

EEG study of hearing in the common dolphin, *Delphinus delphis*

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Abstract

Auditory brain stem responses (ABR) were recorded from the head surface of a non-anesthetized common dolphin. The ABR waveform and temporal characteristics were similar to other investigated dolphin species. The ABR consisted of a sequence of fast waves lasting less than 1 ms each.

We determined ABR thresholds using tone bursts of varying intensity and frequency as stimuli. So the audiogram (frequency-threshold function) of the dolphin was obtained. The audiogram was U-shaped with a steeper high-frequency branch. The audiogram bandwidth was up to 128 kHz at a level of 100 dB above the minimum threshold. The minimum thresholds were observed at frequencies of 60 to 70 kHz.

Tone-tone masking was used to determine ABR tuning curves at two frequencies (64 and 90 kHz) in a simultaneous-masking paradigm. Tuning curves had the same features as the well-known curves in the other mammals. There was a relatively sharp tip segment, an elongated lower-frequency tail, and a steep rise at higher frequencies. At 64 kHz, Q_{10} value was 10.7, and at 90 kHz it was 9.5.

Temporal resolution of hearing in the common dolphin was estimated by measuring the ability of ABR to follow rhythmic clicks, amplitude-modulated tones, and to recover in conditions of double-click stimuli. Very high frequency and temporal resolution in the studied dolphin may be associated with echolocation.

Introduction

Auditory brain stem response (ABR) is widely used for studies of hearing. The ability to record the activity of auditory nerve centers without any surgery makes this evoked response useful for investigation of auditory physiology and pathology, in particular, for comparative studies of hearing in various groups of animals.

Ridgway *et al.* (1981) described ABR recorded from the bottlenosed dolphin skull and studied a number of its features. Our own experiments have

shown that ABR in dolphins can also be recorded from the animal's head surface (Popov & Supin, 1985, 1990a). ABR recording provides a rapid and convenient technique for assessing dolphin auditory perception. ABRs have been described in a number of dolphin species: the bottlenosed dolphin (Ridgway *et al.*, 1981; Popov & Supin, 1985); harbor porpoise (Bibikov *et al.*, 1986); beluga whale (Popov & Supin, 1987); Amazon river dolphin, Tucuxi dolphin (Popov & Supin, 1990b), and killer whale (Szymanski *et al.*, 1995). The ABR method was used to study certain mechanisms of dolphin hearing: frequency and temporal resolution (Supin & Popov, 1990b, 1995a,b; Supin *et al.*, 1993); sound conduction to the dolphin ear (Popov & Supin, 1990d; 1990c); and binaural interactions (Popov & Supin, 1991; Supin & Popov, 1993). These features of the dolphin auditory system were investigated mainly in the bottlenosed dolphin and Amazon river dolphin. It is very important to expand the list of studied dolphin species. We came upon such an opportunity in the summer of 1994.

A sick male common dolphin (*Delphinus delphis*) 1.54 meters long was found not far from the Utrish Marine Station (Black Sea). Since it could not swim normally, it was delivered to the station and placed on a stretcher in a bath (4 × 0.6 × 0.6 m) with sea water. A constant monitoring was arranged over the dolphin's condition by recording its ECG and body temperature. In spite of the intensive course of medical treatment it died 4 days later. Postmortem examination revealed pulmonary edema and purulent lesions of the internal organs.

Along with ECG recording, we were able to record ABR by using the same electrodes. This method enabled us to measure certain auditory characteristics of this dolphin.

Methods

Evoked responses were recorded by using 1 cm plate electrodes attached to the skin by adhesive electric-conductive gel. The active electrode was placed at the dorsal part of the head, 6-8 cm behind the blowhole, where ABR has been shown to have

the maximal amplitude (Popov & Supin, 1990a,b). The reference electrode was placed on the back near the dorsal fin. The recorded signals were amplified, bandpass filtered between 200 Hz and 5 kHz and averaged using the Neuropack-II evoked-response recorder. The averaging of 512 to 1024 responses was enough to measure response parameters with satisfactory precision.

