

Respiratory changes during adaptation to stress induced by movement restriction in rabbits

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Abstract

Background

Confinement inside a restricted space causing movement restriction is a stressful condition, potentially leading to spontaneous changes of respiratory parameters that are expected to return to normal values as the stress subsides. Barometric plethysmography is a non-invasive method to study surrogates of pulmonary mechanics in conscious animals enclosed into a plexiglass chamber. This chamber greatly restricts the animal movements but does not cause its immobilization.

Methods

Respiratory parameters from six rabbits were recorded during 90 min/day for 5 days while the animal was confined inside a plethysmographic chamber. Modifications of respiratory parameters were evaluated by dividing the total length of the recording in three 30-min periods.

Results

During the 90-min recording, enhanced pause (Penh, a lung resistance surrogate) showed a decreasing trend, coinciding with a decline of the mid-expiratory flow (an airway obstruction surrogate), and time of braking (an end-inspiratory glottis closure surrogate). Respiratory frequency increased from 346 to 363 breaths/min, coinciding with a progressive decline of tidal volume and minute ventilation. Because rabbit responses to stressful situations are predominantly parasympathetic in nature, an increased parasympathetic tone during the first minutes of confinement might explain the initially augmented lung, airways and glottis resistances, and these, in turn, could be responsible for the low initial respiratory frequency. Subsequent changes of these variables probably reflect progressively lower level of stress due to adaptation to the new environment. This pattern did not change in the 5 days studied.

Conclusions

We concluded that respiratory parameters in rabbits display subtle changes during the first 90 min of movement restriction, probably driven by an initially augmented parasympathetic tone due to stress, with subsequent normalization as stress diminished due to adaptation to the new environment.

Background

The term “stress” denotes a condition where an environmental demand exceeds the natural regulatory capacity of an organism, with absence of an anticipatory response (unpredictable) or a reduced recovery (uncontrollable) of the neuroendocrine reaction [1]. Animal models of stress may utilize different stressful maneuvers such as handling, exposure to noise, foot shock, mother separation, water immersion, restraining, transportation, etc. The physiological responses following exposure to a stressor have been relatively well characterized. These responses include activation of several cerebral nuclei and triggering of the hypothalamo-pituitary-adrenal axis and sympathoadrenal and parasympathetic responses, which

together lead to a rapid response to face the stressful condition [2]. After the initial physiological responses to stressors have peaked, a progressive phase of adaptive changes ensues, characterized by reversal of the stress-related changes. In humans and most laboratory mammals, during the acute response to stressful conditions a sympathoadrenal response predominates [1, 3]. However, in rabbits it has been documented that stress produces a response that is predominantly parasympathetic in nature [4, 5].

Several non-invasive techniques have been developed for the assessment of pulmonary mechanics in laboratory animals, including barometric plethysmography, a technique in which the animal is enclosed into a plexiglass chamber that greatly restricts its movements, but does not cause its immobilization [6–9]. This last method is remarkably useful due to its simplicity and because it allows the animal to stay in an unrestrained state and even to eat and drink at libitum.

Some years ago, it was reported that guinea pigs submitted to barometric plethysmography have an initial period of adaptation to the confinement inside the plethysmographic chamber, which takes up to 90 min to stabilize [10]. Changes observed during this period were probably due to stress and consisted of a decreasing trend of respiratory frequency that was mirrored by an increasing trend in a surrogate of lung resistance. Recently, this same phenomenon has been described for mice [11]. However, as far as we know, the existence and characteristics of such spontaneous changes have never been described in rabbits.

Thus, the aim of the present study was to determine respiratory variables in rabbits, as measured through barometric plethysmography, during the process of adaptation of the animal to movement restriction when confined into a closed environment.

Methods

Animals

A group of six healthy male New Zealand white rabbits (*Oryctolagus cuniculus*) weighing approximately 3 kg were purchased from the Animal Production Center of the Faculty of Veterinary Medicine, National Autonomous University of Mexico (UNAM). We selected this rabbit breed because it is often employed as laboratory animal and, due to the descriptive nature of the study, we considered that six animals were enough to characterize their respiratory parameters. The animals were quarantined in our institutional laboratory animal care facility under conventional conditions until their study (12/12 h light/dark cycles, filtered conditioned air, 21 ± 1 °C, 50–70% humidity, sterilized bed, and fed ad libitum with pellets and sterilized water). The study protocol was reviewed and approved by our institutional scientific and ethics committee with the approval number B-12-16. All animals were returned alive to the laboratory animal facility after completion of the study.

