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# Cardiovascular and respiratory changes during slow-wave sleep in rats are associated with electrocorticogram desynchronization

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## Abstract

In awake rats a single recurrent larger tidal volume (deep breaths) occurs at regular intervals, followed by oscillations in arterial pressure and heart rate. In the present study we recorded the changes in blood pressure, heart rate and ventilation during the wakefulness-sleep cycle identified by electrocorticographic records in order to determine whether the deep breaths and cardiovascular oscillations were associated with changes in the electrocorticogram. During several episodes of slow-wave sleep (SWS) in 7 rats the deep breaths and oscillations in arterial pressure and heart rate were preceded by SWS desynchronization. The interval between deep breaths during SWS was 71 ± 4 s, the period between initial desynchronization and the generation of deep breaths was 3.98 ± 0.45 s and the duration of SWS desynchronization was 11 ± 0.65 s. Hypotension (-16 ± 1 mmHg) and tachycardia (+15 ± 5 bpm) were observed during deep breaths in the SWS state. These data indicate that the oscillations in arterial pressure and heart rate during SWS are associated with deep breaths, which in turn are preceded by desynchronization of the electrocorticogram in this state of sleep.

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- Wakefulness-sleep cycle
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- Cardiovascular regulation
- Deep breaths
- Ventilatory patterns
- Arterial pressure oscillations
- Whole body plethysmography

## Introduction

Studies on cardiovascular changes in the wakefulness-sleep cycle have shown that short episodes of electrocortical desynchronization occur during slow-wave sleep (SWS) in which arterial pressure exhibits rapid oscillations (1). In previous studies we observed that awake normal rats present deep breath events at regular intervals associated with oscillations in arterial blood pressure and heart rate (2,3). These studies also showed that in rats with sino-aortic deafferentation the deep breath events were consistently accompanied by substantial and abrupt reductions of arterial pressure, whereas this effect was markedly lower in intact rats. These data indicate that the oscillations in arterial pressure are secondary to the ventilatory changes (deep breaths) and that the arterial baroreceptors play a key role in the prevention of major cardiovascular changes during the deep breath episodes. The breathing pattern characterized by a single regularly recurrent larger tidal volume has been described as a deep breath in intact animals by Bartlett Jr. (4) and as gasping by St. John et al. (5-8) in decerebrated cats.

In addition to the studies by Junqueira and Krieger (1), other reports have shown that several changes in blood pressure, heart rate and respiratory frequency occur during the sleep-wake cycle (9,10), but no studies have determined whether the cardiovascular and respiratory changes occurring during the deep breath events in rats are associated with electrocorticographic changes during the different states of the wakefulness-sleep cycle. For this reason, in the present study we recorded pulsatile arterial pressure, heart rate and ventilation during the wakefulness-sleep cycle identified by the electrocorticogram in normal rats.

## Material and Methods

Male Wistar rats weighing 250-300 g were used. Three days before the experiments two bipolar electrodes were implanted into the skull of the rats under Nembutal anesthesia (40 mg/kg, *ip*) by means of two small nickel-chromium screws (100 µm in diameter) fixed into two small holes drilled in the skull. The electrodes were implanted on the dura mater in order to cover a small portion of the parietal cortex (areas 3 and 7). The electrodes were soldered to the two small screws which were fixed 1 mm left to the median suture and 1 mm from the bregmatic suture in the parietal bone and 1 mm from the median suture and 1 mm from the lambda suture on the left side (1,11). The entire system was fixed to the skull with acrylic cement. Electrocorticographic recordings were obtained three days after electrode implantation. The different steps of the sleep-wake cycle were identified by electrocorticographic analysis according to the method of Timo-Iaria et al. (12) but with no measurement of the cervical electromyogram. The characterization of desynchronized sleep (REM) was performed by visual observation of the position of the head under the trunk (12) as well as by the increase in respiratory frequency and reduction in tidal volume, as described by Remmers (13). The electrocorticogram was recorded with a Narcotrace 40 physiological recorder using a Universal Coupler (Narco Bio-Systems, Austin, TX).

