RESEARCH ARTICLE

Dolphin hearing during echolocation: evoked potential responses in an Atlantic bottlenose dolphin (*Tursiops truncatus*)

Songhai Li*, Paul E. Nachtigall and Marlee Breese

Marine Mammal Research Program, Hawaii Institute of Marine Biology, University of Hawaii, P.O. Box 1106, Kailua, HI 96734, USA

*Author for correspondence (songhai@hawaii.edu)

SUMMARY

Auditory evoked potential (AEP) responses were recorded during echolocation in an Atlantic bottlenose dolphin (*Tursiops truncatus*) trained to accept suction-cup EEG electrodes and detect targets by echolocation. AEP recording was triggered by the echolocation clicks of the animal. Three targets with target strengths of –34, –28 and –22 dB were used at a target distance of 2 to 6.5 m for each target. The results demonstrated that the AEP appeared to both outgoing echolocation clicks and echoes during echolocation, with AEP complexes consisting of alternative positive and negative waves. The echo-related AEP amplitudes were obviously lower than the outgoing click-related AEP amplitudes for all the targets at the investigated target distances. However, for targets with target strengths of –22 and –28 dB, the peak-to-peak amplitudes of the echo-related AEPs were dependent on the target distances. The echo-related AEP response amplitudes increased at further target distances, demonstrating an overcompensation of echo attenuation with target distance in the echo-perception system of the dolphin biosonar. Measurement and analysis of outgoing click intensities showed that the click levels increased with target distance (*R*) by a factor of approximately 10 to 17.5 log*R* depending on target strength. The results demonstrated that a dual-component biosonar control system formed by intensity compensation behavior in both the transmission and receiving phases of a biosonar cycle exists synchronously in the dolphin biosonar system.

Key words: AEP response, hearing, outgoing click, automatic gain control, biosonar.

INTRODUCTION

Dolphins and toothed whales (odontocetes) possess highly developed sound production systems and hearing capabilities (Au, 1993; Au et al., 2000; Nachtigall and Moore, 1988). Because sound is transmitted through water much more efficiently than most other energy forms, such as light, heat and electromagnetism, hearing plays a fundamental role as a primary sensory modality in dolphins and toothed whales and functions to aid in navigation, orientation, foraging and communication (Au, 1993; Nachtigall and Moore, 1988; Richardson et al., 1995). Since the first audiogram was measured in a bottlenose dolphin by using a pioneering psychophysical method (Johnson, 1967), the auditory sensitivity of odontocete species has been investigated for over 40 years (reviewed in Au et al., 2000; Nachtigall et al., 2007). However, there are still many gaps in our knowledge of auditory processes in odontocetes. Most of the hearing and biosonar research on odontocete species has emphasized their auditory response to external signals or hearing capabilities (Au et al., 2000). Little is known about how dolphins hear their own echolocation clicks and corresponding echoes during echolocation, how or whether they control their hearing or both sound production and hearing during echolocation.

Despite the fact that echolocation in dolphins and toothed whales has been studied for a few decades, most of our knowledge of odontocete echolocation focuses on their sound production (Au, 1993). Though an attempt was made to record auditory evoked potentials (AEPs) to voluntary click emission from a dolphin by Bullock and Ridgway several decades ago (Bullock and Ridgway, 1972), the quantitative investigation of odontocete ability to hear

their own echolocation clicks and corresponding echoes has only been performed and reported in a single false killer whale (*Pseudorca crassidens*) (Nachtigall and Supin, 2008; Supin et al., 2003; Supin et al., 2004; Supin et al., 2005; Supin et al., 2009; Supin et al., 2010).

By using a non-invasive evoked-potential method, Supin et al. recorded brain AEPs containing responses to both the outgoing echolocation clicks and the echoes in a false killer whale during echolocation (Supin et al., 2003). Their results indicated that both the responses to the outgoing clicks and the echoes were of comparable amplitudes in spite of a more than 40 dB intensity difference of these two sounds near the animal's head. Supin et al. also demonstrated that the amplitudes of the echo-related AEPs were almost independent of target distance and strength (or echo intensity) (Supin et al., 2004; Supin et al., 2005; Supin et al., 2009). These results suggest that: (1) some sort of acoustical or/and neurophysiological gain control mechanisms exist in the hearing of the investigated false killer whale (Nachtigall and Supin, 2008), (2) partial forward masking of the echoes by the outgoing echolocation clicks may serve as a kind of automatic gain control in the auditory system of echolocating odontocetes (Supin et al., 2009) and (3) the evoked-potential method may be an effective and productive method to investigate hearing mechanisms in an actively echolocating animal (Nachtigall and Supin, 2008; Supin et al., 2003).