Confinement maneuver (barometric plethysmography)

Each rabbit was submitted to barometric plethysmography during 90 min/day for 5 days. All studies were initiated in the morning, at approximately 7–8 am. On the day of the study, the rabbit movements were greatly restricted by introducing the animal into a ~ 16 L plexiglass plethysmographic chamber (~ 34 cm in diameter, 17.5 cm in height, Whole Body Plethysmograph, Buxco Research Systems, Wilmington, NC, USA). This chamber was provided with two ventilation ports through which a bias air flow of 2.5 L/min was continuously entering the chamber (Fig. 1A and 1B). The equipment was calibrated daily following the manufacturer's instructions by introduction of 10-ml of air. Respiratory variables were recorded and analyzed with the software supplied by the manufacturer (FinePointe, v2.1.0.9, Buxco). The software was adjusted to record respiratory parameters in a time-based mode, averaging data every 2 s. Raw data from all parameters were transferred to an Excel worksheet, where further analyses were performed. We discarded all 2-s periods with a rejection index (Rinx) higher than 50%, yielding an average of 28.4% (from 12.3–46.5%) periods discarded. From the valid 2-s periods, we obtained the median value of each 5-min period. We preferred the use of medians instead of means in order to exclude occasional extreme values.

Data analysis

After corroborating the normal distribution of variables (Kolmogorov-Smirnov test), including the 5-min periods of each barometric plethysmographic variable, data were expressed as mean \pm standard error. For each variable, the 90-min recording was divided in three time-periods (0–30 min, 30–60 min, and 60–90 min), and differences among them were evaluated through analysis of variance for repeated measures, followed by paired Student's t-test with the Bonferroni correction to adjust for multiple comparisons. Statistical significance was set at two-tailed $p < 0.05$. Finally, in order to visually assess the temporal trend of each respiratory variable during the entire 90-min recording, a straight line- or logistic-fit was made for all of the values in each study day (CurveExpert v1.40, Daniel G. Hyams©, Madison, AL, USA).

Results

The time trend of each respiratory parameter during the initial 90-min period of barometric plethysmography is depicted in Fig. 2, and the mean values in the last 30 min are summarized in Table 1. After averaging the results of the six rabbits, we were unable to identify consistent differences in the time trend patterns among the 5 days of recording, and thus the results were analyzed jointly.

Table 1

Values of respiratory parameters measured by barometric plethysmography in rabbits

Abbreviations and units	Variable name	Median (Q1 - Q3)*	Mean \pm S.E.M.*	CI95%*
EF50 (ml/s)	Expiratory flow 50	2.48 (2.43–2.50)	2.47 \pm 0.014	2.44–2.50
f (breaths/min)	Respiratory frequency	363 (351–374)	363 \pm 2.4	358–368
MV (ml)	Minute ventilation	480 (470–487)	481 \pm 2.8	476–486
PAU (unitless)	Pause	0.666 (0.660–0.675)	0.666 \pm 0.0022	0.662–0.67
PEF (ml/s)	Peak expiratory flow	24.1 (23.6–24.4)	24.1 \pm 0.14	23.8–24.4
Penh (unitless)	Enhanced pause	0.66 (0.64–0.67)	0.66 \pm 0.004	0.65–0.67
PIF (ml/s)	Peak inspiratory flow	26.4 (25.6–26.6)	26.2 \pm 0.18	25.8–26.6
Rpef (unitless)	Peak expiratory flow rate	0.533 (0.527–0.537)	0.533 \pm 0.0013	0.530–0.536
TB (%)	Time of braking	15.42 (15.24–15.69)	15.45 \pm 0.061	15.33–15.57
Te (s)	Expiratory time	0.0832 (0.0806–0.0860)	0.0837 \pm 0.00074	0.0822–0.0852
Ti (s)	Inspiratory time	0.0875 (0.0841–0.0919)	0.0883 \pm 0.00084	0.0867–0.0899
TP (%)	Time of pause	17.49 (17.39–17.62)	17.51 \pm 0.041	17.43–17.59
Tr (s)	Relaxation time	0.0514 (0.0500–0.0534)	0.0518 \pm 0.00045	0.0509–0.0527
TV (ml)	Tidal volume	1.36 (1.32–1.42)	1.39 \pm 0.020	1.35–1.43
*Data correspond to the last 30 min of the 90-min period recording, i.e., once the rabbit has spent 1 h of adaptation to the plethysmographic chamber. CI95%=confidence interval at 95%; Q1 = quartile 1; Q3 = quartile 3; S.E.M = standard error of the mean.				

