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PHYSIOLOGY

The Cardiorespiratory Function and Electrical Activity of the Brain of the Ringed Seal during the Transition from Wakefulness to Sleep

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Although the behavior and physiology of marine mammals are of high scientific interest, our understanding of higher nervous activity of some species remains incomplete. Studies on the bioelectric phenomena in the central nervous system of different groups of pinnipeds are well represented in the literature [1–5]; however, there is no information about the rhythmical characteristics of the total brain electrical activity in the ringed seal during wakefulness and the development of sleep. In recent decades, methods and equipment for studying animals under natural conditions have been intensely developed; at the same time, laboratory experiments still remain important.

The urgency of this study comes from the necessity for further investigation of the behavior and activity of the central nervous and cardiorespiratory systems of the ringed seal. This is important for monitoring the condition of seals after their capture, transportation, and keeping in captivity, as well as for veterinary control.

The study was performed from September 2010 to May 2011 at the open-cage unit of the Experimental Test Ground of the Murmansk Marine Biological Institute of the Kola Science Center of the Russian Academy of Sciences. We studied two male ringed seals (*Pusa hispida* Schreber, 1775, the family Phocidae) aged two and three years, with a body mass of 36–42 kg, living in captivity for more than a year. The seals were kept in a bath (2000 × 2000 × 700 mm) without water. Sedation was performed by an intramuscular injection of 5–6 mL of 20% xylazine (Interchemie Werken "de Adelaar," the Netherlands), an α-2 adrenergic receptor agonist that has analgesic, anesthetic, and mild myorelaxant effects.

Polygraph study provided recording an electroencephalogram (EEG) with leads in three symmetrical regions of the seals' brain, namely the central (C), parietal (P), and occipital (O) points of the left (s) and right (d) hemispheres; electrooculogram (EOG); electrocardiogram (ECG); oronasal flow; breath noise; and thoracic perimetry. The ground electrode was located near the nasal bones; the reference electrodes were located in the postaural region above the mastoid process. The bioelectric activity was recorded using intradermal leads, by means of needle electrodes, as well as using epidermal leads, by means of gold electrodes fixed with an adhesive to the head of the animal. ECG was recorded using a software-and-hardware system developed at the Southern Scientific Center of the Russian Academy of Sciences [6]; polygraphic parameters were recorded using Leonardo C59, S12 (MKE Medizintechnik, Germany) [7]. The beginning of the inhalation and exhalation was determined using the video frames visualizing the opening of the nostrils, taking into account the recorded oronasal flow. The delineation and measurement of the opening area of the nostrils was performed using the ITEM 5.0 TECNAI FEI software. The widening of the nostrils (by 40% or more) together with a negative oronasal flow after the exhalation was considered to be the beginning of inhalation; an increase in the nostril area together with a positive oronasal flow following the respiratory pause or inhalation was considered to be the beginning of exhalation. The interval between successive breaths was considered to be the breathing period; a respiratory pause of 10 s or more was regarded as sleep apnea. The experimental protocols were approved by the Ethics Commission in accordance with the Helsinki Declaration. Numerical series analysis was

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carried out using the Encephalan–131–03 11m digital software environment (Medicom MTD, Taganrog, Russia) after digital filtering in the band 0.5–50 Hz.

Observing the ringed seal in captivity gave us the general idea about the influence of the feeding regime, the presence of humans, and the changes in circadian, climatic, and seasonal factors on seals (Fig. 1). In particular, it was found that, before feeding, the seals prefer the surface of the water and display orientation responses waiting for a trainer who brings some fish. The character of respiration, as measured by the average duration of apnea before feeding (16.9 ± 3.79 s) and after feeding (25.4 ± 1.45 s) changed significantly (Student's test: $t = 1.16$, $p = 0.042$), which means a calmer condition of the animals after feeding. When the seals were at the surface of the water, their nostrils were usually open only long enough to perform exhalation and inhalation (1–2 s). After feeding, only in a few cases, the nostrils of a seal floating on the surface of the water were opened for about 20 s.