In the present study, we recorded the evoked potential responses of an Atlantic bottlenose dolphin (*Tursiops truncatus*) during echolocation by using the same AEP protocol in the same experimental facility as that in the Supin et al. studies (Supin et al.,

2003; Supin et al., 2004; Supin et al., 2005; Supin et al., 2010). We investigated whether the brain of the studied animal, as well as the previously investigated false killer whale, responds to both the outgoing clicks and echo sounds. Amplitudes of the proposed outgoing click-related and echo-related AEPs at different target types and target distances were measured and compared.

MATERIALS AND METHODS
Subject
The experimental subject was an adult female Atlantic bottlenose dolphin [*Tursiops truncatus* (Montagu 1821)] named BJ, who was 24 years old at the time of the experiment. She was housed in a wire-net enclosure in the facilities of the Hawaii Institute of Marine Biology, Marine Mammal Research Program, Kaneohe Bay, Hawaii. The animal was trained to wear soft latex suction cups containing human EEG electrodes to pick up the evoked potentials. The animal performed an echolocation task in which she swam into a hoop, ensonified and detected targets by echolocation, and reported the target presence or absence using a go/no-go reporting paradigm [see detailed description in Schusterman (Schusterman, 1980)]. Three targets were used separately in this study. Targets were hollow aluminium cylinders with an outer diameter of 38 mm, an inner diameter of 25.4 mm and varying lengths of 46, 90 and 180 mm, corresponding to target strengths of –34, –28 and –22 dB, respectively, as measured by a short pulse produced by excitation of a 60 mm spherical piezoceramic transducer with 10 μs rectangular pulses (Supin et al., 2004). AEP audiograms for this animal collected in 2001 and 2005 showed that her hearing began to rapidly decline above 40 kHz (Ibsen et al., 2009). However, the animal was still able to accept echoes with a performance level over 95% (Ibsen et al., 2009).

Experimental facilities and setup for data recording during echolocation
The experimental facilities and setup are shown in Fig. 1. The experimental enclosure consisted of two parts: the experimental pen and the target section. The experimental pen was an $8 \times 10 \, m^2$ floating pen frame that had a wire-net bottom and was used to house the experimental subject. The target pen was $6 \times 8 \, m^2$ in size, which served only to mount targets and did not bear wire-net to avoid the production of extra confounding echoes from the wire during echolocation. In the net divider separating the two parts of the experimental enclosure, there was an opening bounded by a 40 cm diameter hoop (1 in Fig. 1), which served as a hoop station for the animal. In front of the hoop, a Reson TC4013 hydrophone (Reson, Slangerup, Denmark; 2 in Fig. 1) was mounted on a beam at a horizontal distance of 1.6 m to the hoop (Fig. 2) to record sounds emitted by the animal during her positioning in the hoop station. A target (3 in Fig. 1) was hung from a thin monofilament line in the target section with a horizontal distance of 2.15, 3.65 or 6.65 m to the hoop, corresponding to a distance of approximately 2, 3.5 or 6.5 m, respectively, to the nasal sacs (the echolocation click generator) of the echolocating animal (Fig. 2). The target could be pulled up out of the water and lowered into the water. The hoop station, the recording hydrophone and the lowered target were all in a longitudinal straight line at approximately the same depth (approximately 80 cm). Between the recording hydrophone and hoop station there was a movable acoustic screen (4 in Fig. 1, AS in Fig. 2) and a fixed visual screen (5 in Fig. 1, VS in Fig. 2). The acoustic screen was made of 3.2 mm aluminum and was used to interfere with the dolphin's ability to echolocate into the target section prematurely. During a trial, the screen was lowered, which opened the space in front of her for echolocation. The visual screen was made of thin acoustically transparent plastic material and was placed behind the
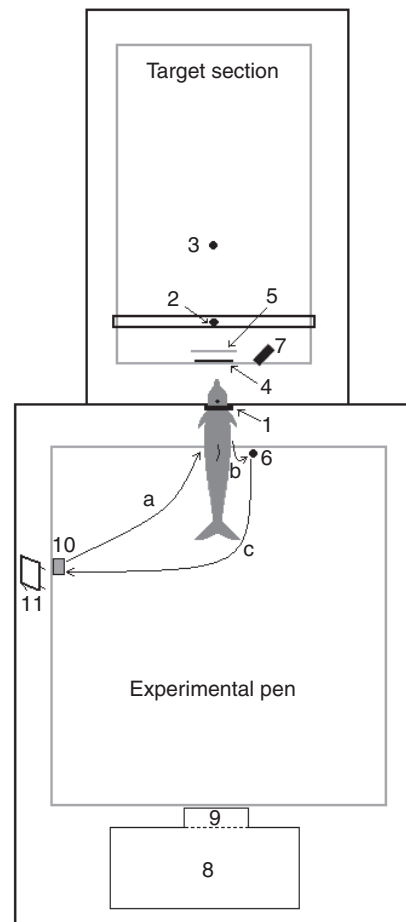