Surprisingly, the conscious rabbit had a much higher-than-expected respiratory frequency, initiating with 347 ± 3.8 breaths/min (mean \pm SEM) in the first 30 min, and increasing up to 360 ± 3.2 and 363 ± 2.4 breaths/min in the two following 30-min periods (Fig. 2A). This increase in respiratory frequency was associated with a progressive decline in tidal volume (TV), which changed from 1.57 ml in the initial 30-min period to 1.44 and 1.39 ml in the following periods (Fig. 2B). Although the behavior of these last two parameters was in a mirror fashion, the resulting minute ventilation (MV), which is the product of

respiratory frequency by TV, still showed a progressive decrease in the 90-min period (Fig. 2C), probably reflecting that the rabbit was progressively less stressed due to its adaptation to the new environment inside the plethysmographic chamber and therefore had a lower metabolic rate.

The pause (PAU), which is the time it takes to expel the final 36% of the total expiratory pressure compared with the time it takes to expel the first 64% (this last time is called time of relaxation [Tr]), did not change over time (Fig. 2D), which was in agreement with the lack of modification of expiratory time (Te) and Tr (Fig. 2E and 2F, respectively). Compared with the initial 30-min period (0.674 ± 0.003), values of Penh (a parameter that has been considered as a surrogate of airway obstruction) showed a relative decrease in the two following periods (0.657 ± 0.003 and 0.657 ± 0.004 , respectively, Fig. 2H). Because Penh is the product of PAU multiplied by the ratio of PEF to PIF, and PAU was stable, changes in Penh were due to a more pronounced decrease of PEF than the corresponding decrease of PIF (Fig. 2I and 2J, respectively). The behavior of Penh contrasted with the expiratory flow at 50% of tidal volume [TV] (EF50) and the peak expiratory flow rate (Rpef, which is the time to PEF related to Te). The latter two parameters are also surrogates of airway obstruction [12, 13], but EF50 showed a decreasing trend in the 90 min period, indicating progressive airway obstruction (Fig. 2K), and Rpef did not change (Fig. 2L).

The two potential pauses of the breath cycle (i.e., when there is almost zero flow), occurring between the end of the expiratory (TP, time of pause) or inspiratory (TB, time of braking) phases and the beginning of the next inspiratory or expiratory phases, respectively, showed contrasting modifications during the 90-min recording. Thus, while the TP did not show modifications (Fig. 2M), the TB significantly decreased in the last 30-min of the recording, probably reflecting a progressively lower laryngeal resistance (Fig. 2N).

As can be seen in the above-mentioned illustrations, five parameters had a relatively stable pattern throughout the 90-min period (PAU, Rpef, Te, TP, and Tr), respiratory frequency had an ascending trend, and the remaining eight variables (EF50, MV, PEF, Penh, PIF, TB, time of inspiration [Ti, Fig. 2G], and TV) showed a diminishing trend. Specifically, Penh, perhaps the most important parameter of barometric plethysmography, required only 30 min to reach a steady state. Table 1 shows average values obtained for each variable in the last 30-min of recording, i.e., once the rabbit has spent one hour into the plethysmographic chamber and has probably been adapted to confinement.

Discussion

In the present study, we described variations in respiratory parameters measured in clinically healthy rabbits during the adaptation process to an enclosed environment, and, secondarily, provided normal values for barometric plethysmography in this animal species.

Animal models are of paramount importance for a comprehensive study of human diseases. Compared with other animal species, the rabbit has been scarcely employed as a model of human respiratory diseases. Certainly, its use has some limitations, such as the higher cost and lesser availability of reagents, but rabbits have proved to be suitable for the study of certain disorders. For example, the rabbit asthma model shares some important pathophysiological mechanisms with the human disease, such as

the IgE production after sensitization and the development of the early- and late-phase responses to an antigenic challenge, with the advantage that these animals are large enough as to better study the pulmonary mechanics [14]. Unfortunately, respiratory parameters of rabbits have been scantily investigated.