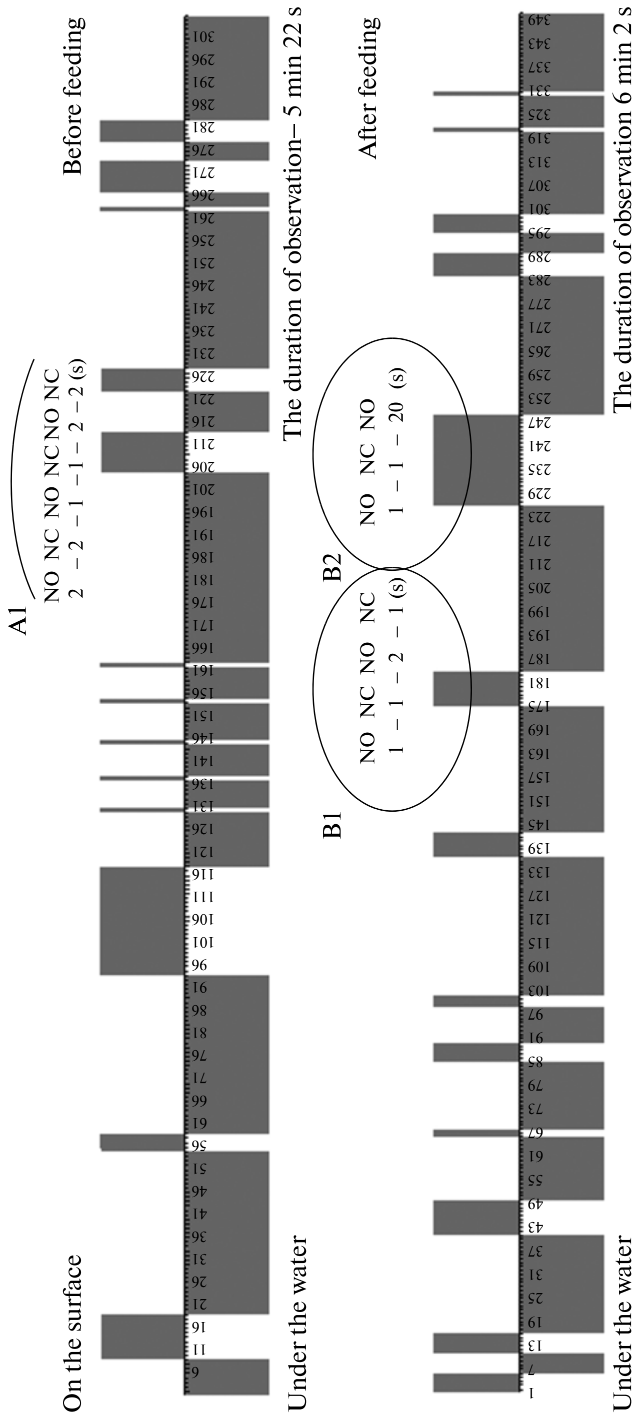


Fig. 1. The dynamics of respiration and behavior of ringed seals on the surface of the water and under the water. The exposure of the nostrils. Fragments A1, B1 and B2: NO, nostrils open, exhalation–inhalation; NC, nostrils closed.

Observing the animals, we found a great variety of respiratory movements of the thorax and types of opening the nostrils, including the transitions from rhythmic to nonrhythmic breathing. For example, rhythmic breathing lasted for 2–5 minutes, and then changed to nonrhythmic breathing, which consisted of patterns with a highly probable prolonged apnea. The transitions between these types of breathing are shown in Fig. 2. The period of breathing with a long apnea (52 s) is followed by rapid rhythmic breathing (14.3 breaths per min); then, it is followed by a slow rhythmic pattern (5.4 respiratory cycles per min). It is interesting that the heart rate significantly decreased (to 20 beats per min) against the background of prolonged apnea, with a subsequent increase in it (up to 55 beats per min) at the end of apnea. Doublets of systoles observed after prolonged apnea are noticeable as well. In general, against the background of apnea, the R-wave amplitude often decreases, subsequently increasing after the return to rhythmic breath. Probably, the change in the R-wave amplitude is due to the change in the heart axis resulting from the tension of the diaphragm.

