged, among groups, from 1.5x to 3x. However, it must be also noted that baseline respiratory values were calculated with largely different approaches among studies (Table 1), spreading the inter-study variability and limiting data comparisons.

In the present experiment, we analyzed the hypnic and breathing pattern of 22 wild-type mice left to sleep undisturbed for 8 hours inside a WBP chamber. Individual percentages of time spent in each wake-sleep state during these recordings are reported in Table S1. The baseline values of T_{TOT} and V_T during NREMS and REMS in the mice included in the present study (Table S2) were in line with those reported in previous studies (Marcouiller et al., 2014, Nakamura et al., 2003, Nakamura et al., 2007, Terada et al., 2008). The analyses performed on our WBP data showed, as expected, that the occurrence rates of apneas and sighs were significantly lower if calculated applying the more conservative 3x cutoff value than those calculated with the 2x cutoff value (median values are shown in Table 2 whereas individual values are reported in Table S3). Similarly, the median Apnea Index (AI) value, computed dividing the overall number of sleep apneas by the Total Sleep Time (TST), was higher when we applied the 2x cutoff (28(59) apneas/h of TST) than that obtained with the 3x cutoff (4(11) apneas/h of TST). Noteworthy, using the 2x cutoff value, all the mice included in the present study (22/22) showed an AI $\geq$ 10 apneas /h of TST (Table S3).

Fig. 3 shows that only in the 1st and 2nd bin (corresponding to 8 s) following a sigh the apnea occurrence rate was significantly higher than that calculated before the sigh. This result, unrelated to the applied cutoff value (2x or 3x), indicated that apneas should be categorized as post-sigh only if they start within 8 s from a sigh or as spontaneous if they start after this interval. To be more specific, our analysis indicates that when the distance between the upward WBP deflection peak of a sigh (considered as the zero time point) and the upward WBP deflection peak of the last breath before an apnea is less than 8 s, then that apnea should be categorized as post-sigh (Fig. 2). Raw tracings showing apneas starting within or following 8 s from a sigh are shown in Fig. 2 and more examples are reported in Fig. S2 and S3. With this 8-s threshold criterion, we found that in wild-type mice only a small % of apneas was categorized as spontaneous when applying either the 3x (0.0(12.5)%) or the 2x (1.2(28.6)%) criterion while most apneas were scored as post-sigh apneas (individual values are reported in Table S3).

DISCUSSION

In the present work we first conducted a systematic review of the criteria used to discriminate post-sigh from spontaneous apneas in mice. Results of this analysis indicated that highly different criteria were used for this categorization even if only few studies investigated the different physiology and pathophysiology of the 2 apnea subtypes (Table 1). Considering this lack of standardization, which invariably increases inter-study variability limiting experimental reproducibility, we decided to provide a robust and objective data-driven definition of post-sigh apneas in mice. The analyses of the present experiment objectively demonstrated that during NREMS, apneas can be categorized as post-sigh only if they start within 8 s from the preceding sigh (Fig. 3). In fact, the occurrence rate of apneas following a sigh was significantly higher than that calculated for spontaneous apneas (before the sigh) only in the 1st and 2nd 4s-bins after the sigh (overall corresponding to 8 s).

Considering our finding, it is possible to hypothesize that previous works using shorter time windows to define post-sigh apneas (i.e. 1 breath or "up to several breaths", Table 1) likely significantly underestimated their occurrence rate (Boukari et al., 2016, Marcouiller et al., 2014, Nakamura et al., 2003, Nakamura et al., 2007, Samarasinghe et al., 2015, Terada et al., 2008). Conversely, slight or no overestimation of post-sigh apneas may have been introduced when longer time windows were applied (i.e. between 10 and 20 s, Table 1) (Lena et al., 2004, Real et al., 2007, Real et al., 2009, Yamauchi et al., 2008), because the % of apneas occurring after this 8-s interval is very small. Previous studies showed divergent coupling between apnea subtypes and either the sleep state or the inspired gas composition. In particular, post-sigh apneas mainly occur during NREMS and their rate of appearance increases in conditions of hypoxia while it decreases in conditions of hyperoxia or hypercapnia (Nakamura et al., 2003). On the other hand, spontaneous sleep apneas mainly occur during REMS and they seem not to be affected by changes in inspired gas composition (Nakamura et al., 2003). These experiments likely indicated that different yet still unknown mechanisms might underlie the generation of these events (Nakamura et al., 2003, Yamauchi et al., 2008). This

