



Respiratory pattern in awake rats: Effects of motor activity and of alerting stimuli

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ABSTRACT

Our aim was to assess the impact of motor activity and of arousing stimuli on respiratory rate in the awake rats. The study was performed in male adult Sprague–Dawley (SD, $n = 5$) and Hooded Wistar (HW, $n = 5$) rats instrumented for ECG telemetry. Respiratory rate was recorded using whole-body plethysmograph, with a piezoelectric sensor attached for the simultaneous assessment of motor activity. All motor activity was found to be associated with an immediate increase in respiratory rate that remained elevated for the whole duration of movement; this was reflected by: i) bimodal distribution of respiratory intervals (modes for slow peak: 336 ± 19 and 532 ± 80 ms for HW and SD, $p < 0.05$; modes for fast peak 128 ± 6 and 132 ± 7 ms for HW and SD, NS); and ii) a tight correlation between total movement time and total time of tachypnoea, with an R^2 ranging 0.96–0.99 ($n = 10$, $p < 0.001$). The extent of motor-related tachypnoea was significantly correlated with the intensity of associated movement. Mild alerting stimuli produced stereotyped tachypnoeic responses, without affecting heart rate: tapping the chamber raised respiratory rate from 117 ± 7 to 430 ± 15 cpm; sudden side move – from 134 ± 13 to 487 ± 16 cpm, and turning on lights – from 136 ± 12 to 507 ± 14 cpm ($n = 10$; $p < 0.01$ for all; no inter-strain differences). We conclude that: i) sniffing is an integral part of the generalized arousal response and does not depend on the modality of sensory stimuli; ii) tachypnoea is a sensitive index of arousal; and iii) respiratory rate is tightly correlated with motor activity.

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1. Introduction

The rat is widely used for physiological studies, but two aspects of its respiratory physiology have received little attention: first, its pattern of respiration while at rest, but awake and freely behaving; and second, the impact of arousal on its respiration. With regard to the first issue, it is generally assumed that we have a good knowledge of the rat's respiratory activity under “resting” conditions. However, most previous studies investigating respiration in awake rats have focussed on homeostatic (chemoreflex-induced) changes, and did not address the possibility that respiration could be also influenced by motor activity; consequently, respiratory data either comprised an average values including undiscriminated periods of motor activity (e.g. [1–3]) or were collected when the animals were immobile (e.g. [4,5]). In reality, immobility is just one component of an animal's “resting state”; indeed, even when “at rest”, animals move about, groom, shift body position, and so on. This issue is significant because it has been established in studies of larger species, including humans, that respiration is closely linked to motor activity, with motor-related

increases in respiratory rate being triggered by feed-forward (“central command”) and maintained by feed-back mechanisms [6,7]. The possibility of motor–respiratory interaction was also out of scope of studies that examined respiratory activity during various behaviors in rats (e.g. [8–11]). Accordingly, the present experiments sought to document the pattern of respiratory rate in awake and freely behaving rats; in particular, its variability, how fast it changes in association with a motor act, and whether it depends on the intensity of motor activity.

With regard to the second issue, i.e. the impact of arousal on the rat's respiration, there have been many studies of the rat's respiratory response to slow onset, relatively long-lasting physiological challenges, such as hypoxia or hypercapnia [1–5]. However, there have been no systematic investigations of this species' respiratory response to spontaneous intrinsic or externally triggered fluctuations in arousal state (with two exceptions; see next paragraph). Arousal can be viewed as a continuum of brain states; it may range from coma and drowsiness to normal wakefulness to hyperarousal and seizures. These alterations in levels of arousal, whether as a result of external sensory stimuli, or as occur in association with internally-generated shifts, such as circadian rhythms or cognitive activity, are a fundamental feature of animal physiology. A variety of methods have been used to measure changes in arousal, ranging from direct recording of the electrical activity of the brain through to the observation of

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changes in gross behavior, such as spontaneous or evoked motor activity. Arousal-related changes in physiological variables under autonomic control have been particularly well characterized; indeed, the elevated blood pressure, tachycardia, mydriasis, piloerection and cutaneous vasoconstriction associated with the fear response is widely accepted as a classic picture of hyperarousal (see [12–14] for reviews). In contrast, arousal-related changes in respiration have received little attention. In his seminal review, Hilton [12] stated: “The respiratory changes which are a constant feature of the defense reaction, and of rapid onset in the alerting stage, have hardly been given any serious consideration”; this statement is still valid. This is somewhat paradoxical, as a close association between respiration and heightened emotions is well established in humans [15,16]. It is currently unknown whether, and how, naturally occurring rapid changes in the state of arousal are reflected in the respiratory pattern in rodents.

Olfaction is one of principal sources of sensory information in rats, and respiratory pattern during exploratory sniffing had received substantial attention starting from the pioneering work of Welker [8]. The landmark feature of sniffing is dramatic and rapid change in the respiratory rate associated with rapid protraction/retraction of the nose; this may or may not be combined with protractions/retractions of mystacial vibrissae and head movements [8]. Later it was found that sniffing is associated not only with odor-sampling but also occurs when animals anticipate reward [9–11]; see Discussion for more detail. Odor-sampling sniffing may be elicited by olfactory stimuli [8,17] but it is currently unknown whether sensory stimuli of other modalities could also provoke this respiratory response.

In the present study we began by examining, in two strains of conscious, freely behaving rats, the relationship between respiratory rate, spontaneous motor activity, and heart rate, the latter two being among the most commonly recorded indices of arousal due, at least in part, to their ease of measurement. This was followed by a comparison of respiratory and heart rate responses to alerting/arousing sensory stimuli. In designing our study we were confronted with several challenges, the main being the lack of adequate framework for analysing respiratory–motor relations in small laboratory animals. We have developed such a framework and present it below in details.

While there are several techniques for assessing respiratory indices in conscious unrestrained rodents, we consider that the whole-body plethysmography is the method of choice as it is entirely non-invasive and thus does not introduce any confounding factors. Using this method, we tested two hypotheses: firstly, that the intensity of motor activity is associated with changes in respiratory frequency; and secondly, that alerting stimuli of various sensory modalities would elicit tachypnoeic responses. A detailed description of cardio-respiratory relationships observed here will be presented in an accompanying article.

2. Methods

2.1. Ethical approval and preliminary surgery

Male Hooded Wistar ($n=5$) and Sprague–Dawley rats ($n=5$) weighing 280–320 g were used in all experiments. All efforts were made to reduce animal pain or discomfort. The experimental protocol was approved by the University of Newcastle Animal Ethics Committee, and is in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). Preparatory surgery was conducted under isoflurane (1.5% in 100% oxygen) anaesthesia, with carprofen (5 mg/kg) being used as a post-surgery analgesic. Telemetric ECG radio-transmitters (TA11CA-F40, Data Sciences International) were implanted in the peritoneal cavity, with electrodes positioned in accord with the method described by Sgoifo [18]: one electrode on the internal surface of the xiphoid process, another one in the mediastinum, along the trachea at the level of the left ventricle. This placement dramatically reduces movement artefacts,

even in vigorously moving animals. Upon recovery from anaesthesia, animals were returned to the animal house for at least one week before experiments began. They were held on a reverse 12 h/12 h light–dark cycle (lights on at 8 pm), with food and water *ad libitum*.