Different kinds of signals (clicks, pure tone bursts, amplitude-modulated tones) were used as acoustic stimuli. They were delivered through spherical piezoceramic transducers immersed in water. To produce a click, the transducers were activated by 5 μ s long rectangular pulses. The frequency of tone signals varied within a range of 5 to 150 kHz. Tone pips had a cosine-wave envelope, i.e. the envelope was one period of a function $1-\cos(t)$. This form of signal provided a narrower spectral band than triangular or rectangular signals of the same duration. Amplitude-modulated tones were produced by multiplying the tone carrier by the sum of a sinusoidal and DC signal, so that the sound amplitude was modulated as $1+m \sin 2\pi ft$, where t is time, m is the modulation index ($0 < m < 1$), and f is modulation rate. The amplitude-modulated bursts were of 12 ms duration with a linear rise time of 2 ms and modulation imposed on the carrier throughout all the burst, beginning with minimal amplitude.

The transducer was immersed in water at a depth of 30 cm, 0.7 m in front of the animal's head. To increase the regularity of the acoustic field, the bath walls and free water surface in front of the animal were covered with sound absorbing rubber. Intensity and frequency spectra of signals were monitored through a probe hydrophone with a passband of 150 kHz, located near the animal's head. The relative level for click intensity indication in decibels was taken to be 1 μ Pa of peak sound pressure and for tone burst, 1 μ Pa of effective sound pressure.

Results

ABR waveform and characteristics

Acoustic stimuli, such as clicks, or tone bursts evoked ABR recorded from the body surface of the common dolphin (Fig. 1). They consisted of the sequence of waves, each lasting below 1 ms. The response latency without the acoustic delay (i.e., from the instant the acoustic pulse reached the animal's head) constituted 0.75 ms for the 120-dB click. The main positive and negative waves of ABR were designated as P1 to N5 (P—positive, N—negative), as shown in Fig. 1. Wave peak latencies for the 120 dB click had the values as follows: P1—1.35 ms; N2—1.8 ms; P3—2.3 ms; P4—2.95 ms; and N5—3.35 ms. The ABR of the common dolphin and those of other investigated

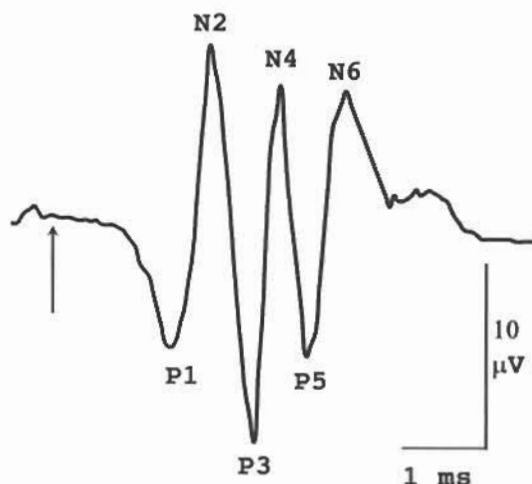


Figure 1. ABR waveform evoked by a click (120 dB) as recorded from the dolphin's head surface. Active electrode negativity is upward. Arrow indicates the instant when acoustic pulse reaches the animal's head. P1–N6: designation of the main waves (P—positive, N—negative).

dolphins are very similar in the waveform (Ridgway *et al.*, 1981, Popov & Supin, 1990b), except that the most prominent component in the ABR of the common dolphin was an N2–P3 wave, whereas in other dolphins it was P5–N6.

ABR dependence on click intensity

Fig. 2 shows ABRs evoked by clicks of varying intensity. There is an increase in the response amplitude with increase in the stimulus intensity. The shape remained almost unchanged. Shortening of latencies of the main waves was observed with intensity increase.

The dependence of the response amplitude measured between N2 and P3 peaks on the click intensity is shown in Fig. 3. The threshold of click-evoked ABR was near 60 dB. The plot of ABR amplitude dependence on click intensity consisted of four distinct sections: (i) the response amplitude growing within a range of 60 to 100 dB; (ii) a plateau within a range of 100 to 110 dB; (iii) the amplitude sharp rise again within a range of 110 to 130–135 dB; (iv) finally, a second plateau at intensities higher than 135 dB. We demonstrated similar ABR dependence on click intensity in the Amazon river dolphin (Popov & Supin, 1990b).