Fig. 1. Experimental facilities and setup (top view). 1, hoop station; 2, recording hydrophone; 3, target during echolocation sessions or transducer during AEP recording to external dolphin-like clicks; 4, acoustic screen; 5, visual screen; 6, response ball; 7, video camera; 8, instrument shack; 9, window; 10, stationing pad; 11, trainer position. Traces: a, the animal swam to the hoop station from the stationing pad; b, the animal got out of the hoop station to touch the response ball to report that the target was present; c, the animal swam back to the stationing pad.

acoustic screen to prevent visual cues from the target section but allow unobstructed acoustical access. Near the hoop station, a response ball (6 in Fig. 1) was mounted above the water surface; this served as a target-present response indicator. The animal position in the station hoop was monitored using an underwater video camera (7 in Fig. 1) by both the experimenter in an instrument shack (8 in Fig. 1) and the trainer (position 11 in Fig. 1) to give instructions to the animal and to reward her with fish for correct responses. When not in the hoop, the animal stationed at a foam stationing pad (10 in Fig. 1) to await instructions from the trainer. The experimenter and the trainer communicated during experimental sessions through a window (9 in Fig. 1) in the instrument shack.

Experimental procedure, equipment and data collection
Data recording during echolocation
Each experimental session was typically conducted in the morning when fish was first offered, and one session was conducted per day. Each session consisted of 50 trials, 25 target-present and 25 target-absent, randomly alternated.
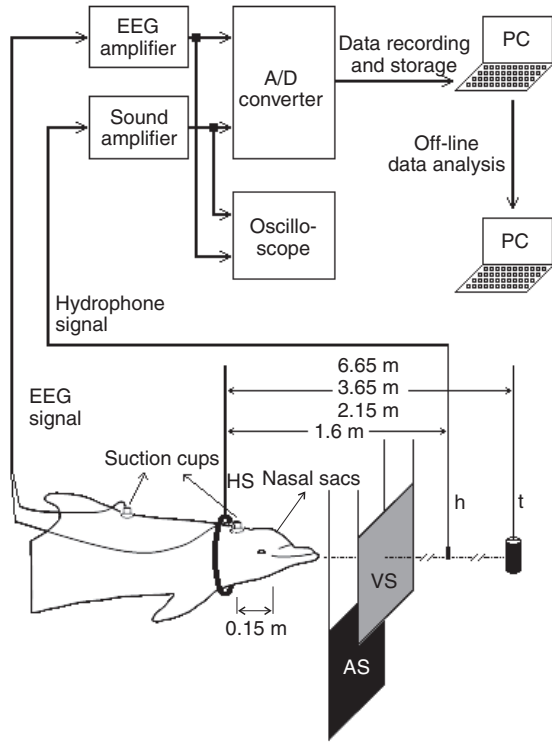
Fig. 2. Schematic of the dolphin's relative position, data recording equipment and flow chart. AS, acoustic screen; h, recording hydrophone; HS, hoop station; PC, laptop computer; t, target during echolocation sessions or transducer during AEP recording to external dolphin-like clicks; VS, visual screen.