2.2. Recordings of respiration and gross motor activity

Respiratory movements were detected using a custom-built whole-body plethysmograph. This consisted of a transparent Perspex cylinder (i.d. 95 mm, length 260 mm, volume 1.84 l, wall thickness 3 mm) that had both ends closed with removable plugs, and compressed medical air was constantly flushed through it at a flow rate of 2.5 l/min. Both input and output lines were fabricated from polyethylene tubing (o.d. 1.45 mm, i.d. 0.75 mm, about 1 m length) that were tightly fitted into the plugs. A plastic T-connector was inserted 20 cm away from the start of the output line and then linked to one input of a differential pressure amplifier (model 24PC01SMT, Honeywell Sensing and Control, Golden Valley, MN, USA), the second input being opened to the room air. An additional plastic tubing inserted into the “output” plug connected the plethysmograph chamber to the input of the CO₂ monitor (Normocap, Datex Instrumentarium Corp., Helsinki, Finland). In preliminary experiments we have established that keeping airflow at 2 l/min is sufficient to prevent any rise of CO₂ in the plethysmograph.

For semi-quantitative assessment of animals' motor activity, a piezoelectric pulse transducer (MLT1010/D, ADInstruments, Sydney, Australia) was placed under the plethysmograph. The transducer was sensitive enough to detect even minor movements (e.g. turning the head), while locomotion produced large oscillatory responses.

2.3. Experimental protocol

All experiments were carried out in the first half of the dark phase of the light/dark cycle, with just sufficient levels of red light to permit observation of the animals. Importantly, to limit the stressfulness of their transfer, plastic cylinders of the same size as the plethysmograph chamber had been placed in all home cages, allowing animals to habituate to them. Indeed animals often sat or slept inside these cylinders. On the day of experiment, rats were placed in a plethysmographic chamber while still in their home cage. Presumably as a result of their habituation process, animals entered the chamber willingly, without being forced. The ECG probes were then switched on and recording started. The experimental protocol consisted of a 40-min basal level recording period followed by presentation of three alerting stimuli separated by 5-min intervals: a) a tap to the chamber with a remotely released metal rod (sound level in the chamber 65 dB), b) sudden lateral displacement (~5 cm) of one side of the chamber; c) turning on bright light for 10 s (a 60 W light globe located just above the chamber). All stimuli were presented when animals were in a quiet but awake state (eyes opened, no motor activity, heart and respiratory rate at steady rest levels).

2.4. Data acquisition and analysis

Analogue ECG, respiratory and motion signals were digitized at 1 KHz and acquired using a PowerLab A/D converter and ChartPro 6.0 software (ADInstruments, Sydney, Australia). Heart rate was calculated from the ECG records using the same software.

Custom written software developed with MATLAB® was used for the analysis of the respiratory pattern and its dependence on motor activity. We first computed time series for respiratory intervals, the inspiratory onsets being determined as the zero-crossings of the first derivative of the respiratory signal. Mean respiratory interval duration and coefficient of variation of respiratory interval were computed from the obtained breath-to-breath time series and respiratory interval histograms constructed with a bin width of 10 ms. These histograms

were bimodal for all animals, and we characterized them by measuring mode values for the low- and high-frequency peaks; the best non-linear regression fit for these data was then determined using Prism (GraphPad Software, San Diego, CA, USA).

We calculated three measures of motor activity after appropriate thresholding (30% above the noise level) of the movement sensor signal: i) total duration of activity (both in seconds/min and as a percentage of total time); ii) number of movements/min; and iii) average duration of individual movement (in seconds).

Analysis of relationship between respiratory rate and motor activity required us to develop a novel framework. The first task was to find a method of characterising the intensity of a movement. For this purpose we initially computed, for each respiratory interval, the movement power index (MPI):

$$MPI_n = \frac{1}{(t_{n+1} - t_n)} \sum_{i=t_n \Delta f}^{t_{n+1} \Delta f} p_i^2$$