The dependence of P1 wave latency on click intensity is shown in Fig. 3. Latency fell with stimulus intensity, and the latency vs. intensity relationship was around 4 μ sec/dB for clicks. This latency vs. intensity function in the common dolphin is the same as in the bottlenosed dolphin (Ridgway *et al.*, 1981) and almost twice as shallow

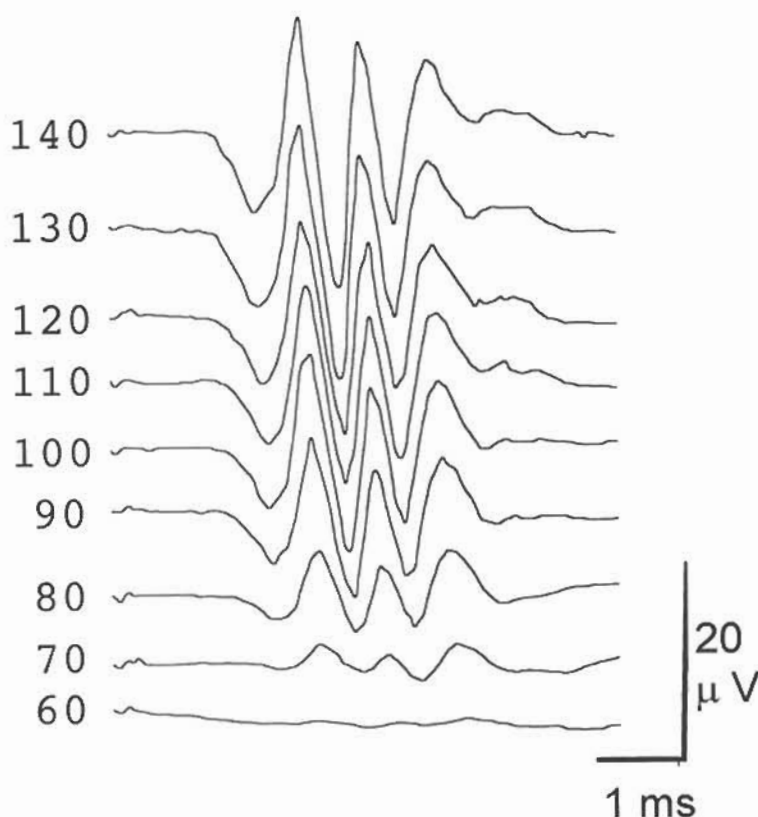


Figure 2. ABRs evoked by clicks of different intensities. Click intensity indicated in dB re 1 μ Pa of peak sound pressure.

as that for the Amazon river dolphin (Popov & Supin, 1992).

ABR dependence on tone intensity and frequency

The onset of a tone burst led to an ABR of the same shape as that evoked by clicks. The ABR amplitude in this case depended both on the tone intensity and frequency. We determined ABR thresholds using tone bursts of varying intensity and frequency as stimuli. A rather high amplitude of ABRs and their consistence in the common dolphin made it possible to determine thresholds with an accuracy of 3–5 dB. Therefore the ABR threshold measuring could be used to determine an audiogram. The ABR audiogram presenting the dependence of the ABR thresholds on the frequency of tone bursts is shown in Fig. 4. The lowest threshold of about 53 dB was at a frequency of 64 kHz. A sharp rise of ABR thresholds took place at higher frequencies; at low frequencies the threshold values increased rather slowly. The maximum frequency at which the ABR appeared was as high as 152 kHz. At this frequency, the response threshold was near 127 dB re 1 μ Pa.

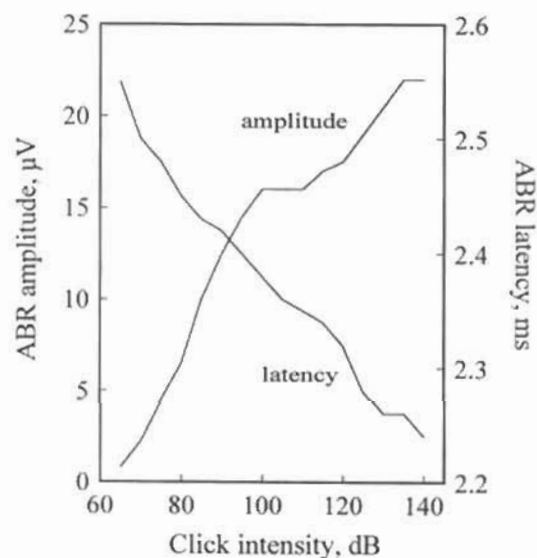


Figure 3. Effects of stimulus intensity on ABR amplitude (left scale) and peak latency (right scale).