Traditionally, it has been claimed that respiratory frequency of rabbits varies around 30–80 breaths/min [15–17]. In sharp contrast with this concept, in our study we found that adult New Zealand rabbits had a much higher frequency during plethysmography, reaching approximately 340–370 breaths/min, and this is clearly illustrated in Fig. 1C. Some authors have also reported relatively higher breath rates than the previously assumed. For example, Schroedder et al. [18] and Finzi [19] described an average of ~ 182 and ~ 185 breaths/min in adult New Zealand rabbits, while Richter et al. [20] reported an average respiratory frequency of 198 breaths/min in newborn rabbits (hybrids of New Zealand and Dendermore). By contrast, Maskrey and Nicol [21] used barometric plethysmography (like us) and their results clearly showed a high basal breath frequency (235 ± 46 breaths/min, mean \pm SEM) in the first 30 min of recording, which increased up to 297 ± 48 breaths/min in the second 30-min period. These breath frequency and time-trend fully agree with our results and were made in control rabbits at 35 °C ambient temperature. Moreover, in order to verify this high respiratory rate, we introduced a rabbit into a home-made plexiglass chamber that contained the body excluding the head with a latex seal at the neck. This hermetic chamber allowed us to record changes in the in-box pressure caused exclusively by thoracic respiratory movements, and we confirmed that rabbits reached indeed such high breath frequency (Fig. 3).

The high level of respiratory frequency observed in spontaneously breathing rabbits was comparable to respiratory frequencies reached in humans during the ventilator mode known as high frequency oscillation [22]. In this sense, the rabbit might be considered as a natural model of this type of mechanical ventilation. In fact, Piva et al. [23] found that in rabbits anesthetized and deprived from surfactant by repeated bronchoalveolar lavage, the PaO₂ and oxygenation index could be restored to normal levels when animals were submitted to high frequency ventilatory oscillation (300 to 900 breaths/min). Thus, it seems that these high rates of breathing are within physiological ranges for rabbits.

Perhaps the most used parameter in barometric plethysmography is Penh, a unitless index that has been employed as a surrogate of lung resistance and airway obstruction [24, 25]. We found that Penh in rabbits had a progressive decrease in the first 60 min of recording. This is contrary to Penh changes occurring in guinea pigs during barometric plethysmography [10]. Thus, in guinea pigs, Penh values were relatively low immediately after the animal entered the chamber, but these were followed by a progressive increase in the next 90 min. The initially low Penh values in guinea pigs were probably due to the stress generated by the new environment and the ensuing release of catecholamines and nitric oxide, as they were avoided by administration of propranolol or L-NAME [10]. Contrasting with this adrenergic response of guinea pigs to stress, it has been demonstrated that in rabbits a stressful maneuver is accompanied by parasympathetic activation causing, for example, transient bradycardia and hypotension that can be

abolished by a muscarinic antagonist [4, 5]. Thus, guinea pigs and rabbits seem to trigger different physiological responses to stressful situations.

Figure 4 describes the possible sequence of events occurring in the rabbit during the first minutes of confinement inside the plethysmographic chamber. The stress generated by the new environment probably triggers an increment in the parasympathetic tone, causing an end-inspiratory glottis closure through activation of laryngeal nerves and a tracheobronchial narrowing through activation of vagus nerves. Both phenomena would lead to prolongation of TB and Ti, and to an increment in Penh, respectively. Combination of these alterations, in turn, would be reflected by a decrease in respiratory frequency. On the other hand, the stressful situation would enhance the metabolic demands and thus increase MV. It is reasonable to suppose that all these respiratory parameters will return to more physiological (normal) values once the rabbit is adapted to the confinement inside the plethysmographic chamber.

In addition to the “short-term” adaptation described above, we had also hypothesized that rabbits will display a “long-term” adaptation during the course of the 5 days of barometric plethysmography, i.e., that at day 5 they would be less stressed than on day 1. However, we did not identify consistent differences in the time trend pattern among the 5 days of recording. Thus, it is possible that 5 days were not sufficient for the rabbit to become familiarized with the plethysmographic chamber.

A potential limitation of our study is that validity of barometric plethysmography, and especially Penh, has been questioned by some authors [26, 27]. However, this last parameter has proved to be correlated with acute changes in lung resistance during a cholinergic challenge in some animal species such as guinea pigs and mice [24, 25]. Thus, barometric plethysmography seems to be an appropriate tool to measure acute modifications of pulmonary mechanics, such as those expected to occur during confinement of rabbits inside the plethysmograph box.

Conclusions

In conclusion, we found that respiratory parameters in rabbits display subtle changes during the first 90 min of adaptation to confinement inside the plethysmographic chamber (a progressive decline of Penh, EF50, TB, Ti, MV, TV, PIF, and PEF; a progressive increase of respiratory frequency). These changes were probably driven by an initially augmented parasympathetic tone due to stress, with subsequent normalization as stress diminished due to habituation to the new environment inside the plethysmographic chamber.