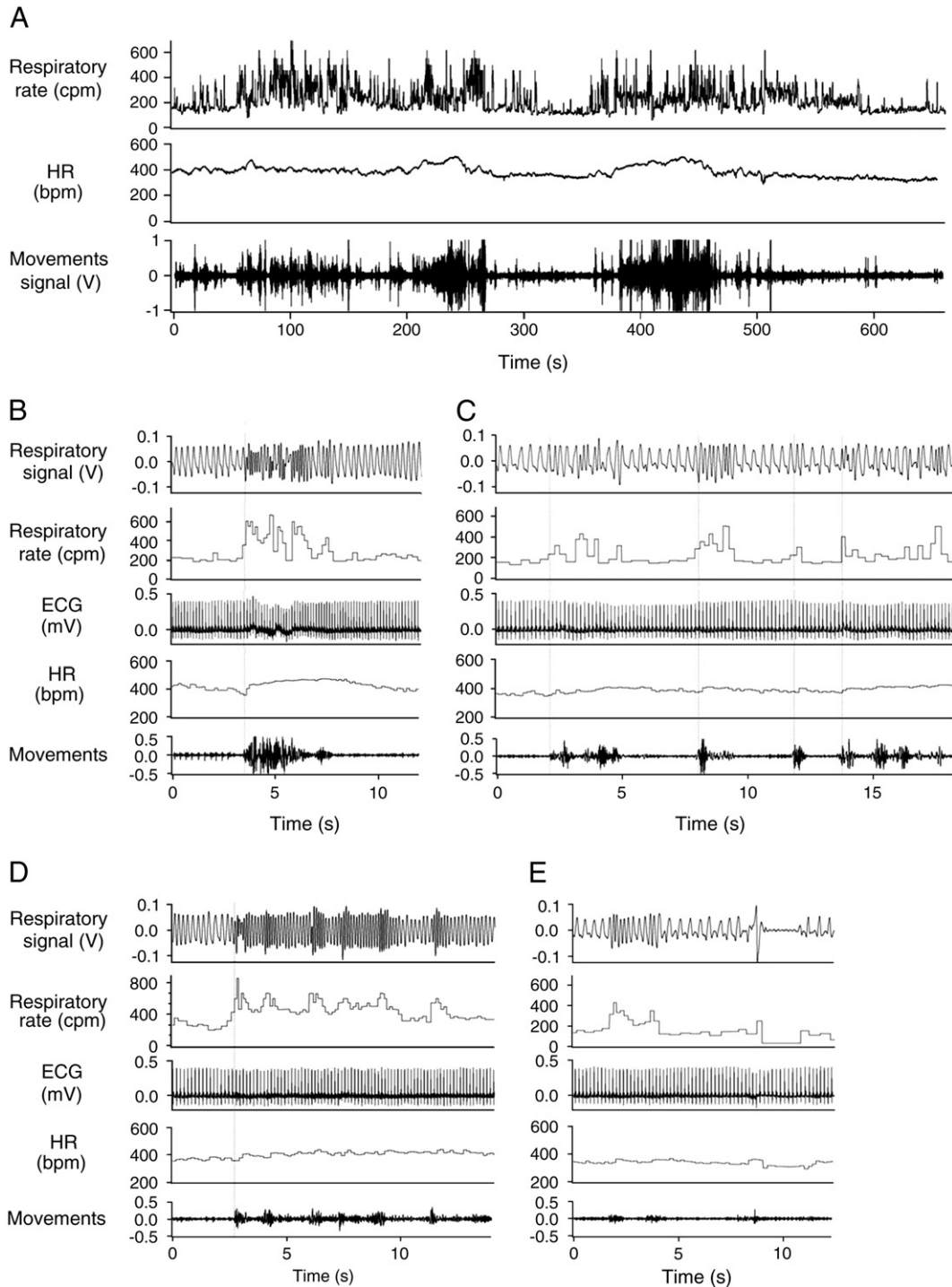


Fig. 1. Record of respiration, ECG and gross spontaneous motor activity. Respiratory rate and heart rate were computed online; the movement signal is from a piezoelectric sensor positioned under the plethysmographic chamber. A — compressed 10-min record containing periods of rest intermingled with periods of spontaneous motor activity (raw respiratory and ECG signals are removed as they could not be resolved at this time scale). Panels B–E are expanded segments of (A) showing respiratory and cardiac changes during various individual motor acts. B — the rat turned around; C — several short grooming episodes; D — sniffing; E — tachypnoea without any motor activity (left) and "augmented breath" (right).

where P is the piezoelectric sensor signal (V), t_n is the time of the n -th inspiratory onset ($n = 1, 2, 3, \dots, N - 1$) and Δf is the sampling frequency (s^{-1}). We then plotted each respiratory interval against the corresponding MPI value for the whole record in each animal. To characterize these plots, and to permit inter-group comparisons, we dichotomized the respiratory interval time series based on the median, computing the average MPI value of respiratory intervals shorter than the median value and the average value of respiratory intervals longer than the median value. To further explore the relationship between the movement intensity and respiratory interval, we sorted all measured MPI values into bins of $0.01 V^2 s^{-1}$ widths, computed the mean and the standard deviation of all respiratory intervals within each bin, plotted these values against corresponding MPI values and determined the optimum regression function fit for these data (GraphPad Software, San Diego, CA, USA).

To investigate the presumed relationship between the duration of motor activity and the duration of elevated respiratory rate, we initially derived from the whole 30-min recording period 15–20 artefact-free segments, and calculated, for each such segment, the total movement time and the total time of tachypnoea. From these, we computed the total movement time and the total tachypnoea time for each record.

Finally, to assess the relationship between motor activity and the heart rate, we measured $R-R$ intervals before and during a movement, computed the difference (Δ), and plotted it against the duration of the associated movement. To characterize and compare this relationship we computed, for each record, mean values for $\Delta R-R$ interval changes during short (<1 s), intermediate (1–5) and long (>5 s) movements.

Group data were analysed by ANOVA with Fishers' protected t -tests, significance threshold being set at the 0.05 level. All data are presented as mean $\pm$ SEM; where possible, data values have been embedded in accompanying figures.

3. Results

3.1. Respiration during basal conditions – qualitative observation

Behaviors displayed by animals after entering the plethysmographic chamber did not differ noticeably from those displayed in the home cage. Periods of motor activity were intermingled with periods of relative quiescence. Sometimes the rats curled up and closed their eyes, suggesting that they were asleep. We could clearly distinguish quiet resting state (eyes open, no motor activity), sniffing (head up, frequent movements of vibrissae), grooming (repetitive touching facial area with both front limbs) and exploring (Fig. 1). As could be seen, even minor body movements (e.g. turning the head) were readily detectable by the piezoelectric sensor.

Observations made during experiments, and subsequent visual inspection of our records, revealed a high extent of variability in the respiratory rate and a strong association between respiration and motor activity (Fig. 1). Indeed episodes of tachypnoea always occurred when an animal performed a motor act, whether or not it involved locomotion. Occasionally tachypnoeic episodes were observed without any detectable movements (see Fig. 1E for an example). The duration of a tachypnoeic episode was generally related to the duration of associated motor events. The onset of respiratory rate acceleration either coincided with the onset of a movement or even slightly preceded it, but was never delayed (see dashed lines in Fig. 1B–D). In the following sections we present the results of our attempt to quantify these qualitative observations.

3.2. Assessment of respiratory indices and motor indices

The mean values for respiratory intervals differed between the two strains ($F = 22$, $n = 5$, $p < 0.05$), being 421 ± 76 ms and 285 ± 18 ms for SD and HW rats, respectively. As seen in Fig. 1A, respiratory

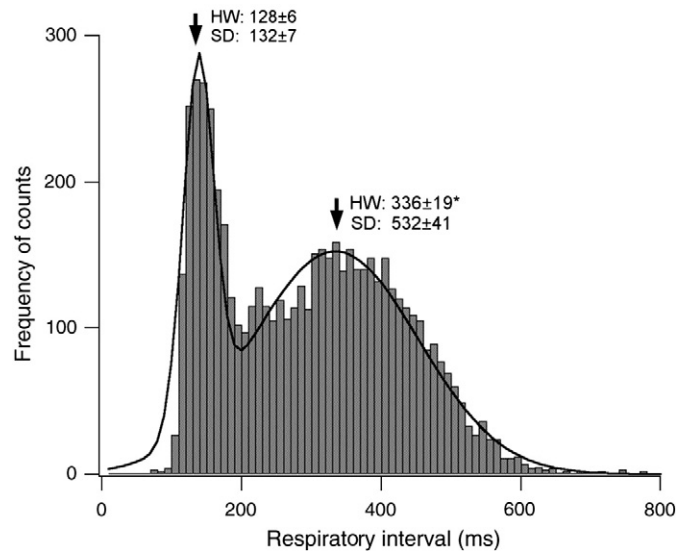


Fig. 2. Histogram of respiratory intervals recorded during spontaneous behavior in one rat (HW3) showing a bimodal distribution. These data could be well approximated by the sum of two Gaussian functions (superimposed curve). Bin width – 10 ms; total number of intervals – 7213; duration of record – 30 min. The values near the arrow-heads are the mean group data for the modes of the shorter and of the longer peak. HW – Hooded Wistar; SD – Sprague–Dawley; *different from another strain, $p < 0.05$.