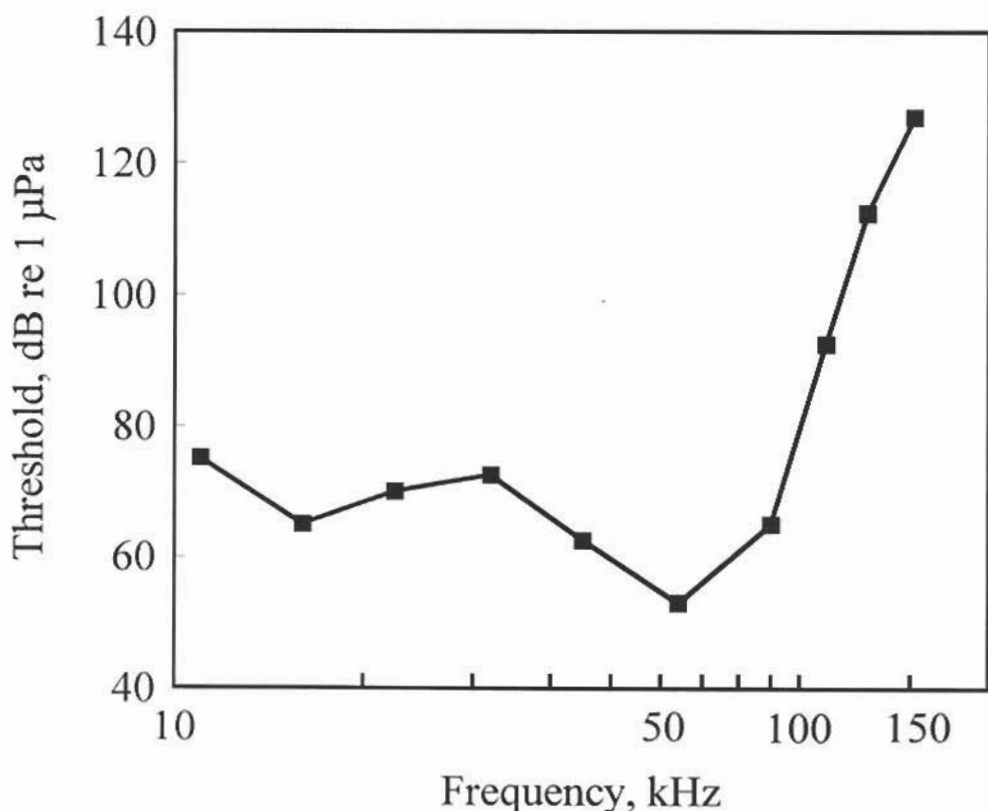


Figure 4. Dependence of ABR threshold on sound frequency.

The only attempt to determine the low and high frequency limit of hearing in the common dolphin was made by Belkovitch & Solnzeva (1970). According to that study, the upper frequency limit in the common dolphin was 280 kHz. Unfortunately, the authors did not provide a description of the methods used, so we cannot explain this large difference in estimations of the high frequency limit.

ABR dependence on stimulus rate

The ability of responses to follow rhythmic stimuli appears to be an important parameter of the auditory system. In order to measure this characteristic in the dolphin, we recorded ABR evoked by rhythmically repeated clicks of various rates. Fig. 5 presents an example of ABR to rhythmic clicks of varying rate. The response waveform and amplitude changed slightly at stimulus rates up to 200/s. Further increase in the rate resulted in a gradual decrease in the amplitude and in smoothing of the response; individual response components fused together forming a sinusoidal response. This response could follow the stimulus rate up to 1700/s.

Fig. 6 presents plots of the amplitude of auditory responses vs. rate of rhythmic stimuli. The ABR of the common dolphin demonstrated a very high ability to follow rate up to 2000 Hz. Thus our data point to the ability of the auditory nuclei in dolphins to a fast responsiveness recovery. This feature was described in some dolphin species (Bullock *et al.*, 1968; Ridgway *et al.*, 1981; Popov & Supin, 1990a,b) and it may be associated with echolocation.