One day before recording a catheter (PE-10 connected to PE-50, Clay Adams, Parsippany, NJ) was inserted into the abdominal aorta through the femoral artery under ether anesthesia, for measurement of pulsatile arterial pressure (PAP) and heart rate (HR). The catheter was tunneled and exteriorized through the back of the neck to be connected to the pressure transducer under freely moving conditions inside a chamber for ventilatory measurements. PAP and HR were recorded with a Narcotrace 40 physiological recorder (Narco Bio-Systems). PAP was measured with a pressure transducer (model CDX III, Cobe Laboratories, Lakewood, CA) and diastolic pressure was quantified. HR was also quantified with a Narco Biotachometer Coupler (model 7302).

Ventilation was measured by the whole body plethysmographic method described by Malan (14), which is based on monitoring small pressure changes within a closed animal chamber. During inspiration, a volume of gas inside the chamber is heated from room to body temperature, which increases total pressure within the chamber. Conversely, expiration decreases total pressure. A highly sensitive differential pressure transducer (Statham PM 979) connected to the recorder was used for these measurements. The pressure transducer of the chamber was also connected to the physiological recorder for measurement of tidal volume and respiratory frequency. A limitation of the plethysmographic approach is that the ventilation measurements must be interrupted at 5- to 7-min intervals to flush the chamber with fresh air, which precludes performance of uninterrupted measurements of ventilation during long periods of time. Therefore, the rats were maintained inside the plethysmographic

chamber which was carefully closed and opened at intervals of approximately 7 min. When the chamber was closed PAP, HR, electrocorticogram and ventilation were recorded continuously.

Despite the limitations of the whole body plethysmographic method for long-term measurement of ventilation, the method was reliable for the purpose of the present study because a) no significant changes in basal respiratory frequency were observed during the period of 5-7 min in which the chamber was closed (2,3); b) the deep breath event was not an artefact of this method because it was also observed in the rats outside the chamber; c) after the rats were trained to stay inside, the chamber could be carefully opened and closed with no major disturbance of the sleep-awake cycle of the rats, and d) the advantage of this method in relation to the diaphragm electromyogram or records of electrical activity of the phrenic nerve is that it can be used in unanesthetized rats without implanting additional electrodes.

For 2 days prior to the experiments the rats used in the present study (N = 7) were trained to stay inside the plethysmographic chamber for periods of 2-3 h. On the day of the recordings the rats were maintained inside the chamber for at least 2 h before starting the procedure. The recordings were performed between 2:00 and 5:00 p.m. in an acoustically isolated room. The duration of the recordings varied for each rat because it depended on the occurrence of a complete sleep-wake cycle. The cables from the skull for the EKG recordings and the catheter from the femoral artery were exteriorized through a small hole in the plethysmographic chamber to be connected to the transducers and physiological recorder. This small hole was then filled with silicone grease in order to seal the plethysmographic chamber.

The changes in mean arterial pressure and heart rate during the deep breath episodes between different states of the wakefulness-sleep cycle and the awake state were compared by the unpaired Student *t*-test and the level of significance was set at $P<0.05$.

## Results

Deep breaths occurred at intervals of 71 ± 4 s during the SWS in the 7 rats studied, in which 29 episodes were analyzed. The time between the initial changes in the electrocorticogram and the appearance of the deep breath episodes was 3.98 ± 0.45 s (33 episodes analyzed) and the duration of the changes in the electrocorticogram pattern during SWS was 11 ± 0.65 s (33 episodes analyzed). Figure 1 is a tracing obtained from one rat representative of the group showing 2 episodes of deep breaths with the corresponding changes in electrocorticogram, PAP and HR during the SWS.

Table 1 shows the changes in diastolic arterial pressure and heart rate associated with deep breaths during the awake state (-7 ± 2 mmHg; +4 ± 3 bpm) and SWS (-16 ± 1 mmHg; +15 ± 5 bpm) and in the deep breaths observed during the transition from SWS to the awake state (-10 ± 3 mmHg; +12 ± 6