intervals varied greatly during the recording period and this was reflected by high coefficient of variation values (44% and 38% for SD and HW, respectively); there was no significant group effect ($F = 1.2$, $n = 5$, $p > 0.05$). The histograms of respiratory intervals constructed for each animal revealed a bimodal distribution that could be well approximated by the sum of two Gaussian functions (Fig. 2). While the mode values for the shorter peak did not differ between the strains (128 ± 6 ms vs. 132 ± 7 ms, $n = 5$, $p > 0.05$), the mode values for the longer peaks were larger in SD rats (532 ± 80 ms) compared to HW (336 ± 19 ms, $n = 5$, $F = 18$, $p < 0.05$). The small number of long (>1 s) respiratory intervals were not an experimental error; they originated from the apnoeic periods that followed augmented breaths (Fig. 1E).

Temporal characteristics of motor activity recorded in our rats are presented in Table 1. The indices comprised the average duration of an individual movement and the total movement time. While SD rats tended to be less active, there was no significant difference between the two strains.

3.3. Relationship between motor activity and respiratory pattern

We first determined whether the extent of the tachypnoea depends on the intensity (power) of associated movements. To quantify motor activity, we developed the “movement power index” (see Methods for details) and computed it for each respiratory interval. We then plotted the value of each respiratory interval against the intensity of movement associated with (i.e. occurring during) this interval. An example of such a plot is presented in Fig. 3A and B; this relationship was similar in the other nine rats as evidenced by the fact that the mean values of the movement power index for respiratory

Table 1
Temporal characteristics of motor activity.

Rat strain	Mean movement duration (ms)	Total movement time	
		(s/min)	(%)
Hooded Wistar ($n = 5$)	683 ± 62	11.7 ± 0.7	19.4 ± 1.2
Sprague–Dawley ($n = 5$)	552 ± 28	10.3 ± 1.0	17.2 ± 1.7

Data presented as mean $\pm$ SEM.

intervals shorter than the median were significantly higher than corresponding index for all intervals longer than the median (see inset in Fig. 3A). To further analyze these data we sorted all measured MPI values into bins of $0.01 \text{ V}^2 \text{ s}^{-1}$ width, computed the mean respiratory interval and the standard deviation of all respiratory intervals within each bin, and plotted these results against corresponding MPI values. Examples of these plots are presented in Fig. 3C and D. Data points with MPI values below $0.2 \text{ V}^2 \text{ s}^{-1}$ were fitted with parabolic curve. Note that data points for $\text{MPI} > 0.2 \text{ V}^2 \text{ s}^{-1}$ that could be not satisfactory fitted represent a small fraction of all analysed respiratory intervals; this is evident from panels A and B. The variation of respiratory intervals with regard to MPI bins followed an inverse linear trend (Fig. 3D). Thus, at rest or at low-intensity movements respiratory rate was lower and more variable compared to high-intensity movements when it was invariably high.

The next question we addressed was whether there was an association between the duration of motor activity and the duration of tachypnoea. To address this issue, data records from each animal were first split into 15–20 artefact-free segments, and movements and respiration were quantified individually in each of these segments. This analysis revealed that, for each such segment, duration of movements (total movement time) and of rapid breathing (total time of tachypnoea) are highly correlated. An example of such analysis is presented in Fig. 4A; the results were very similar in the nine other animals, with an R^2 range of 0.96–0.99 ($p < 0.001$). We then computed the total movement time and the total time of tachypnoea for the whole observation period in each animal; again, we found a strong correlation between the two indices (Fig. 4B). Importantly, the value of total tachypnoea time ($348 \pm 12 \text{ s}$) was slightly but significantly higher than the total movement time ($316 \pm 12 \text{ s}$; $p < 0.001$, $n = 10$).