Analysis of the breathing of the seals showed that a rapid exhalation (about 1 s) is followed by longer inhalation (about 2 s). Then, in the case of rhythmic breathing, a new cycle starts, or apnea of a different duration follows (Fig. 3). The figure shows the effect of the respiratory arrhythmias on the nature of heart contractions. Tachycardia during the inhalation is followed by bradycardia during the exhalation, which is particularly evident against the background of apnea. These features of the respiratory function contribute to the data on the respiratory system of marine mammals and clarify some phenomena that we have described [8, 9].

Analysis of the behavior and EEG parameters allowed active wakefulness (AW) and quiet wakefulness (QW), as well as the beginning of slow-wave sleep (SWS), to be distinguished from the sleep–wake cycle.

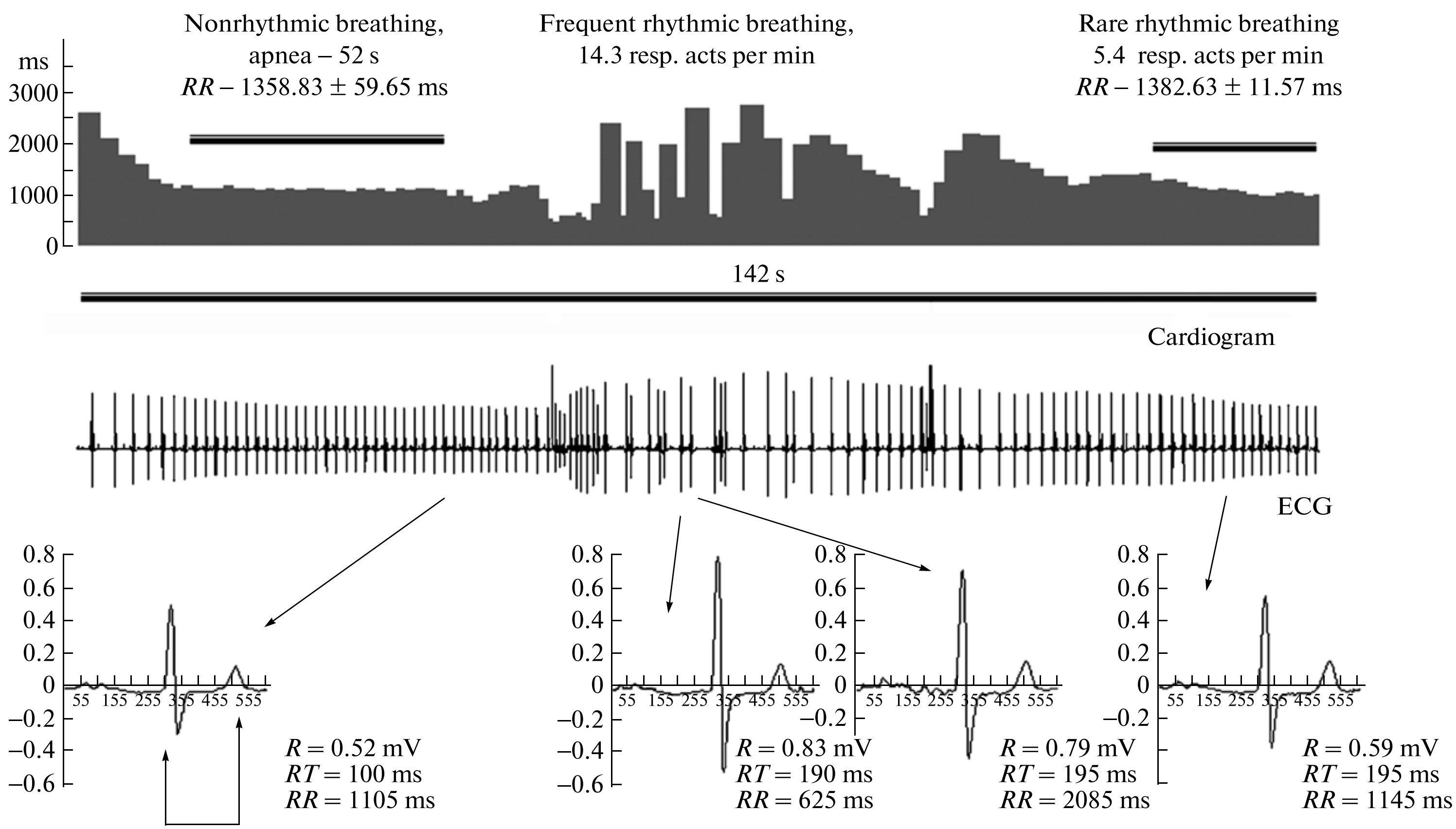
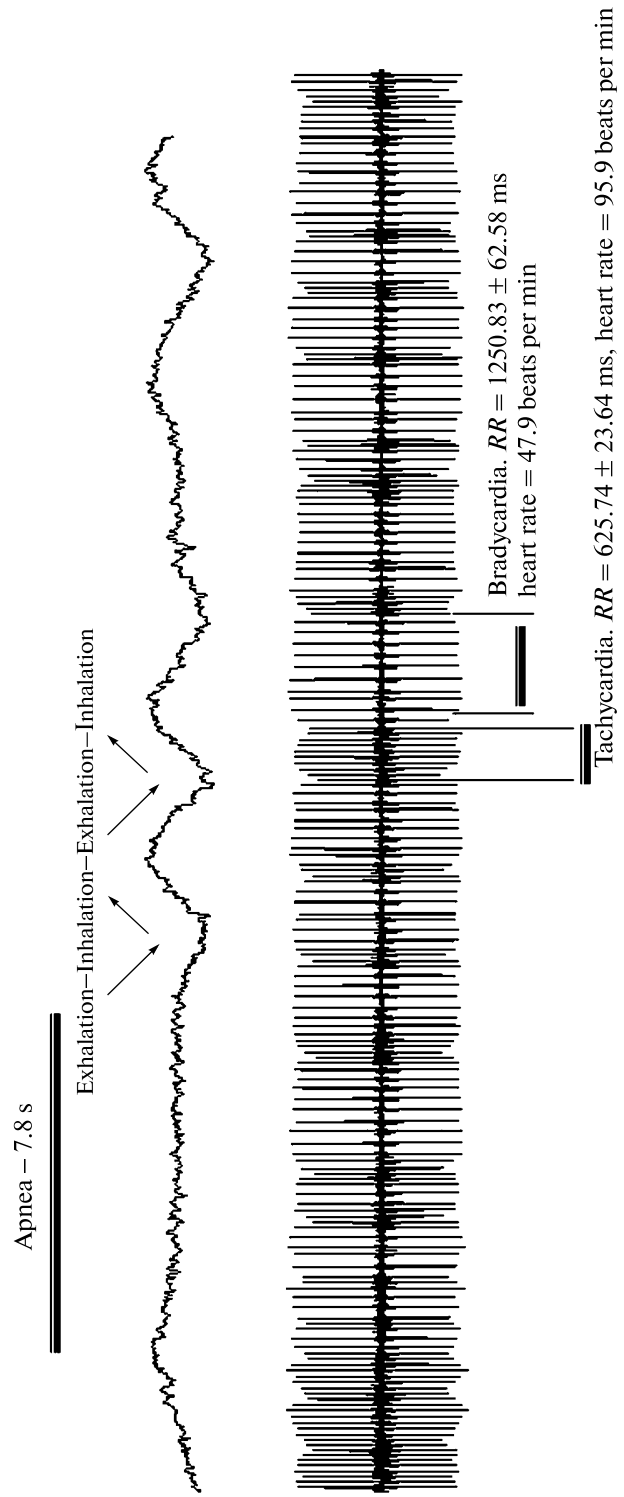


Fig. 2. The cardiorespiratory function of ringed seals during rhythmic and nonrhythmic breathing. Top–down: the periodogram of *RR* intervals, ECG, and the changes in ECG cardiac cycles.