hypothesis is also supported by the fact that only the occurrence rate of post-sigh apneas was altered in Leight Syndrome patients (Yasaki et al., 2001) and in mice lacking the progesterone receptor (Marcouiller et al., 2014) or recapitulating the Rett Syndrome disease (Voituron et al., 2010); on the other hand, only the occurrence rate of spontaneous sleep apneas was affected in a mouse model of CDKL5 disease (Lo Martire et al., 2017). All these differences highlight the importance of discriminating between post-sigh and spontaneous sleep apneas when respiratory functional genomic studies are performed on mice.

Two main hypotheses have been proposed for the determinants of post-sigh apneas during NREMS: the CO_2 threshold and the mechanoreceptor hypothesis. According to the first hypothesis, sigh appearance produces a drop in blood CO_2 level (hypocapnia) below the apnea threshold thus reducing the CO_2-driving stimulus and causing one or more central apneas. The other hypothesis posits that sigh appearance induces activation of lung stretch receptors which, through vagal afferences, centrally inhibit the generation of the following breath. The fact that almost all post-sigh apneas did not occur immediately after a sigh (Nakamura et al., 2003) but within 8 s from it (present study, Fig. 2 and 3) hardly fits with the mechanoreceptor hypothesis of apnea generation. At the same time, the fact that the probability of finding an apnea between 8 and 24 s following a sigh was significantly lower than that of having an apnea before the sigh (applying the 2x cutoff value, Fig. 3A), suggests the existence of an apnea refractory period possibly linked to the CO_2 rebound in the blood after an apneic event.

Cutoff values used to generally define apneas and sighs, in mice, are not uniform in the literature (Table1). Unfortunately, as highlighted by our systematic review, this lack of standardization is still an open issue. However, the criterion we identified for the definition of post-sigh apneas (i.e., start of the apnea within 8 s from a sigh) did not change with the thresholds (2x or 3x) for the definition of apneas and sighs, thus proving the robustness of our findings. On the contrary, similar cutoff values are well consolidated both in adults and newborns. Similarly, the cutoff values to discriminate between healthy and pathological subjects are known and widely accepted among clinicians. Such

indications are still missing for rodents. However, the fact that the totality of mice included in this study showed an AI ≥ 10 apneas/h of TST (applying the 2x cutoff value, Table S3), suggests that mice have a stronger predisposition to develop sleep apneas compared to humans. Nowadays, there is growing interest in the study of the pathophysiology of sleep apneas (anatomical characteristics favoring upper airway obstruction, loop gain, arousal threshold…) in order to develop personalized therapies and, in this regard, mice might represent a useful feature for translation studies and for a better comprehension of respiratory control during sleep.

Finally, some limitations of the present work should be acknowledged. First, we performed our analyses on adult male mice with a C57Bl/6 genetic background. Although this is the most common mouse strain in functional genomic studies, it must be kept in mind that some genetic mutations or different genetic backgrounds might affect the time window for discriminating post-sigh and spontaneous sleep apneas. Moreover, it is possible that the criteria for the definition of sleep apnea subtypes, at least in part, depend on mouse gender or age. Second, our sleep and breathing data were recorded only during the first 8 hours of the light period which, in mice, corresponds to the resting phase. We made this choice because long WBP recordings might be stressful in mice and because, in this specific study, we focused on the analysis of the breathing pattern during sleep rather than on the characterization of mouse circadian sleep phenotype (which requires at least 24 hours of recordings). Third, the experimental setup used in this study did not allow us to categorize sleep apneas according to their origin (central vs obstructive) and, thus, to relate them with sigh occurrence. To date, both post-sigh and spontaneous sleep apneas in mice have been anecdotally classified as central sleep apneas (Nakamura et al., 2003) but extensive and exhaustive studies on this topic are still missing.