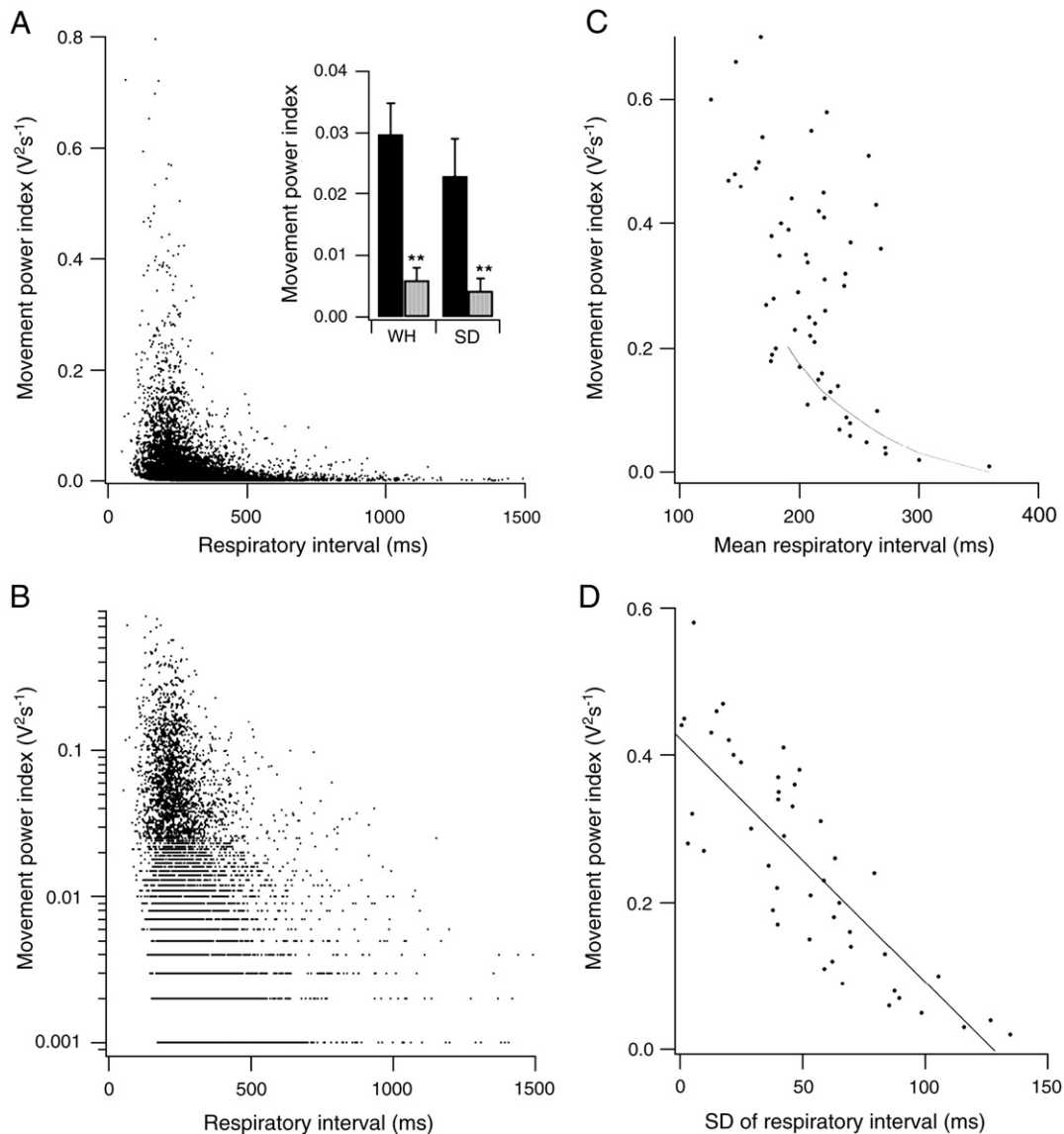


Fig. 3. Relationship between respiration and motor activity in a spontaneously behaving rat; example of analysis performed on the data from a single animal. A — each data point represents the duration of a respiratory interval (abscissa) and an index reflecting the power of movement that occurred during this respiratory interval (ordinate; see Methods for the details of computing this index). Inset in A shows group data for the mean movement power index computed separately for respiratory intervals that were shorter than the median (black) and longer than the median (grey). HW — Hooded Wistar; SD — Sprague-Dawley; **different from “below the median” value, $p < 0.05$. Panel B shows the same data set as in A, with logarithmic y-axis. C — dependence of the mean respiratory interval (computed from data shown in panel A, with bin width of $0.01 \text{ V}^2 \text{ s}^{-1}$) on the movement power index. Data points with MPI values below $0.2 \text{ V}^2 \text{ s}^{-1}$ were fitted with parabolic curve ($R^2 = 0.56$, $p < 0.01$). Note that data points for $\text{MPI} > 0.2 \text{ V}^2 \text{ s}^{-1}$ that could be not satisfactory fitted represent a small fraction of all analysed respiratory intervals; this is evident from panels A and B. Panel D — variability of tachypnoea is inversely correlated to the intensity of associated movement. Data points are standard deviations computed for each binned respiratory intervals. Data were fitted with linear equation ($R^2 = 0.62$, $p < 0.01$).

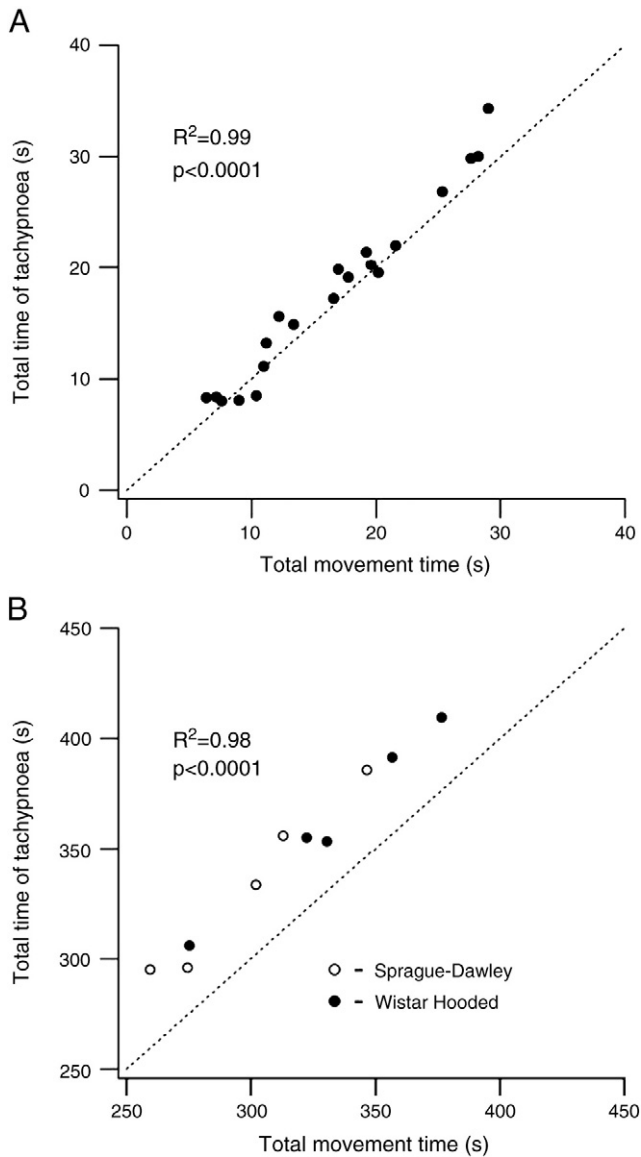


Fig. 4. Relationship between the total movement time and total time of tachypnoea. A – example from an individual rat where the recording was split into 20 segments, and the pairs of movement and respiratory indices were plotted against each other for each such segment. B – group data; each point represents the pair of movement and respiratory indices derived from the whole recording period in a given animal. Note that in (A) most data points, and in (B) all of them are located above the line with the slope = 1, indicating that the total time of tachypnoea consistently exceeded the total movement time.

consistent with the fact that in some instances (in $7.1 \pm 1.6\%$ of total tachypnoea time), the rise in the respiratory rate occurred without any detectable movement. There was no effect of strain on this motor–respiratory relationship.

3.4. Heart rate during basal conditions and its association with motor activity

The values for the mean R–R intervals differed significantly between the two strains ($F=17$, $p<0.05$), being shorter for HW (155 ± 3 ms) compared to SD (187 ± 8 ms). There was, however, no between-strain difference in the coefficients of variation for R–R intervals ($1.0 \pm 0.1\%$ and $0.7 \pm 0.1\%$ for HW and SD, respectively; $F=2.7$, $p>0.05$).