Abbreviations

EF50, expiratory flow at 50% of TV; f, respiratory frequency; MV, minute ventilation; PAU, pause; PEF, peak expiratory flow; Penh, enhanced pause; PIF, peak inspiratory flow; Rpef, peak expiratory flow rate; TB, time of braking; Te, expiratory time; Ti, inspiratory time; TP, time of pause; Tr, relaxation time; TV, tidal volume.

Declarations

Ethics approval and consent to participate

The protocol was approved by the Research and Ethics Committees of the Instituto Nacional de Enfermedades Respiratorias with the approval number B-12-16, and the study was performed in strict accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors participated in the conception and design of the study. JLAR & JAM contributed in the acquisition of data and, along with MHV, PSM and VC in its analysis and interpretation. All authors participated in the drafting of the manuscript and its critical review for important intellectual content, and approval of the final version of the manuscript. JLAR & JAM agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Authors assure that all of us qualify for authorship.

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Figures

Fig. 1
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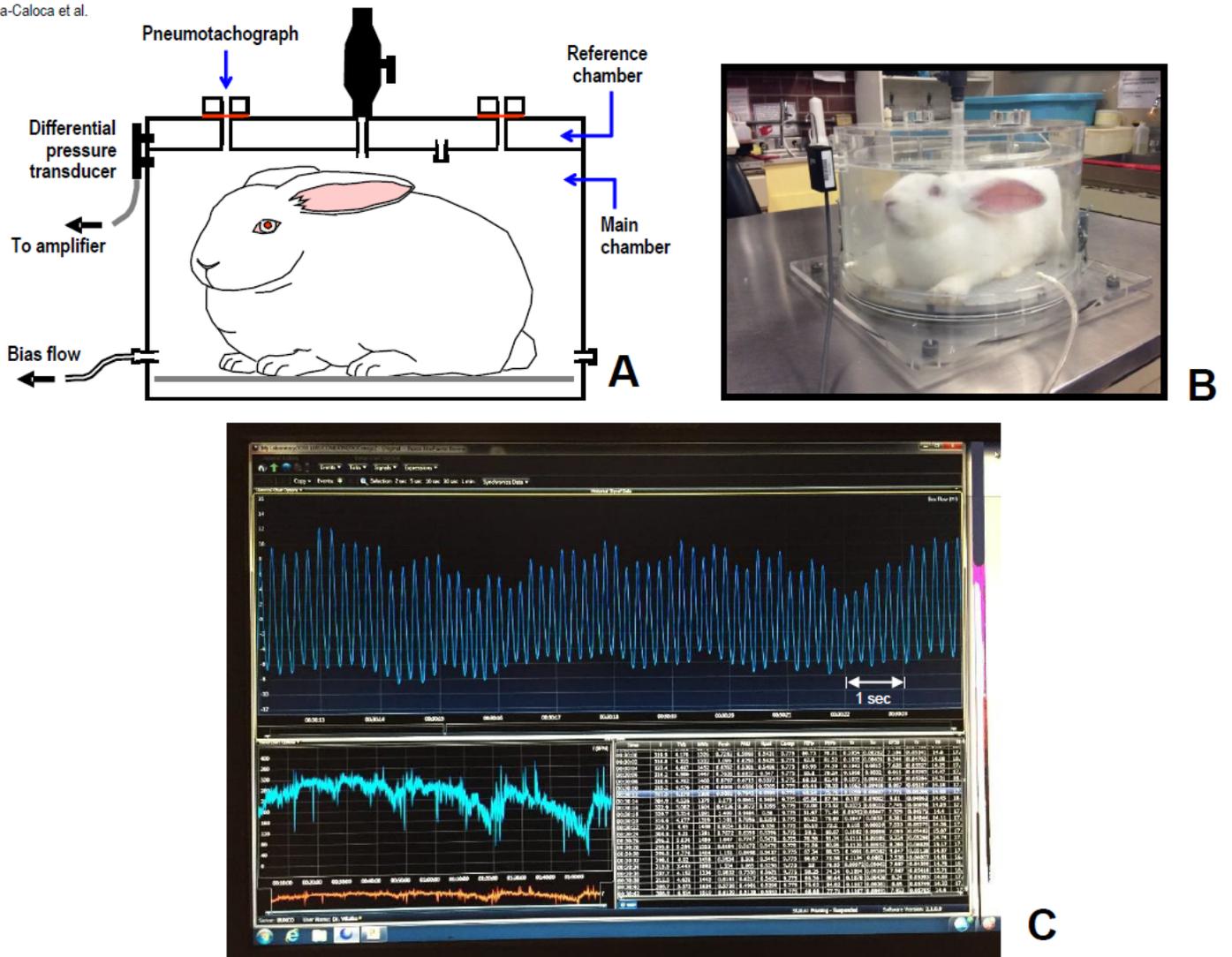


Figure 1

Set up for barometric plethysmography (A) for conscious non-restrained rabbits (B), and a representative recording of pressure changes inside the chamber and data acquisition (C).