ABR dependence on delay between paired stimuli

These measurements were made using double clicks of varying interstimulus intervals (ISI). Intensities of both clicks in a pair were always equal. In this stimulus condition, the second (test) response was smaller in amplitude than the first (conditioning) one. Fig. 7A shows several examples of ABR records obtained at a few of the tested ISI. At ISIs shorter than 2 ms, the first and second responses were partially superimposed. In such cases, the second response was isolated from a combined paired response by a routine point-to-point subtraction procedure: response to a pair of clicks minus

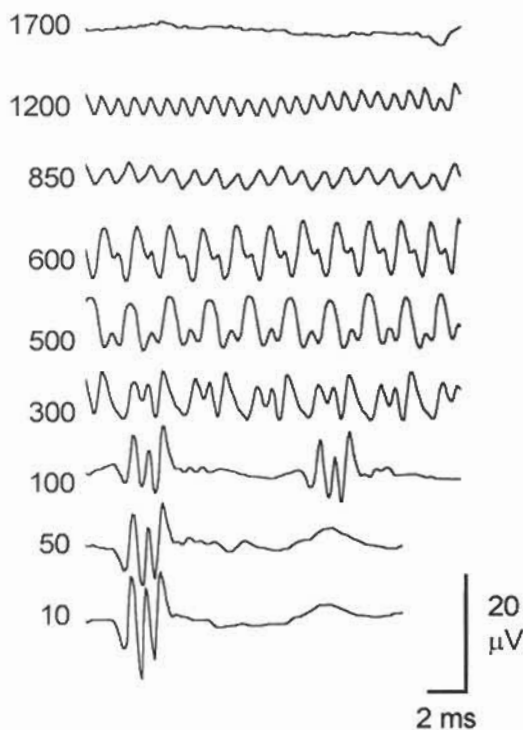


Figure 5. ABRs to rhythmic clicks of various rates. Click intensity 120 dB. Stimulus rate (clicks per s) is indicated near the oscillograms.

that to the single click which was the same as the first click of the pair (Fig. 7B). Just detectable response can be seen at ISI down to 0.2 ms. 50% recovery of response amplitude was observed at ISI of 0.8–1 ms.

In accordance with previous data obtained on other dolphin species (Bullock *et al.*, 1968; Bullock & Ridgway, 1972; Popov & Supin, 1990*a,b*), these results show a very quick recovery of excitability in the auditory centers of the common dolphin.

ABR frequency tuning curves

An effective way to study frequency selectivity of hearing is the evoked potentials method in conjunction with tone-tone masking. Tuning curves were obtained in humans and some experimental animals using evoked potentials such as cochlear APs (Dallos & Cheatham, 1976; Eggermont, 1977; Harrison *et al.*, 1978) and ABRs (Mitchell & Fowler, 1980; Gorga *et al.*, 1983). Our previous work (Supin *et al.*, 1993) has shown that the ABR method is also effective to investigate frequency tuning in the bottlenosed dolphin.

Just as in the previous work, we used the simultaneous masking paradigm and chose the near-complete masking criterion to obtain tuning curves.

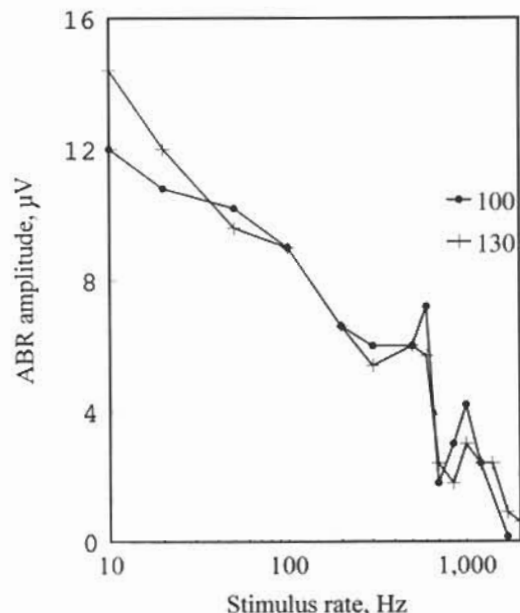


Figure 6. Dependence of the ABR amplitude on rhythmic clicks rate. Clicks intensities 100 and 130 dB.