As illustrated in Fig. 1, the association between changes in heart rate and locomotion was not as obvious as it was for the respiratory–

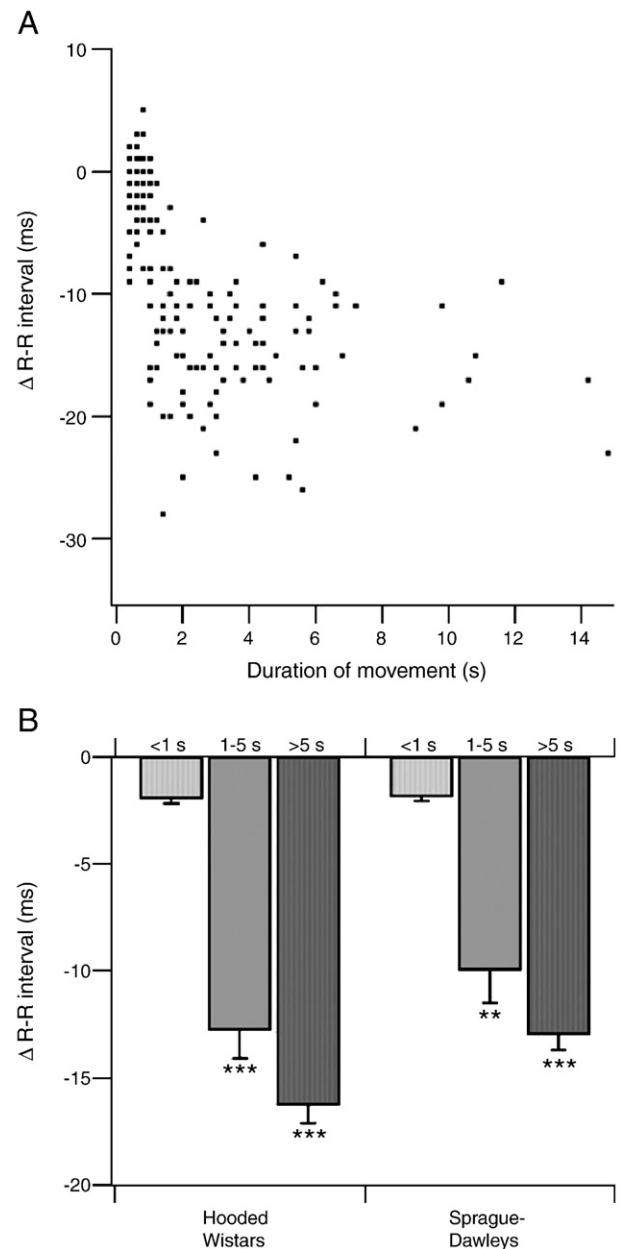
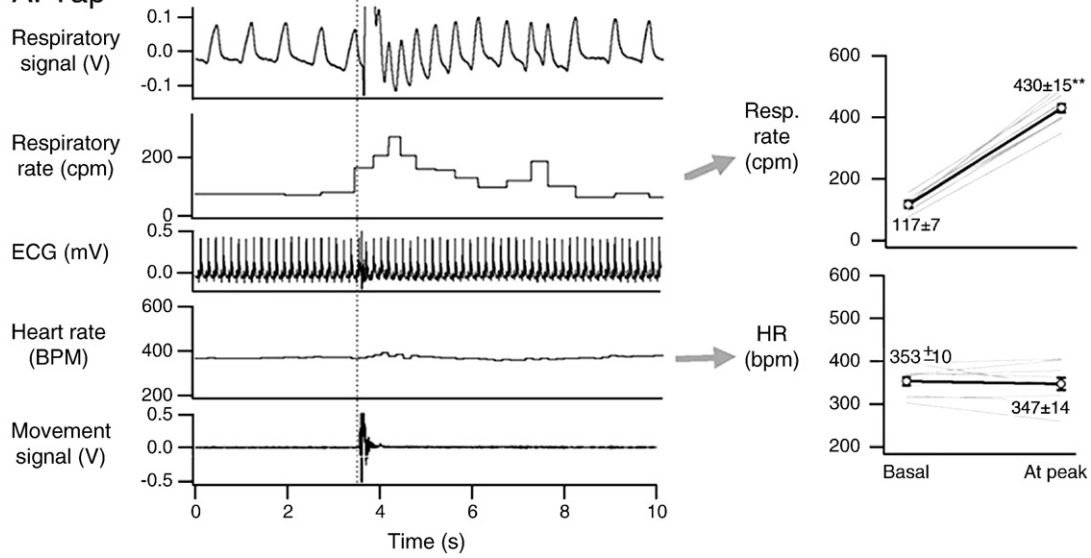
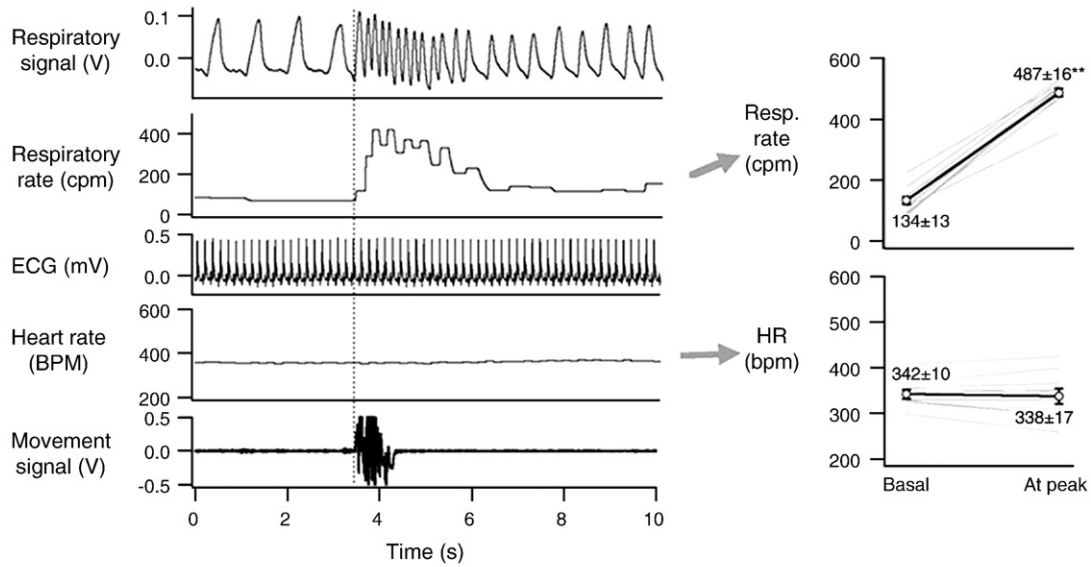
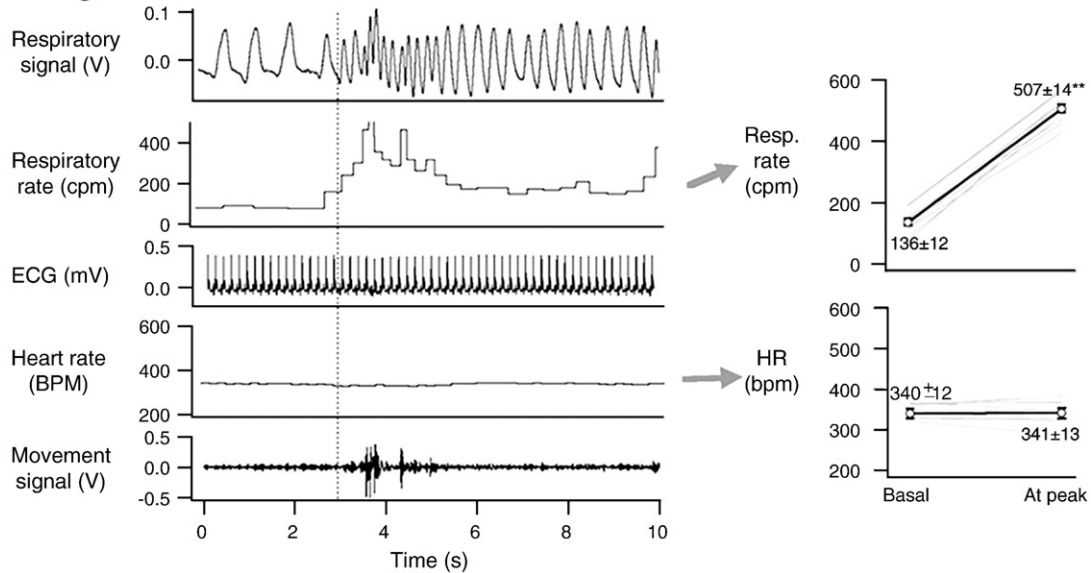