Each session began with the trainer attaching suction-cup electrodes to the dolphin for AEP recording. Each trial was initiated with a hand signal to cue the animal to swim from the stationing pad to the hoop along trace 'a' in Fig. 1. During the time when the animal was positioning herself in the hoop, the acoustic screen was up and screened the target from the animal. The target was either lowered into the water for target-present trials or pulled up out of the water for target-absent trials. As soon as the subject took the position in the hoop station, the acoustic screen was lowered, thus opening the space in front of her and cueing her to begin echolocation. The screen remained in the lower position for 3 s and was then raised back. The animal typically emitted 20 to 40 echolocation clicks immediately after the acoustic screen was lowered down. The echolocation clicks and click-triggered AEPs were recorded by the click- and AEP-acquisition system (see details below) operated by the experimenter in the instrument shack. With the go/no-go paradigm, the go response was required when the target was present and the no-go response was required when the target was absent. For the go response, the animal was required to back out of the hoop and follow trace 'b' in Fig. 1 to touch the response ball with her rostrum. The animal received a bridge whistle if her response was correct. The subject would then follow trace 'c' in Fig. 1 back to the stationing pad, receive the fish reward and wait for the hand signal to begin the next trial. For the no-go response, instead of touching the response ball, the animal was required to stay in the hoop for additional 3 s after the acoustic screen was raised back. If the response was correct, she received a bridge whistle and was required to follow trace 'c' in Fig. 1 back to the stationing pad,

where she received her fish reward and waited for the hand signal to begin the next trial. If the dolphin was incorrect in either a go or no-go trial, she was not given a fish reward and was required to return to the stationing pad waiting for the next trial.

The data recording equipment and flow chart are presented in Fig. 2. AEP responses were picked up by EEG electrodes (Grass Technologies, West Warwick, RI, USA), gold-plated disks 10 mm in diameter mounted within rubber suction cups 60 mm in diameter. The recording electrode was attached with conductive gel to the dorsal head surface, located midline, approximately 5–7 cm behind the blowhole. The reference electrode was also attached along with conductive gel to the animal's back near the dorsal fin. AEP responses were led by shielded cables to an EEG amplifier (Grass CP511 AC Amplifier, Grass Technologies) and amplified 20,000 times within a frequency band of 300 to 3000 Hz. The amplified signal was monitored by an oscilloscope (Fluke 196C ScopeMeter, Fluke Corporation, Everett, WA, USA) and input to a 16 bit analog-to-digital converter of a data acquisition card (DAQmx USB-6251, National Instruments, Austin, TX, USA) that was connected to and installed on a standard laptop computer. Signals from the click-recording hydrophone were input to a sound amplifier (Krohn-Hite Model 3362 filter, Krohn-Hite Corporation, Brockton, MA, USA) and amplified by 20 dB within a frequency range of 1 to 200 kHz, monitored by the same oscilloscope, and led to another analog-to-digital converter of the same data acquisition card. Sampling rates were 25 kHz for the EEG-recording channel and 500 kHz for the click-recording channel.

The data-collection process was controlled by the experimenter with a custom-made program designed using LabVIEW software (National Instruments). The program continuously monitored the click-recording input, and each time the signal exceeded a predetermined triggering level (approximately 157 dB re. 1 μPa peak-to-peak), a 15 ms window of the EEG-recording channel and a 0.1 ms window of the click-recording channel were recorded and stored in the memory of the laptop computer for off-line analysis. The click-recording window included 0.02 ms pretrigger time.

AEP recording to an external dolphin-like click

For comparison, the AEP responses to external dolphin-like clicks were also recorded and analyzed. The experimental facilities and setup were same as in the echolocation sessions (Fig. 1), except that the target was replaced by an ITC-1032 spherical transducer (International Transducer Corporation, Santa Barbara, CA, USA) (3 in Fig. 1) at a distance of 2.15 m to the hoop, and the hydrophone and response ball were removed.