In conclusion, with the present study we provided robust and useful standards for the definition of post-sigh and spontaneous sleep apneas in mice. Altogether, our data underline the importance of reporting sigh occurrence rate concomitantly with the categorization of apneas into different subtypes and their occurrence rate in each sleep state.

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FIGURES LEGEND

Figure 1. Flow chart of systematic literature revision.

The present diagram was modified from the model provided in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) website (http://prisma-statement.org/). It schematizes the decisional process we followed to select the articles included in the present systematic review.

Figure 2. Example of raw tracings including sigh and apneas during sleep in mice.

Representative raw tracings of the whole-body plethysmography signal (WBP, upwards deflection indicating inspiration) during non-rapid-eye-movement sleep (NREMS) in an adult male C57Bl/6 mouse. EMG, electromyogram; EEG, electroencephalogram. Blue arrow indicates a sigh; yellow arrow indicates an apnea occurring within 4 s from the preceding sigh (see the insert); green arrow indicates an apnea occurring before the sigh; red arrows indicates apneas occurring after 8 s from the sigh. The insert shows the magnification of the WBP signal in the 15 s following the sigh. Black dot marks WBP upward deflection peak of the sigh (representing the zero time point for the calculation of apnea distance from the sigh); yellow dot marks the upward deflection peak of a post-sigh apnea; red dot marks the upward deflection peak of a spontaneous apnea (see Results for definition of post-sigh apnea and spontaneous apnea). The latter sleep apnea begins within 12 s from the sigh (red dot) and it ends after 12 s from the sigh. Based on our definition, this apnea occurs in the third 4-s bin from the sigh.

Figure 3. Apnea occurrence rate before and after NREMS sighs in wild-type mice.

The 32 s interval following each sigh during non-rapid-eye-movement sleep (NREMS) was split into 4- or 8-s bins and the occurrence rate of apneas in each bin was compared to that calculated in the 8 s preceding the sigh (i.e. the boxplot before the vertical dotted line). Apneas and sighs in 22 wild-type mice were first assessed as breaths with T_{TOT} (apneas) or V_T (sighs) > 3 times (3x, panel b) the average T_{TOT} or V_T for each sleep state and then, they were also calculated as breaths with T_{TOT} or V_T > 2 times (2x, panel a) the average T_{TOT} or V_T for each sleep state. ʌ or v indicate that apnea occurrence calculated in that time bin was, respectively, significantly ($p < 0.05$) higher or lower than that calculated in the 8 s preceding the sigh. Data are shown as median, quartiles and min/max values of each distribution.

Figure S1. Percentage of NREMS episodes available for subsequent analyses based on the duration of baseline pre-sigh time window

The 2 panels show the Kaplan-Meier estimator of the distribution of non-rapid-eye-movement sleep (NREMS) episodes as function of the distance (seconds) between the beginning of that specific sleep episode and the occurrence of an augmented breath (sigh) during this episode. Upper and lower panels show this analysis performed applying, respectively, the 2x or the 3x cutoff value in sigh detection. In each panel, the green dot corresponds, on the x-axis, to a distance of 8 s between the beginning of NREMS episodes and the sigh and, on the y-axis, to the % of residual NREMS episodes available for subsequent analyses applying a pre-sigh window of at least 8 s. The red dots indicate the % of residual NREMS episodes available for subsequent analyses for a pre-sigh window of 32 s or longer.

Figure S2. Example of raw tracings including unrelated sighs and apneas during sleep in mice.

Representative raw tracings of the whole-body plethysmography signal (WBP, upwards deflection indicating inspiration) during non-rapid-eye-movement sleep (NREMS) in an adult male C57Bl/6 mouse. NREMS is characterized by low electromyographic (EMG) tone and by electroencephalographic (EEG) signal with high voltage and prominent δ frequency components. In the upper panel, the red arrow indicates an apnea (breath pause) with no augmented breaths (sighs) in the preceding 8 s thus categorized as spontaneous apnea. In the lower panel, the blue arrow indicates a sigh with no apnea in the following 30 s.

Figure S3. Example of raw tracings including spontaneous apneas during rapid-eye-movement sleep.