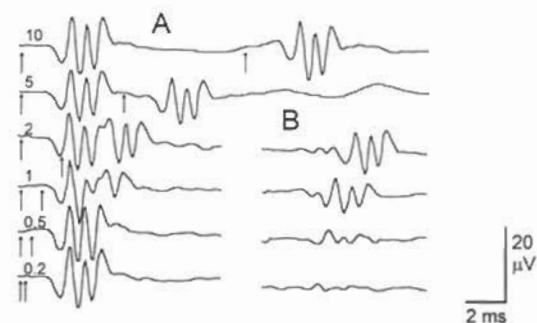


Figure 7. A—ABR to paired clicks of equal intensity (120 dB). B—Response to the second click in pure form obtained by point-to-point subtraction of the ABR to the first click from that to the paired click. Instants of stimulation indicated by arrows. ISI is indicated in ms.

Probe stimuli were tone bursts with a cosine-wave envelope. Burst duration was 0.5 ms. Masking signals were continuous tones of various frequency and level. The conditions of the experiment allowed us to obtain tuning curves only at two frequencies, 64 and 90 kHz.

To obtain a tuning curve, the probe frequency and intensity were fixed and masker frequency and intensity were varied. For each masker frequency, the level required for near-complete masking of the probe response was determined with a 2.5-dB step. This procedure was repeated at each frequency of the masker to yield a complete tuning curve. Tuning

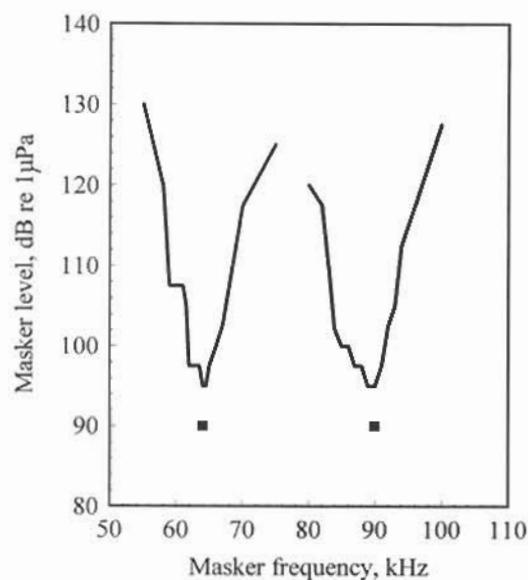


Figure 8. Tuning curves obtained at two probe frequencies (64 and 90 kHz). Square symbols represent probe frequency and level (90 dB re 1 μ Pa).

curves at probe frequencies of 64 and 90 kHz are shown in Fig. 8. These tuning curves possessed the same features as curves in the bottlenosed dolphin and as well-known curves in other mammals. There was a relatively sharp tip segment and elongated low-frequency tail as well as steep rise at high frequencies. All the curves peaked at the probe frequency. At this frequency, the near-complete masking required a masker level of 5 dB above the probe level.

A convenient measure of the sharpness of tuning is the Q_{10} values (the center frequency divided by the bandwidth at a level of 10 dB above the tip of the curve). In the common dolphin, at 64 kHz, the Q_{10} value was 10.7, and at 90 kHz it was 9.5. This is just half as much as Q_{10} in the bottlenosed dolphin at the same frequencies (Supin & Popov, 1993), but near Q_{10} values in the majority of studied terrestrial mammals and humans (Dallas & Cheatham 1976; Mitchel & Fowler 1980; Harrison *et al.*, 1981).

Envelope following response (EFR)

In psychophysical and physiological studies, sinusoidally modulated sounds are widely used to estimate the temporal resolution of the auditory system. In such studies, the temporal resolution is evaluated by the ability to transfer various modulation rates; i.e., by the modulation rate transfer function (MTF). In physiological studies, either neuronal responses or evoked potentials can be used to obtain MTF. In humans and in some

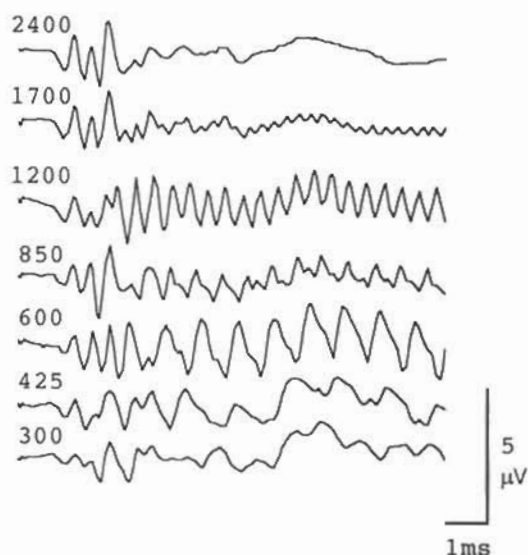


Figure 9. EFR evoked at various modulation rates. Tone carrier: 64 kHz, 120 dB. Modulation depth 1. Modulation rate is indicated near the curves (Hz).