Active wakefulness (Fig. 4) was characterized by desynchronized EEG with a low amplitude, presented in a wide frequency range (0.5–30 Hz) typical of mammals, without a distinct dominance of any range with a small amount of slow waves (0.5–2 Hz). Variation from animal to animal were not significant, they differed on the nature of the domination of a narrow strip of α-like rhythm (9–11 Hz). During the rhythmic breathing, the average duration of the respiratory cycle was 4.75 ± 0.29 s. According to our observations, the apnea (a breathing pause more than 10 s) accounted for 4.17%; the mean ECG RR interval duration was 698.8 ± 39.76 ms, which corresponded to the pulse of 85.9 beats per min. The eyes of the seals were opened, and its head was raised. The muscle tone allowed the seals to attempt to support on their flippers.



In contrast, QW (Fig. 4) was characterized by the appearance of dominant ECG frequencies of 8–14 Hz with a modal frequency of 10.2 Hz for one seal and 11.4 Hz for the other one, as well as by slow components in the range of 0.5–4 Hz. During the dynamic transition from AW to QW, the duration of the respiratory cycle increased to 6.0 ± 0.47 s. The representation of apnea increased to 8.0%; the average duration of ECG RR interval increased to 808.6 ± 45.30 ms, which corresponded to the pulse of 74.2 beats per min. The seal's eyes were mostly closed, and its head fell onto the platform. The seals did not take attempts to support on the flippers.

Following the QW, SWS became more profound (Fig. 4). In an EEG of SWS, a dominant frequency range of the bioelectric potential oscillations shifted to the low-frequency region of the spectrum (0.5–4.0 Hz) (Fig. 4). It is worth noting that, at all stages of the research and in both animals, no significant phenomena of hemispheric asymmetry were observed in the EEG. As it was shown by a number of researchers, asymmetric SWS is typical of all members of the family of eared seals, however it does not occur in the true seals [1, 10, 11]. The duration of the respiratory cycle increased more and reached 9.12 ± 1.27 s; the probability of apnea increased to 12.5%; and the average duration of ECG RR–interval increased to 950.12 ± 45.77, which corresponded to the pulse of 63.16 beats per min.

Statistical evaluation of data sets using the Wilcoxon test for paired comparisons of dependent variables allowed us to record significant differences between the duration of the respiratory cycle during QW and SWS ($p = 0.001$), AW and SWS ($p = 0.00002$); the duration of RR interval during QW and SWS ($p = 0.0001$), AW and SWS ($p = 0.044$).

The most important finding was that, during dropping-off to sleep, the probability of prolonged respiratory pauses (apnea) significantly increased from 4.17% during AW to 12.5% during SWS. Probably, the preva-

Fig. 3. The change from nonrhythmic to rytmic breathing with changes in the heart rate in ringed seals. At the top: the perimetry of the thorax (marked: apnea and the alternation of exhalation and inhalation). At the bottom: ECG (marked: tachycardia and bradycardia).