Representative raw tracings of the whole-body plethysmography signal (WBP, upwards deflection indicating inspiration) at the transition (dotted yellow line) between non-rapid-eye-movement sleep (NREMS) and rapid-eye-movement sleep (REMS) in an adult male C57Bl/6 mouse. NREMS is characterized by low electromyographic (EMG) tone and by electroencephalographic (EEG) signal with high voltage and prominent δ frequency components. REMS is characterized by low/atonic EMG and by EEG with low voltage with predominant θ frequency components. Red arrows indicate apneas (pause breaths) with no augmented breaths (sighs) in the preceding 8 s thus categorized as spontaneous apneas.

Table 1. Criteria used for the identification of apnea, sigh and post-sigh apnea in mice.

Author and Year	T_{TOT} baseline definition	V_T baseline definition	Threshold change in T_{TOT} vs. baseline to detect apneas	Threshold change in V_T vs. baseline to detect sighs	Maximum time interval from preceding sigh to define post-sigh apneas
Nakamura et al. 2003	10 – 20 s of stable breaths before the apnea	10 s before the apnea	2x	1.25x	"Up to several normal breaths"
Lena et al. 2004	in NREMS	30 respiratory cycles before the apnea	2x	2x	< 15 s
Nakamura et al. 2007	10 s before the apnea		2x	2x	"Up to several normal breaths"
Real et al. 2007	10-s period of stable breaths in the 20 s before the apnea	10 s before the apnea	2x	1.5x	< 10 s

Terada et al. 2008	10 s before the apnea		2x	2x	"Up to several normal breaths"
Yamauchi et al. 2008	6 s before the apnea		2x	2x	< 20 s
Real et al. 2009	10-s period of stable breaths in the 20 s before the apnea	10 s before the apnea	2x	1.5x	< 10 s
Marcouiller et al. 2014	few seconds after a sigh	Not defined	2x	2x	"Up to several normal breaths"
Samarasinghe et al. 2015	10 min of stable recording in the control period		2x	2x	1 breath
Boukari et al. 2016	10-20 s of stable breaths before the apnea	of the 10 s before the apnea	2x	2x	"Up to several normal breaths"

Lo Martire et al. 2017	average of all available data within sleep state (NREMS or REMS)	3x	3x	< 8 s

All these studies were performed on male mice except for the studies by Marcouiller et al. and Lo Martire et al. in which female mice were used. In the paper by Boukari et al. both male and female mice were used, whereas Samarasinghe et al. did not specify mouse gender.

Table 2. Sleep apneas and sighs in wild-type mice.

		2x (Events/h)	3x (Events/h)
Apneas	**NREMS**	22.2 (64.4) *	3.3 (11.7)
	REMS	70.5 (145.7) *	10.1 (17.3)
Sighs	**NREMS**	20.9 (62.3) *	5.5 (2.3)
	REMS	8.4 (86.1) *	0.0 (7.8)

Apnea and sigh occurrence rate recorded during non-rapid-eye-movement sleep (NREMS) and rapid-eye-movement sleep (REMS) in 22 wild-type mice. For each mouse, apneas and sighs were first assessed as breaths with T_{TOT} (apneas) or V_T (sighs) > 3 times (3x) the average T_{TOT} or V_T for each sleep state and then, they were also calculated as breaths with T_{TOT} or V_T > 2 times (2x) the average T_{TOT} or V_T for each sleep state. Data are reported as median(range). *, < 0.05 vs 3x condition.