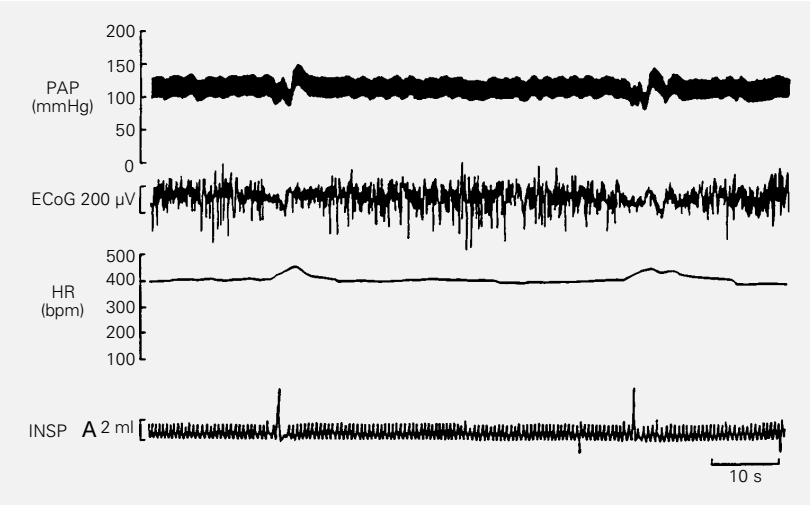


Figure 1 - Tracings of one representative rat of the group showing the simultaneous recordings of pulsatile arterial pressure (PAP), electrocorticogram (ECoG), heart rate (HR) and ventilation. The arrow indicates the direction of inspirations (INSP) during slow-wave sleep (SWS). The tracings show 2 episodes of SWS desynchronization followed by deep breaths and oscillations in PAP and HR. The duration of the tracings was 95 s and the time interval between the two episodes of deep breaths was 53 s.

Table 1 - Peak changes in diastolic arterial pressure (Δ DAP) and heart rate (Δ HR) during episodes of deep breaths in the awake state (deep breaths/wake), in the slow-wave sleep (SWS) state, in the transition from slow-wave state to awake state (SWS/wake) and in the transition from desynchronized to the awake state (REM/wake).

*P<0.05 compared to the changes in deep breaths in the awake state (deep breaths/wake) (Student *t*-test).

| | Δ DAP (mmHg) | Δ HR (bpm) | Number of rats | Number of episodes |
|---|---|---|---|---|
| Deep breaths wake | -7 ± 2 | +4 ± 3 | 6 | 6 |
| Deep breaths SWS | -16 ± 1* | +15 ± 5* | 7 | 21 |
| Deep breaths SWS/wake | -10 ± 3 | +12 ± 6 | 4 | 10 |
| Deep breaths REM/wake | -17 ± 7* | +5 ± 5 | 5 | 5 |

bpm) and from desynchronized sleep (REM) to the awake state (-17 ± 7 mmHg; +5 ± 5 bpm).

The transition from the SWS to wakefulness in the 4 rats was also associated with deep breaths and hemodynamic changes. The transition episodes from REM to wakefulness (5 episodes in 5 different rats) were also recorded and in these cases the transition was also associated with deep breaths and hemodynamic changes. The REM state is characterized by an irregular respiratory rhythm (13), which is normalized after the transition to wakefulness. In 4 rats, the irregular respiratory rhythm during the REM state presented reduction in tidal volume and short apnea, which were followed in many cases by tachypnea.

## Discussion

The results of the present study indicate that all micro-awake episodes during the SWS are associated with deep breaths and that the transition from SWS to wakefulness as well as from desynchronized sleep (REM) to wakefulness is associated with deep breaths and cardiovascular changes. The transition from REM sleep to wakefulness is also associated with a deep breath episode that seems to be a marker for the normalization of the frequency and tidal volume of the ventilatory cycle. The cardiovascular changes observed during the SWS desynchronization and simultaneous to the deep breaths were not greater probably because of the buffering role played by the arterial baroreceptors (2,3).

Studies by Junqueira and Krieger (1) have shown the occurrence of some episodes of desynchronization in the electrocorticogram during the SWS which seem to be coincident with a short period of animal wakefulness. These investigators also observed that immediately after this short desynchronization of the electrocorticogram the rat was again in the SWS state of the sleep-wake cycle. In the present study we observed that this desynchronization is a consistent phenomenon occurring at regular intervals of 71 ± 4 s and all the episodes recorded were associated with deep breaths and with changes in PAP and HR. The deep breaths and cardiovascular changes occurred at regular intervals in all phases of the sleep-wake cycle, but the changes in the electrocorticogram were observed only in the SWS. It is important to note that deep breaths occurred 3.98 ± 0.45 s after the initial desynchronization of the electrocorticogram. The total period of desynchronization during the SWS was 11 ± 0.65 s. We may suggest different possibilities to explain these findings: a) deep breaths may be generated by cortical mechanisms, b) the micro-awake episodes may be related to neurovegetative adjustments activated by a possible reduction in $pO_2$ during the SWS and c) the short period of SWS desynchronization may be the electrophysiological expression of micro-awake episodes and the cardiovascular and respiratory changes observed are part of this event. The micro-awake episodes observed in the present study are similar to the arousal episodes that follow synchronized and desynchronized sleep, in which rostrum and eye movements, vibrissal twitches and tachycardia were observed (15). Since we did not record the cervical electromyogram or eye movements to determine the different steps of the wakefulness-sleep

cycle, we cannot rule out the possibility that desynchronization of the SWS (micro-awake episodes) observed in our study is related to episodes of relaxed wakefulness (15).