For this experiment, the animal was sent to the same hoop station after having the suction-cup electrodes attached for AEP recording by the trainer. As soon as she took the proper position, external dolphin-like clicks were played through the transducer, and AEPs to these click stimuli were collected. After that, the animal was called back to the trainer for a fish reward. External dolphin-like click stimuli were digitally generated by the same National Instruments data acquisition card and played through a 16 bit digital-to-analog converter, amplified by a power amplifier (Hewlett-Packard Agilent 465A, Palo Alto, CA, USA) and projected by the ITC-1032 spherical transducer. The click stimuli were produced with activation of the transducer by short rectangular pulses. Pilot measurements showed that the projected external clicks (Fig. 3A,B) were similar to typical echolocation clicks (Fig. 3C,D) of the experimental subject in both waveform and spectrum.
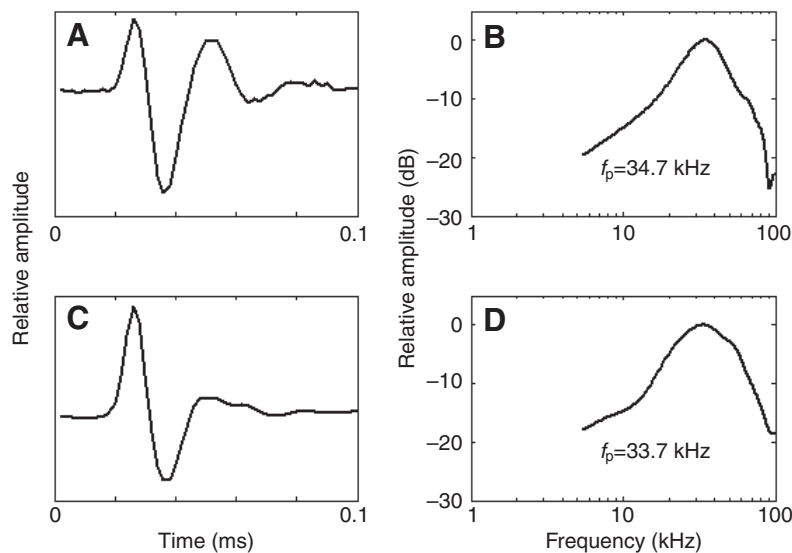
Fig. 3. Comparison of external dolphin-like clicks with the dolphin's echolocation click. (A) Waveform and (B) spectrum of an external dolphin-like click; (C) waveform and (D) spectrum of the subject's typical echolocation click. Note that the external dolphin-like click is very similar to the typical echolocation click of the experimental subject in both waveform and spectrum. f_p, peak frequency.

The equipment for AEP collection included the same electrodes, amplifier and data acquisition card as for data recording during echolocation. The amplifier gain, bandpass and recording window for AEP recording were also same: 20,000 times, 300–3000 Hz and 15 ms, respectively. Unlike the AEP collection during echolocation, the card was programmed for on-line averaging to extract AEPs from background noise. The averaging was triggered by the external dolphin-like clicks presented at a rate of 20 clicks s^{-1}. AEPs were collected by averaging 1000 individual records.

Data analysis
Analysis of AEP response during echolocation
To extract low-amplitude AEPs from background noise, an off-line excluding and averaging procedure was used [described in Supin et al. (Supin et al., 2004)]. In spite of averaging one to several thousand individual records, the averaged AEP record was still contaminated by some low-frequency artifacts. In particular, the record began with a wave without any latency; this was considered an artifact, probably provoked by the recording systems. To reject these artifacts, the first 15 sampling points of the AEP record (0.6 ms in the time scale) were deleted, and a high-pass zero-phase shift digital filtering with a cut-off frequency of 800 Hz was used for the rest of the record. This process slightly changed the AEP waveform and reduced the lowest-frequency AEP components but was considered as acceptable for comparison of responses analyzed in the same way (Supin et al., 2004).

Analysis of AEP response to external dolphin-like clicks
For comparison, the artifacts in the on-line averaged AEP response to external dolphin-like clicks were filtered in the same way as the AEP recording during echolocation, i.e. the first 15 sampling points in the AEP record were deleted, and then the rest of the record was filtered by using the same high-pass zero-phase shift digital filtering with a cut-off frequency of 800 Hz.