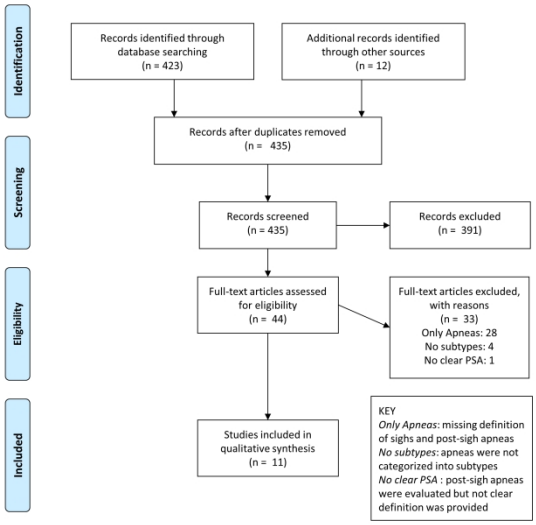

Figure 1. Flow chart of systematic literature revision

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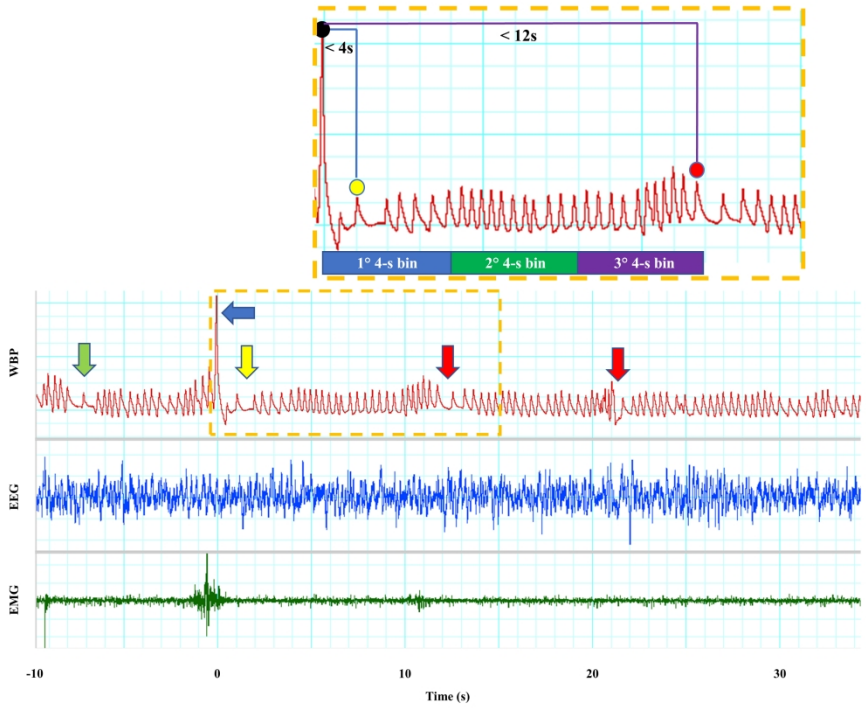

Figure 2. Example of raw tracings including sigh and apneas during sleep in mice.

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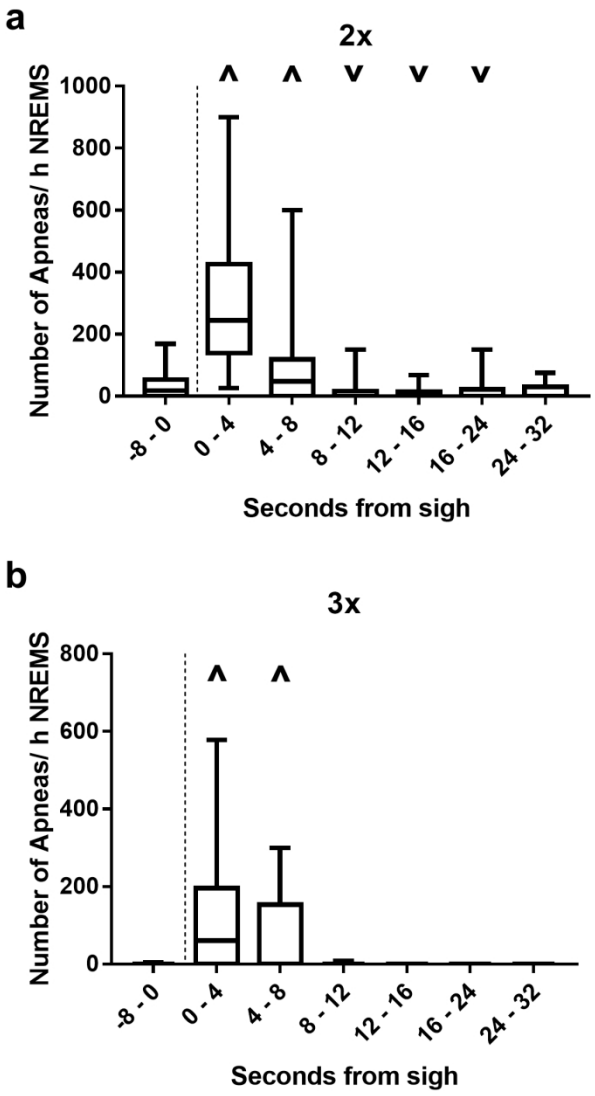

Figure 3. Apnea occurrence rate before and after NREMS sighs in wild-type mice.