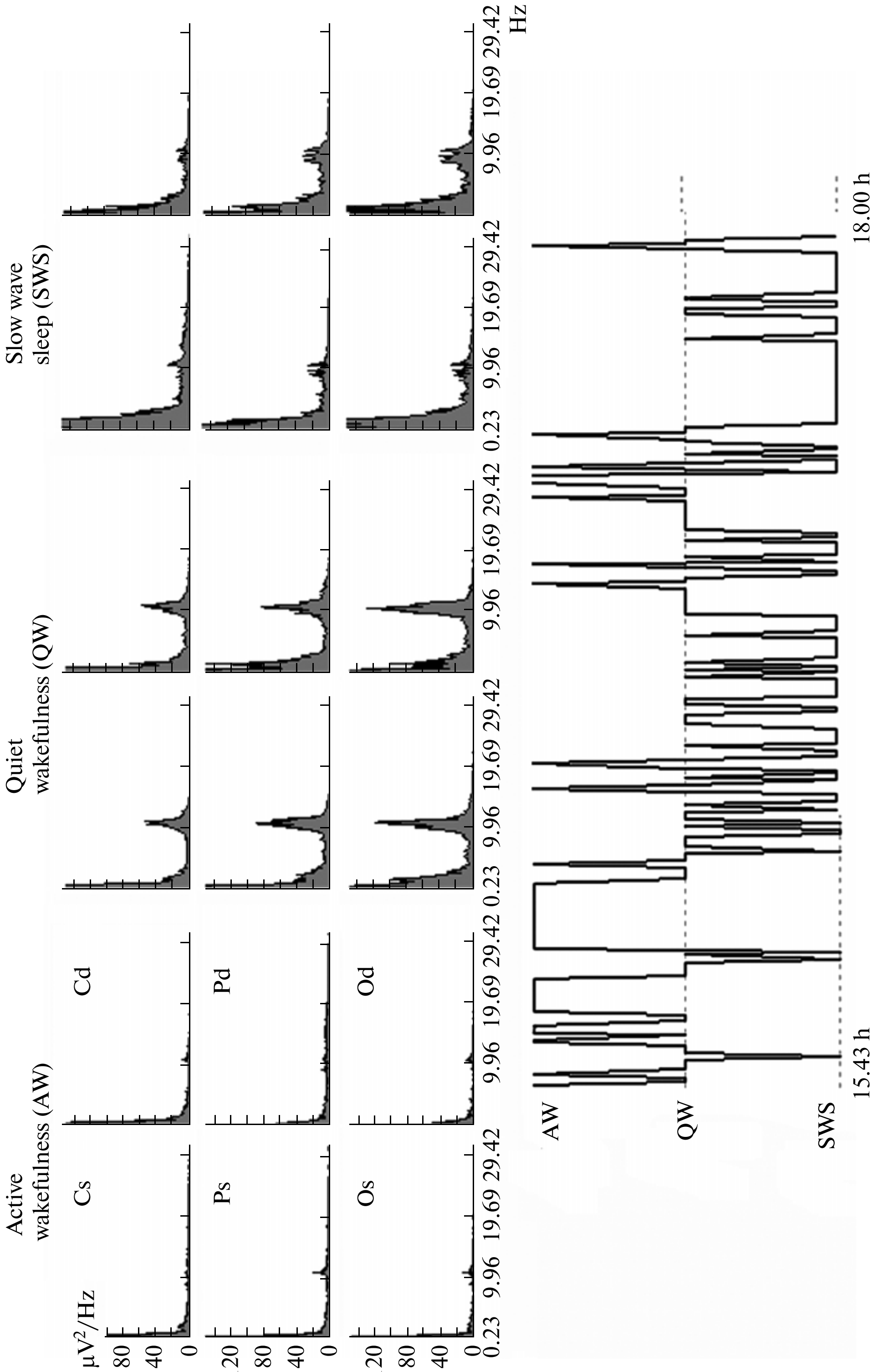


Fig. 4. Averaged EEG power spectra of the central, parietal, and occipital regions of the brain of a ringed seal during active wakefulness (AW), quiet wakefulness (QW), and at the beginning of slow wave sleep (SWS) (at the top); and a fragment of hypnogram characterizing the dynamics of dropping off to sleep (at the bottom).

lence of irregular breathing with a lot of apnea has a reflex nature. Most likely, this type of breathing is necessary for animals not to swallow water during dropping-off to sleep. The strength of this reflex action is demonstrated by the fact that this ability of the animal is preserved in the absence of water in a pool.

The described phenomena clarify the existing information on the electrophysiology of sleep–wake cycles of pinnipeds [2, 3] and show new relationships in the cardiorespiratory function and the changes in the electrical activity of the brain during the transition from wakefulness to SWS in the ringed seal.

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