RESULTS
Echolocation clicks
The peak frequency of the typical echolocation click of this subject is between 30 and 40 kHz (Fig. 3D). The click levels recorded at the position of the hydrophone (approximately 1.45 m to the nasal

sacs, Fig. 2) are within a range of 165 to 215 dB re. 1 μPa peak-to-peak. The histograms of the click levels are presented in Fig. 4 for different target strengths and target distances. Mean values (±s.d.) of the click levels and number of clicks analyzed are indicated in the upper left corner of each panel. The data in Fig. 4 show that the histogram apices of the outgoing click levels moved gradually rightward as target distance changed from 2 to 6.5 m or target strengths changed from –22 to –34 dB, i.e. the animal was inclined to produce louder echolocation clicks to detect further or smaller targets. The averaged values of the click levels as a function of target distance are presented in Fig. 5 for all the investigated targets with different target strengths. A regression curve and its equation and correlation coefficient are also shown for each target along the target distances. The relationship between mean click level and target distance indicates that the mean click levels increased by a factor of 9.98, 11.46 and 17.51 log$R$ (where R is target distance) with high r^2 values for the targets with target strengths of –22, –28 and –34 dB, respectively.

AEP records
The dolphin's AEP response to the external dolphin-like clicks (Fig. 3A,B), after off-line digital filtering, is presented in Fig. 6A. The AEP record is presented in such a way that the zero point of the time scale corresponds to the expected time point when the external dolphin-like clicks arrive at the 'acoustic window' located at the lower jaw of the subject, where the sounds were assumed to travel to the inner ear (Norris, 1968). The complex of AEPs to the external dolphin-like clicks consists of alternative positive and negative short waves, each shorter than 1 ms, and is characterized by a negative-positive-negative-positive-negative pattern (negativity of the active electrode is upward).

Data from eight to 10 experimental echolocation sessions were collected for each target type and target distance. The echolocation performance of the animal during the experimental sessions remained high. Her overall correct performance level remained over 98%. An example of AEP records during echolocation in the target-absent situation (after off-line excluding, averaging and high-pass digital filtering) is presented in Fig. 6B. The zero point of the time scale corresponds to the expected time point when the outgoing echolocation clicks arrive at the 'acoustic window'. AEP records during echolocation at target-present situation, after off-line
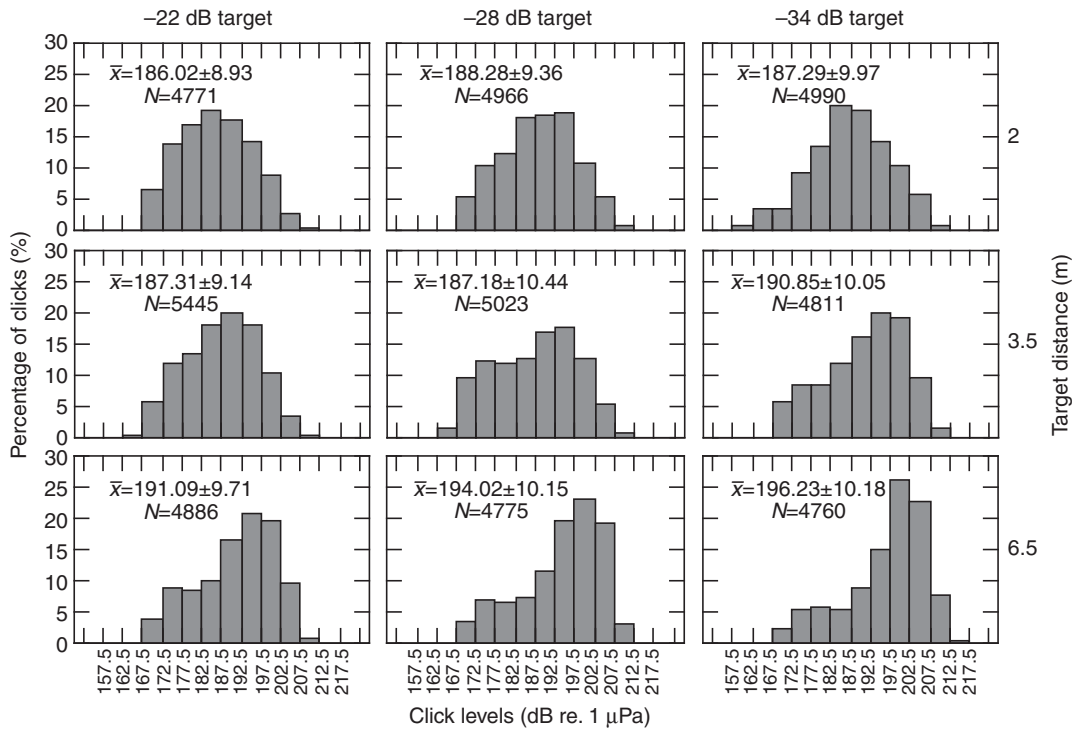
Fig. 4. Outgoing click levels (recorded by the hydrophone at a distance of approximately 1.45 m from the nasal sacs of the subject) for different targets and at different target distances. $\bar{x}$, mean ± s.d. of the click levels; N, number of collected clicks.