Fig. 2 Villalba-Caloca et al.

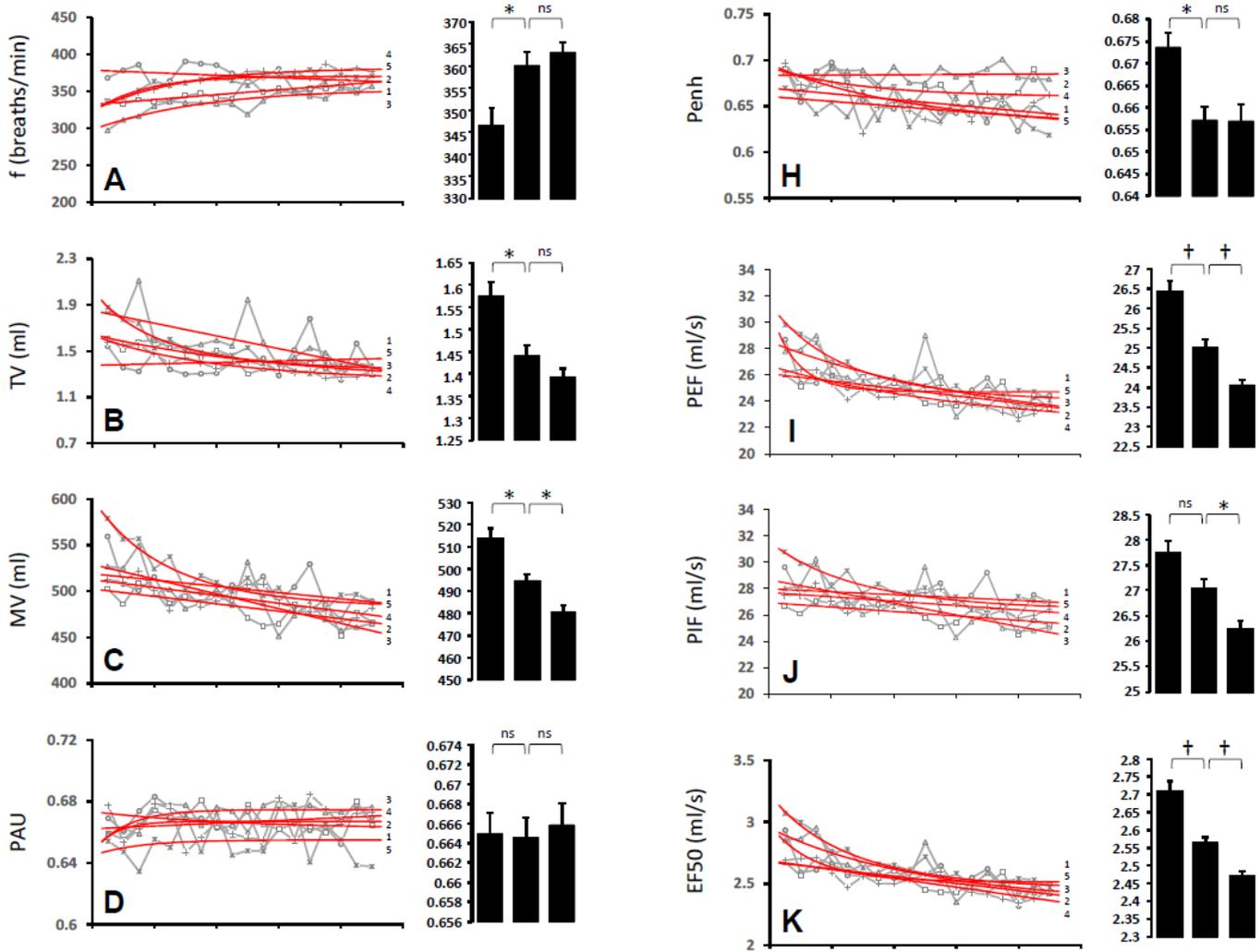


Figure 2

Time trends of respiratory parameters in rabbits during barometric plethysmography. Six rabbits were submitted to barometric plethysmography during 90 min/day for 5 days, and the average of the six animals was calculated every 5 min for each day (symbols). Continuous lines correspond to the straight line- or logistic-fit of the averages recorded in a single day (the sequence of the 5 days is displayed at the right end of lines). Bars correspond to the mean \pm standard error of the three time periods (0-30, 30-60, and 60-90 min). * $p < 0.05$ and † $p < 0.01$, as calculated through paired Student's t-test with Bonferroni correction; ns=non significant.