Fig. 5. Relationship between the duration of an individual motor act and the changes in heart rate associated with it. A – example from an individual rat; each data point represents two values: change (Δ) in R–R interval that occurred during or following a movement (abscissa), and the duration of corresponding movement (ordinate). B – group values for delta R–R intervals computed separately for short (<1 s, light-grey columns), intermediate (1–5 s, grey) and long (>5 s, dark grey) movements. ** and ***significantly different from short movements, $p<0.01$ and $p<0.001$, respectively.

motor relationship. Overall, we found that longer bouts of locomotion were always associated with tachycardia, whereas during short-lasting movements (<1 s), there were no consistent changes in the HR. This relationship is reflected by the plot in Fig. 5A that was constructed from the data obtained in one rat, and by the results of group data analysis as shown in Fig. 5B.

3.5. Cardiac and respiratory responses to alerting stimuli

Examples of records made during the presentation of alerting stimuli are shown in Fig. 6 (left panels). A single tap to the plethysmographic chamber caused rapid startle movement followed by a transient and potent increase in the respiratory frequency,

A. Tap**B. Side move****C. Lights on**

without affecting heart rate. A sudden side move of the plethysmographic chamber provoked a similar response. Turning on the light caused respiratory rate to increase rapidly; heart rate either remained unchanged or fell slightly (no effect overall). Animals remained immobile (“freezing”) for the first few seconds after the lights were turned on. Subsequently, exploration began and usually lasted for the whole “lights on” period. Respiratory and cardiac changes associated with this exploratory behavior were similar to those occurring during spontaneous motor activity (see above). Tachypnoeic responses to alerting stimuli developed with short latencies (<0.5 s); sometimes, however, it was not possible to assess this parameter due to the distortion of respiratory signal by a startle reaction (e.g. Fig. 6A). The duration of tachypnoeic responses was highly variable, ranging between 1–2 and 5–7 s. In contrast, the amplitude of tachypnoeic responses was remarkably uniform (see Fig. 6, right panels for mean group values and statistics). While there was a tendency for slightly larger tachypnoeic responses in Wistar compared to Sprague–Dawley rats, there was no significant group effect, and thus the results of both strains were pooled together.

Changes in the amplitude of the plethysmographic pressure signal were inconsistent – sometimes it increases, sometimes it decreases, and sometimes there was no apparent change. This inconsistency was observed in both motor-related tachypnoea (Fig. 1) and arousal-related tachypnoea (Fig. 6).

4. Discussion

Our most important findings are that motor activity is associated with an increase in the respiratory rate, and that sensory stimuli provoke stereotyped tachypnoeic arousing responses which are independent from the modality of sensory input. Further, we discovered that the respiratory pattern in freely moving rats has several distinctive features including: i) high variability of the respiratory rate; ii) bimodal distribution of respiratory intervals; iii) dependence of motor-related tachypnoea on the intensity of associated movement; and iv) a tight correlation between the duration of motor-related tachypnoea and the duration of motor activity.

4.1. Respiratory rate in spontaneously behaving rats

Most of previous studies investigating respiration in rats have focussed on homeostatic changes [1–5], and did not address the possibility that respiration could be also influenced by motor activity or by sensory stimuli. The range of respiratory rate observed in our rats at rest (2–3 Hz) is in good accord with these previous reports. The slow and regular resting breathing was frequently intermingled with much faster respiratory movements that were sometimes associated with obvious sniffing behavior. The latter was the focus of several studies, and it has been initially regarded as a behavioral component of odor-sampling [8,17,19]. However, as early as in 1971 Clarke and Trowill [9] discovered that sniffing is also a sign of reward anticipation in rats. Recently, addressing this issue, Kepesc et al. [11] demonstrated that this “anticipatory sniffing” occurred at a higher frequency (9–12 Hz) compared to odor-sampling sniffing (6–9 Hz). In our study, tachypnoea rarely exceeded 10 Hz suggesting that only odor-sampling sniffing but not anticipatory sniffing was present in our rats.

We studied our animals in the active phase (lights off), when they were frequently engaged in stereotyped behaviors or moved around the chamber. We found that all motor acts were accompanied by tachypnoea, with the latter being grossly associated with the

movement intensity. The relation between the two appears to be quite complex: while vigorous movements were always accompanied by near maximal rises in respiratory rate, it was lower and varied greatly during low-intensity movements or during immobility. This relationship is reflected in the negative correlation between the movement power index and the dispersion of the mean respiratory interval. A tight correlation between the duration of motor activity and the duration of tachypnoea supports the idea that most respiratory changes observed in our animals were related to the former. All these findings are in a good accord with basic principles of exercise physiology [6,7]. Our current results also shed extra light on phenomena of anticipatory sniffing, as it is evident from the previous studies that animals actually moved in anticipation of their reward. Furthermore, even odor-sampling sniffing does not usually occur in the state of total immobility and thus may be at least in part motor-related.

4.2. Dissociation between respiratory and cardiac responses to alerting stimuli

Several previous studies described sniffing elicited by olfactory stimulation in rats [8,17]. Our novel finding is that tachypnoeic responses could be also triggered by sensory stimuli of other sensory modalities – acoustic (tap), proprioceptor/vestibular (side move of the plethysmographic chamber) and visual/photic (light). Indeed, all these stimuli provoked remarkably robust effects, with up to 6-fold increases in the respiratory rate. In most instances these responses were not associated with any motor activity (apart from a startle) and thus were likely mediated by mechanisms different from those responsible for the motor-related tachypnoea. We suggest that tachypnoeic responses to alerting stimuli are an integral part of a non-specific arousal reaction. In rodents, olfaction is the primary channel of sensory information, and it appears natural that the first behavioral manifestation of their threat-detection strategy is odor-sampling.