excluding, averaging and high-pass digital filtering, obtained for different target strengths are shown in Fig. 7. Fig. 7A presents AEP records after averaging all of the collected AEP sweeps. Fig. 7B presents AEP records after averaging only the AEP sweeps with outgoing click levels between 190 and 200 dB re. 1 μPa peak-to-peak recorded by the hydrophone. The three traces in each panel present AEP records at target distances of 6.5, 3.5 and 2 m from the nasal sacs of the animal. Mean levels of the outgoing clicks that correspond to the AEP sweeps for averaging, as well as the number of AEP sweeps used for averaging, are indicated. The zero point of the time scale in Fig. 7 corresponds to the time point when the outgoing echolocation clicks were picked up by the hydrophone and the AEP recordings were triggered.

One consistent feature of all AEP records during echolocation is the presence of a stereotyped prominent AEP complex located between 1.5 and 3.5 ms after the received echolocation click trigger and independent of target strength, distance and presence/absence. This prominent AEP complex consists of several alternative positive and negative waves (each shorter than 1 ms), the same as the complex of AEPs to the external dolphin-like clicks, characterized by a negative-positive-negative-positive-negative pattern. When the targets were present, all the AEP records contain a second AEP complex consisting of alternative positive and negative waves (Fig. 7), following after the prominent AEP complex with time latency proportional to the target distance. The second AEP complex is characterized by positive-negative-positive-negative waves that are relatively smaller and simpler than the prominent first AEP complex in their wave forms. The negative peak waves of the second AEP complex are designated by arrows in Fig. 7. The time latency of the second complex is plotted as a function of the target distance in Fig. 8. The solid line in Fig. 8 is a linear regression line fitting the data from all the target strengths with a high r^2 value. When the target was absent, no second AEP complex was identified (Fig. 6B).

DISCUSSION
Origin of the AEP complexes
The AEP response of the subject to the external dolphin-like clicks is very similar to those recorded and described previously in the same species, *T. truncatus* (Møhl et al., 1999; Ridgway et al., 1981; Supin et al., 2001), and even in different species, such as the false killer whale, *P. crassidens* (Supin et al., 2003). The data in Fig. 6 indicate that after positioning of the AEP records on the time scale, the AEP complex recorded during the target-absent situation matches the AEP complex to external dolphin-like clicks very well in both waveform shape and time. They both consist of a couple of alternative positive-negative short waves. Comparison of the AEP recordings between the target-present and -absent trials indicates that in both situations there is a stereotyped prominent AEP complex located between 1.5 and 3.5 ms after the recordings were triggered; however, only when the target was present was
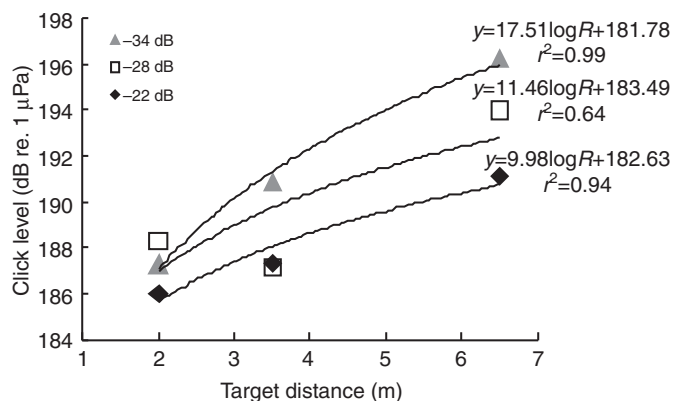