Fig. 3
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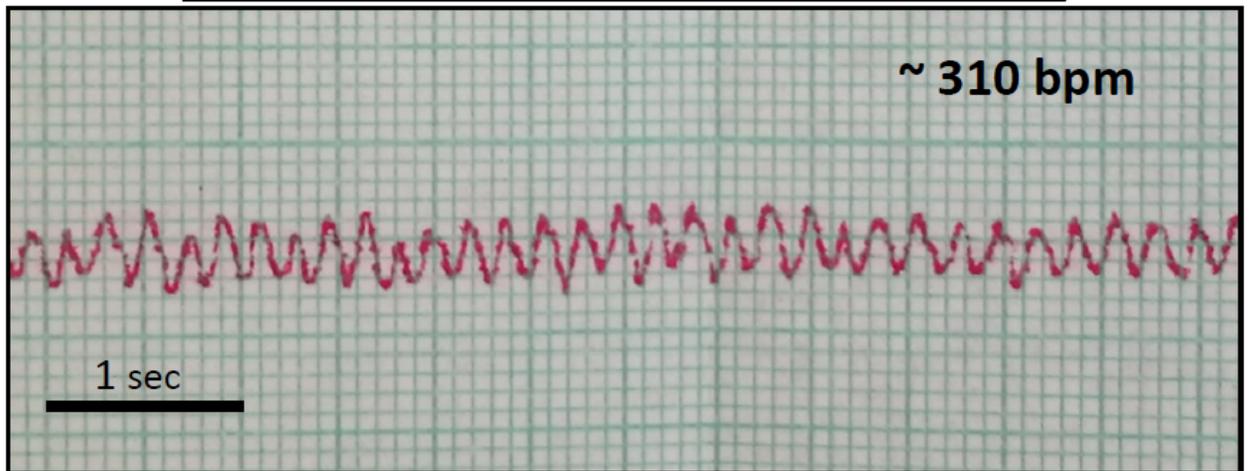
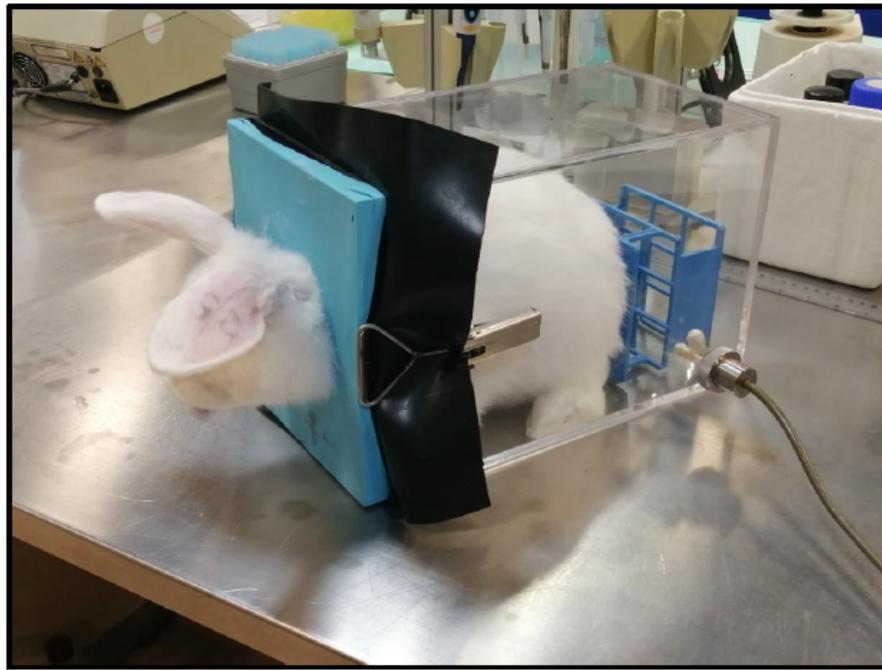


Figure 3

Recording of pressure changes caused by respiratory movements from a rabbit enclosed into a hermetic chamber with head excluded.