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Table S1. Wake-sleep cycle structure of recorded mice.

Mouse	WAKE (%)	NREMS (%)	REMS (%)
1	26	60	6
2	24	60	11
3	29	52	4
4	13	71	5
5	38	50	7
6	18	64	3
7	32	55	4
8	20	64	6
9	30	48	7
10	32	57	8
11	26	63	7
12	38	41	10
13	28	58	8
14	21	69	6
15	27	56	9
16	20	52	9
17	45	49	2
18	19	59	9
19	14	69	5
20	18	64	10
21	19	50	0
22	10	79	6

The table shows the percentage of time spent in the states of Wakefulness, non-rapid-eye-movement sleep (NREMS) and rapid-eye-movement sleep (REMS) by each mouse included in this study. Mice were recorded for 8 hours inside a whole-body plethysmographic chamber.

Table S2. Baseline values of tidal volume and ventilatory period

	T_{TOT} (ms)	V_T (ul)	V_T/weight (ul/g)
NREMS	382 ± 6	183 ± 9	7.7 ± 0.3
REMS	335 ± 7	155 ± 8	6.5 ± 0.3

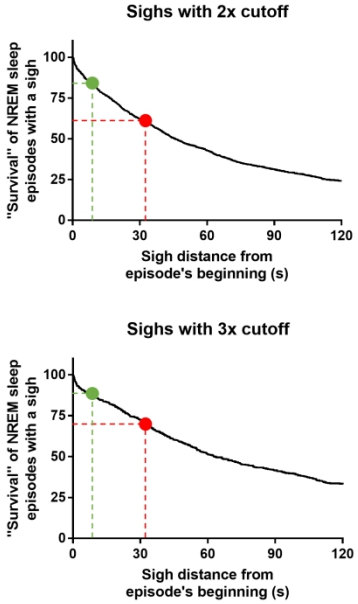
Baseline values of ventilatory period (T_{TOT}), tidal volume (V_T) and V_T per body weight (V_T/weight) during non-rapid-eye-movement sleep (NREMS) and rapid-eye-movement sleep (REMS) in 22 wild-type mice. For each mouse, values were calculated as average within each sleep state and reported as group mean ± SEM.

Table S3. Sleep apneas and sighs in recoded mice

MOUSE	NREMS				REMS				TOTAL SLEEP	
	SIGHS		APNEAS		SIGHS		APNEAS		APNEA INDEX	
	2X	3X	2X	3X	2X	3X	2X	3X	2X	3X
1	47	34	16 (94)	2 (100)	11	0	41	6	14	2
2	57	18	25 (100)	0 (/)	0	0	58	12	23	3
3	13	2	34 (85)	0 (/)	3	0	15	1	16	0
4	92	34	54 (100)	3 (100)	11	1	8	0	16	1
5	35	11	15 (100)	0 (/)	4	0	38	7	20	3
6	53	13	40 (100)	2 (87.5)	1	0	8	0	16	1
7	60	10	51 (98)	16 (100)	6	0	21	3	20	5
8	104	16	220 (98)	36 (100)	6	1	25	3	48	8
9	74	16	48 (88)	23 (100)	11	0	57	5	31	8
10	47	6	52 (100)	11 (100)	1	0	18	3	20	4
11	207	50	91 (99)	27 (100)	8	0	33	4	28	7
12	18	5	28 (71)	3 (100)	0	0	21	3	34	4