Deep breaths induce a 4-5-fold increase in tidal volume (2) and this large expansion of the chest wall may induce cardiovascular changes such as hypotension and tachycardia that may be counterbalanced by cardiovascular reflexes. We have reported that the fall in blood pressure of rats submitted to removal of the arterial baroreceptors was greater than in control rats (2,3). Thus, on the basis of a previous study (2), we may suggest two mechanisms to explain the fall in pressure during deep breaths: 1) activation of cardiopulmonary receptors in response to hemodynamic changes secondary to the large expansion of the chest wall and 2) interaction of neurons in the brain stem associated with the central neural control of the circulation and ventilation, considering that ventilatory and cardiovascular changes occurred at the same time. The activation of cardiopulmonary receptors may result from an increased end-diastolic filling pressure. However, this mechanism may not be important in this case because the fall in pressure seems to be too fast for the characteristics of this reflex mechanism. In addition, the tachycardia observed is not typical of the cardiopulmonary reflex (Bezold-Jarisch reflex), which is characterized by an intense bradycardic response. The second possibility, despite the complex neural mechanisms involved, is plausible if we consider the studies by St. John et al. (5-8) showing that gasping, a deep breath that occurs in decerebrated cats, is generated by neural circuits in the brain stem.

The hypothesis of respiratory and cardiovascular neuron interaction in the brain stem is supported by evidence showing that neurons generating the breathing rhythm in the pre-Botzinger complex (16) are located in the vicinity of the sympathetic vasomotor neurons in the rostral ventrolateral medulla (RVLM) (17) and also that neurons of the central respiratory generator may have an excitatory as well as an inhibitory effect on the sympathetic neurons located in the RVLM (18). Therefore, we suggest that the hypotension that follows the large deep breath seems to be more closely related to a possible sympatho-inhibitory mechanism at the RVLM level than to hemodynamic changes secondary to the large expansion of the chest wall. However, the interaction of these neural mechanisms in the brain stem to generate the respiratory and cardiovascular changes described in the present study requires further experiments to be better understood.

In previous studies (2,3) we observed that deep breaths occurred at intervals of 71 ± 8 s in the awake state and the change in arterial pressure was only -3 ± 2 mmHg. In the present study, in the SWS state the frequency was similar (71 ± 4 s) but the change in arterial pressure was greater than in the awake state (-16 ± 1 *vs* -7 ± 2 mmHg). It is important to note that the fall in mean arterial pressure observed during deep breaths in the SWS state was similar to that observed previously during deep breaths in the awake state in rats with sino-aortic deafferentation (-16 ± 3 mmHg) (2). Therefore, the primary changes consequent to SWS desynchronization seem to be a large increase in ventilation (deep breaths) and changes in cardiovascular parameters, which appear to be very well regulated in the awake state but not during desynchronization of the SWS state. Immediately after the fall in arterial pressure we also observed (Figure 1) a secondary increase in pressure, which may be related to autonomic adjustments in response to arterial baroreceptor deactivation during the fall in pressure. In previous studies it was observed that rats with sino-aortic deafferentation present a greater fall in arterial pressure during the episodes of electrocortical SWS desynchronization (1) and during deep breaths in the awake state (3). These data, taken together, indicate that arterial barore-

ceptor reflex integrity plays a key role in counterbalancing the cardiovascular oscillations during deep breaths and the present data suggest that during SWS desynchronization the sensitivity of the baroreflex may be reduced since the fall in pressure at that time was greater than in the awake state.

The cortical desynchronization during SWS indicates micro-awake episodes, which may induce cardiovascular and respiratory changes. The interaction of rostral brain areas involved in the generation of SWS desynchronization with brain stem structures involved in the autonomic regulation of circulation and ventilation is still a matter for further investigation.

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