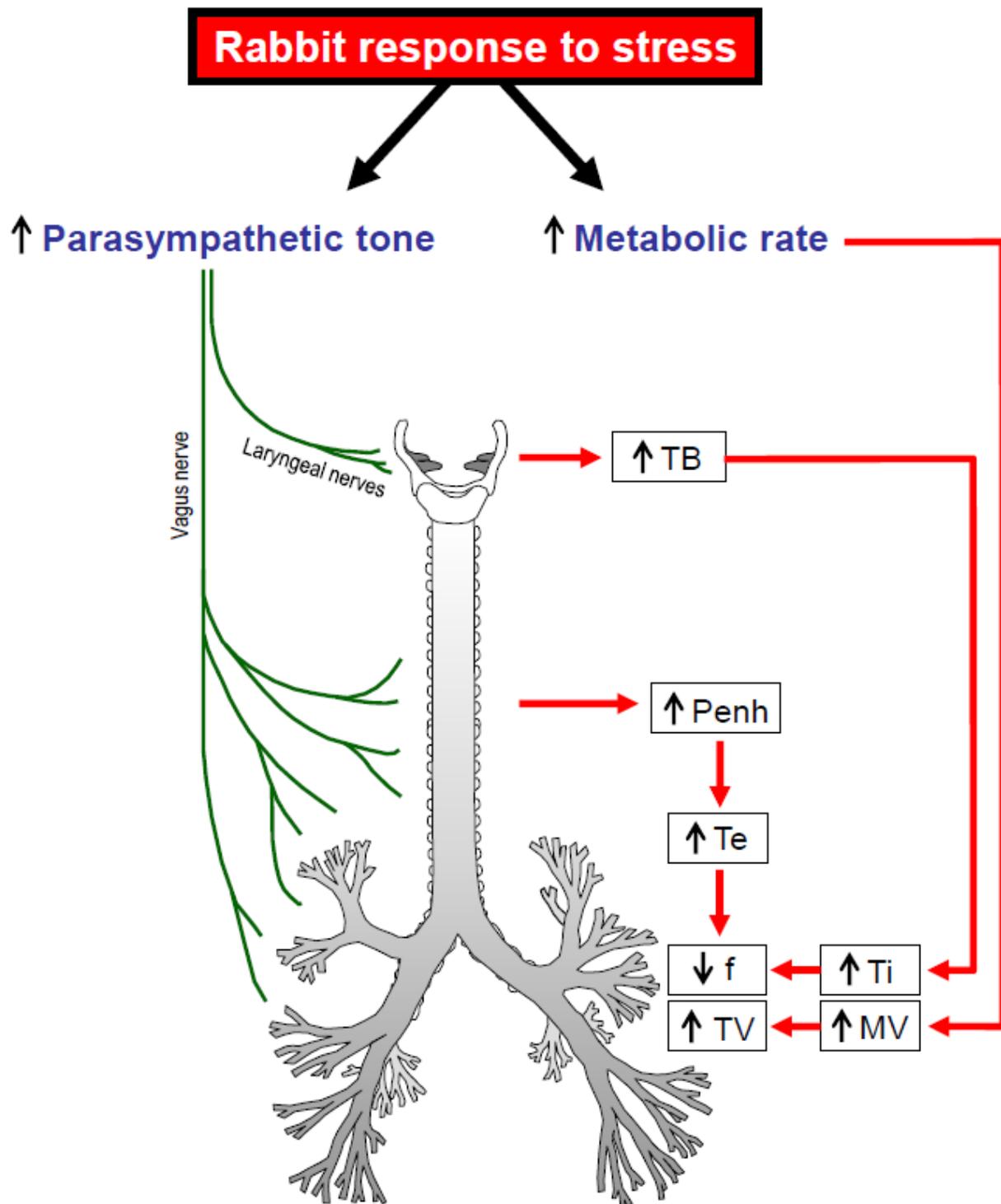


Figure 4

Possible sequence of events occurring in the rabbit during the first minutes confined inside the plethysmographic chamber, a maneuver probably associated with stress. For explanation, see the text. EF50=air flow at 50% of TV during the expiratory phase; f=respiratory frequency; MV=minute ventilation; Penh=enhanced pause; TB=time of braking; Te=expiratory time; Ti=inspiratory time; TV=tidal volume.

Supplementary Files

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