13	20	3	80 (100)	10 (100)	3	0	52	6	33	4
14	131	21	84 (98)	7 (100)	5	0	34	5	28	3
15	27	2	71 (100)	11 (100)	4	0	24	3	27	4
16	27	4	61 (100)	8 (100)	7	1	26	3	36	5
17	84	30	129 (95)	22 (95)	0	0	3	0	69	11
18	64	14	81 (99)	27 (89)	12	0	33	5	38	11
19	249	79	169 (96)	24 (100)	0	0	15	3	46	7
20	136	41	96 (100)	9 (100)	4	1	62	8	39	4
21	134	46	19 (95)	0 (/)	0	0	0	0	10	0
22	277	114	289 (93)	57 (93)	0	0	17	1	58	11

Number of apneas and sighs recorded during non-rapid-eye-movement sleep (NREMS) and rapid-eye-movement sleep (REMS) in 22 wild-type mice. For each mouse, the number of apneas and sighs are reported either applying the 2X or the 3X cut-off value (i.e. breaths with T_{TOT} (apneas) or V_T (sighs) > 2 or 3 times the average T_{TOT} or V_T for each sleep state). In the NREMS apnea column, values between brackets indicate, as percentage, the amount of post-sigh apneas over the total number of apneas (values outside the brackets) recorded with the 2X or the 3X cut-off values. The apnea index is calculated as the total number of apneic events recorded during NREMS and REMS divided by the total sleep time (hours).



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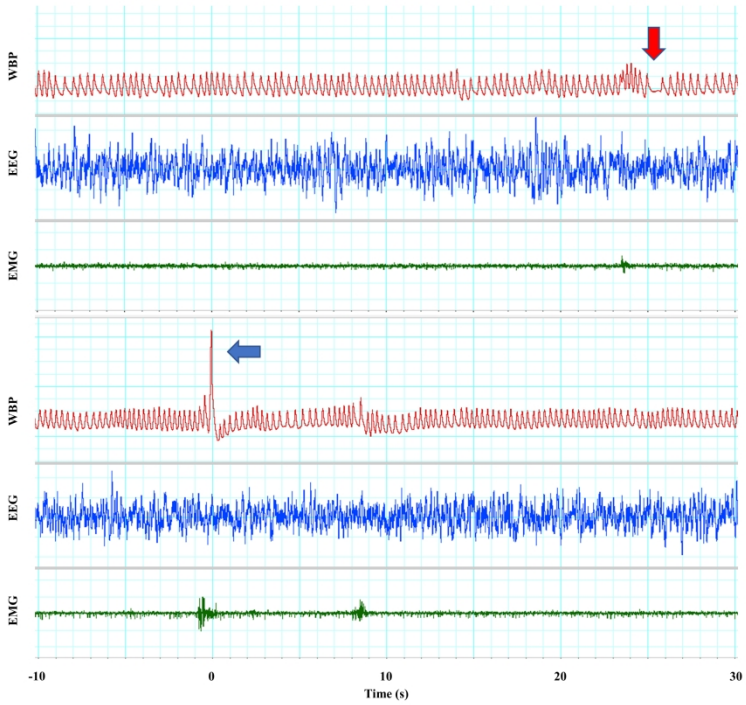

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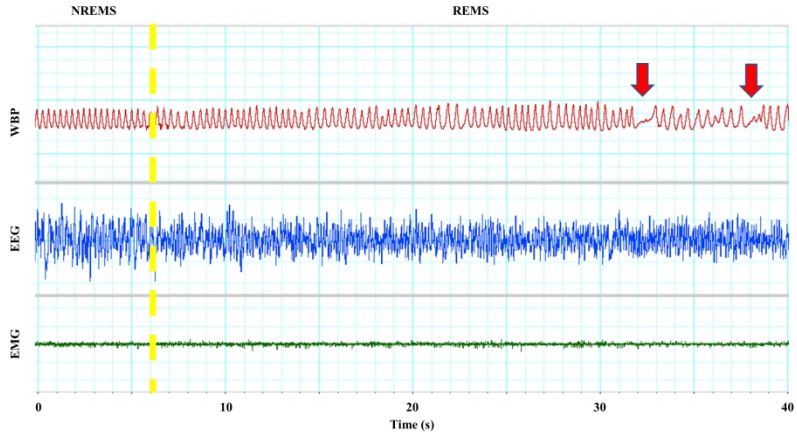

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