animals, potentials recorded from the head surface were shown to follow the envelope of amplitude-modulated carriers (Rodenburg *et al.*, 1972; Hall, 1979; Dolphin & Mountain, 1992). This type of response is termed the amplitude-modulation following response (AMFR) or envelope following response (EFR). The possibility was shown recently to record EFR in dolphins at low-frequency (Dolphin, 1995; Dolphin *et al.*, 1995) and high-frequency carriers (Supin & Popov, 1996).

Amplitude-modulated tone bursts evoked, in the common dolphin, a robust rhythmic response which followed the modulation rate, i.e., EFR. Fig. 9 presents representative EFR examples at various modulation rates. The tone burst with the shallow rise evoked a small transient on-response which after a few milliseconds was replaced by the quasi-sustained EFR. The start of the response appeared with a few ms lag relative to the stimulus. The response-free initial part of records indicates that the records were not contaminated with electromagnetic artifacts. In spite of the sinusoidal stimulus envelope, rhythmic responses to lower modulation rates (below 600 Hz) had more complex waveform than sinusoids. At higher modulation rates, the response waveform became simpler and closer to a sinusoid.

To evaluate EFR magnitude to a given burst stimulus, a number of response cycles in the last 6-ms part of the 12-ms response were Fourier transformed to find the weight of the component at the modulation frequency. Fig. 10 shows EFR as a

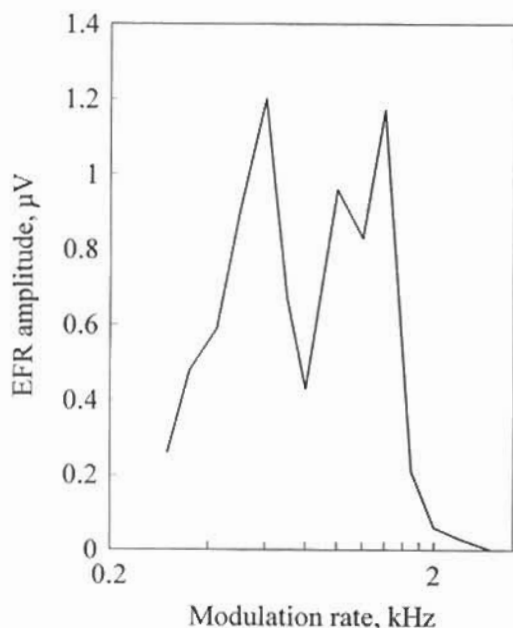


Figure 10. EFR amplitude dependence on modulation rate. Tone carrier 64 kHz, 120 dB. Modulation depth 1.

function of modulation rate. The plot represents a typical form of this function. It was a multi-peak function with higher peaks at modulation rates of 600, 1000 and 1400 Hz, and a trough at 800 Hz. The function declined sharply at rates above 1400 Hz. The response was not detectable at rates higher than 3000 Hz.

The function shown above may be adopted as a first approximation of MTF. Thus, the temporal resolution of the common dolphin's auditory periphery is expected to be not less than the obtained MTF bandwidth (1700 Hz cut-off). These results agree with data obtained using the EFR method in the bottlenosed dolphin (Dolphin *et al.*, 1995; Supin & Popov, 1996), in the false killer whale and beluga whale (Dolphin *et al.*, 1995) and show a very high temporal resolution in dolphins as compared to other mammals (Fay, 1988).

Thus, using the method of ABR recording, we succeeded in measuring a number of auditory parameters in the common dolphin. Unfortunately, the short time with the animal made it impossible to carry out a more detailed investigation. In its basic parameters (thresholds, range of perceived frequencies, response rate), common dolphin hearing is close to the auditory characteristics of other studied dolphins (Ridgway *et al.*, 1981; Popov & Supin, 1990a,b). It is unlikely that the morbid state of the animal greatly affected the measured parameters since ABR reflects the activity of the

auditory periphery which is more resistant to changes in the animal's state (Bobbin *et al.*, 1979; Cohen & Britt, R. H., 1982).

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