One of our most interesting observations is a clear dissociation between the respiratory and the cardiac signs of arousal. Indeed, sensory stimuli employed in this study did not provoke any changes in heart rate, whereas respiratory rate consistently rose by 300–400%. This lack of cardiac changes is most likely attributable to a relatively mild nature of our stimuli as more potent acoustic [20,21], vestibular [22] or photic [23] stimulation do provoke considerable alterations in the heart rate. We thus conclude that there must be a difference between sensory thresholds for eliciting respiratory vs. cardiac responses, the former being lower. This is in agreement with earlier studies on arousing effects of brain stimulation where similar dissociation could be achieved by applying low-intensity stimulation currents [24,25]. Why the threshold should be lower for respiratory changes is an open question. Based on the assumption that alerting-induced tachycardia and tachypnoea represent separate components of the same adaptive anticipatory response in preparation to “fight or flight”, it is tempting to speculate that the rise in the respiratory rate must precede the rise in the heart rate, in order to make this response more efficient.

We did not assess changes in tidal volume in this study mainly because method for its computing requires equilibration of the temperature if inspired air with body temperature [26], which may not be the case during tachypnoea [11]. We do not consider this as serious limitation of our study as it has been explicitly stated by Hilton that tachypnoea, rather than hypernoea, is the usual component of the arousal reaction [25].

Fig. 6. Sudden alerting/arousing stimuli provoke tachypnoea responses, but do not affect heart rate in conscious rats. Respiratory and HR responses to a tap (A), to a side move (B), and to turning on the lights (C). Left panels show examples of recordings obtained during presentation of these stimuli; dashed lines indicate when a stimulus was presented. Right panels represent corresponding group data values (pre-stimulus vs. peak) for changes in respiratory rate and heart rate. Grey lines – individual responses; black lines – grouped values ($n = 10$). **significantly different from basal, $p < 0.001$. Note that in B, the prolonged burst in the movement signal is largely due to the side displacement of the plethysmographic chamber by the experimenter.

4.3. Neural mechanisms underlying motor-related and arousal-induced tachypnoea

The initial increase in minute ventilation during physical activity occurs predominantly due to the “central command” [6,7], and it is likely that motor-related tachypnoea observed in our rats was also initiated in the forebrain rather than triggered by peripheral mechanisms. The principal argument supporting this idea is lack of delay between the onset of motor acts and the onset of tachypnoea. Likewise, the command for tachypnoeic arousal responses was also generated in the brain as they were obviously the consequence of sensory activation. It would be thus of major interest to elucidate which brain structure(s) could be involved in the generation of motor- and sensory-related tachypnoeas. Several studies have indicated that the dorsomedial hypothalamus, the central amygdala and the periaqueductal grey have “central command’s” properties, and that stimulation of these areas can induce tachypnoea (see [6] for review). Of particular interest is recent demonstration of spatially distinct subpopulations of hypothalamic neurons whose activation causes selective increase in either heart rate or respiratory rate [27] as this mechanism could possibly explain dissociation between cardiac and respiratory responses to alerting stimuli in our rats. Obviously, the only way to confirm involvement of these brain regions in the generation of tachypnoeas described in this report is via direct pharmacological inhibition.

Several well-described peripheral mechanism may contribute to the genesis of our tachypnoeic responses; among those are the chemoreflex [28], muscle metaboreflex and muscle stretch reflex [29]. We consider that two former mechanisms could be rather safely excluded from being potential triggers as tachypnoeas developed with a short latency after sensory stimulation, and occurred without delay during motor acts, so that there was clearly not sufficient time for developing any metabolic effects. We currently cannot rule out involvement of stretch reflex in the genesis of our tachypnoeic responses; it is not known whether short-lasting brisk movements such as startle response (that clearly occurred following our acoustic stimulus) may affect respiratory rate. On the other hand, when larger movements occurred, involvement of this mechanism was quite possible, as activation of stretch receptors causes fast and vigorous increase in the respiratory rate [30].

We suggest that bimodal distribution of the respiratory intervals found in our study may reflect two states of the central respiratory controller, with intermodal events representing frequent shifts between the two. Indeed, we found that such shifts do exist, and that they are associated with at least two mechanisms – initiation of motor activity and alerting sensory stimulation. Peak tachypnoea observed during movements or provoked by alerting stimuli corresponded to the fast peaks in the respiratory interval histograms.

4.4. Inter-strain differences

The rationale for employing two rat strains in the current study was our previous observation that in HW rats, tachycardic responses to restraint stress were substantially higher compared to SD rats [31,32], and we thus tested the possibility that there might be also an inter-strain difference in respiratory responses to external stimuli. Our current findings suggest that there may be some genetically determined differences in the control of respiratory rate at rest (with lower basal values in SD compared to HW), but respiratory responses to alerting stimuli do not differ between the two strains.

4.5. Physiological role of tachypnoeic responses

The prevailing view, which we owe largely to Cannon, is that the physiological role of arousal-induced tachypnoea is to reduce the blood CO₂ in anticipation of the “fight or flight” motor reaction (see

also [12] for the discussion of this idea). An alternative possibility is that the arousal-induced rise in respiratory rate is mainly for facilitating olfactory input (i.e. it represents odor-sampling sniffing) and thus is a part of the risk-assessment strategy during exposure to novelty, as olfaction plays a major role in the detection of potential threat in rodents. These two possibilities are obviously not mutually exclusive.

Likewise, more than one option exists for interpretation of motor-related rises in respiratory rate reported here. Close association between minute ventilation and exercise intensity is well established in larger mammals, including humans, and our finding of dependence of tachypnoea amplitude and duration on the intensity and duration of associated movement suggests that its main physiological role is to facilitate gas exchange in the lungs. On the other hand, it is possible that during motor activity animals more actively scan the environment for potential threats, and thus the odor-sampling sniffing is triggered at the same time as the motion starts. Moreover, in some instances (e.g. overt exploratory behavior) odor-sampling is clearly associated with intense body movements, so that tachypnoea in this case possibly has dual function. A possible way to further advance understanding of these complex relations is to perform ethologically-oriented experiments combined with more detailed assessment of the respiratory pattern.

5. Conclusions

We conclude that sniffing is an integral part of the generalized arousal response and does not depend on the modality of sensory stimuli that provoke it; and that voluntary movements in rats are associated with substantial and rapid increases in the respiratory rate. Our results add new information to the growing body of knowledge about forebrain mechanisms that control respiratory rate in awake rats during their natural behavior. It is now evident that these forebrain mechanisms are activated in at least four entirely different behavioral states: odor-sampling, reward anticipation, motor activity and sudden change in arousal level. It must be acknowledged that possibly none of these states occurs in isolation.

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