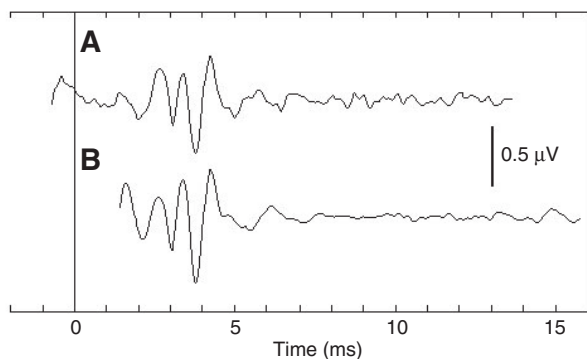Fig. 5. Dependence of mean outgoing click levels on target distance.

Fig. 6. (A) AEP response to external dolphin-like clicks and (B) AEP record during echolocation in the target-absent situation. The zero point of the time scale corresponds to the expected time point when the external dolphin-like clicks or outgoing echolocation clicks arrive at the 'acoustic window' located at the lower jaw of the subject, where the sounds were assumed to travel to the inner ear. Note the match in both waveform shape and time for the AEP complexes shown in A and B.

there a second relatively smaller and simpler AEP complex consisting of alternative positive and negative waves with a time latency proportional to the target distance (Figs 7, 8). AEP records under the target-absent situation exhibit a slow raised wave following the prominent AEP complex (Fig. 6B); however, this raised wave is not characterized by a typical AEP waveform, which consists of alternative positive and negative waves (Supin et al., 2001), and could be a following response of the prominent AEP complex (Supin et al., 2001). The regression equation with a high r^2 value in Fig. 8 indicates that the time latencies of the second AEP complex correspond to the two-way distances between the subject's nasal sacs and the targets. This is supported by the following considerations: (1) the underwater sound velocity is

approximately $1500 \, \text{m s}^{-1}$, at which velocity the factor of $1.33 \, \text{ms m}^{-1}$ corresponds to the double-way delay *versus* distance; (2) the distance between the hoop station and the subject's nasal sacs is approximately 0.15 m; (3) the relative position of the subject to the hoop station could change minutely during echolocation; and (4) the exact position where the echolocation clicks and corresponding echoes travel to the inner ear is actually uncertain. The constant –0.01 in the regression equation implies that the real distance between the hoop station and the subject's nasal sacs is a little bit longer than 0.15 m. The above results confirmed that the second relatively smaller and simpler AEP complex under the target-present situation is an AEP response to the echo sound, and the prominent AEP complex under both the target-present and -absent situations is an AEP response to the outgoing click. We can therefore conclude that, when the target was present, the dolphin responded to both outgoing echolocation clicks and echoes with two definite AEP complexes, the same as the previously investigated false killer whale (Supin et al., 2003; Supin et al., 2004; Supin et al., 2005; Supin et al., 2010). The first AEP complex is outgoing click-related AEP, and the second is echo-related AEP. When the target was absent, she responded to only the outgoing echolocation clicks, as there were no echoes.

Dependence of AEP amplitude on target distance

As shown in Fig. 7A, the echo-related AEP amplitudes are obviously lower than the outgoing click-related AEP amplitudes for all the investigated targets and distances. However, the echo-related AEP amplitude tends to increase with target distance, especially for the targets with target strengths of –22 and –28 dB. The increase of echo-related AEP amplitude could either be attributed to the increasing mean levels of outgoing echolocation clicks, which increase by approximately 5–9 dB within the target distance of 2–6.5 m depending on different targets (Figs 4, 5), or be contributed by the hearing system with some sort of hearing gain control
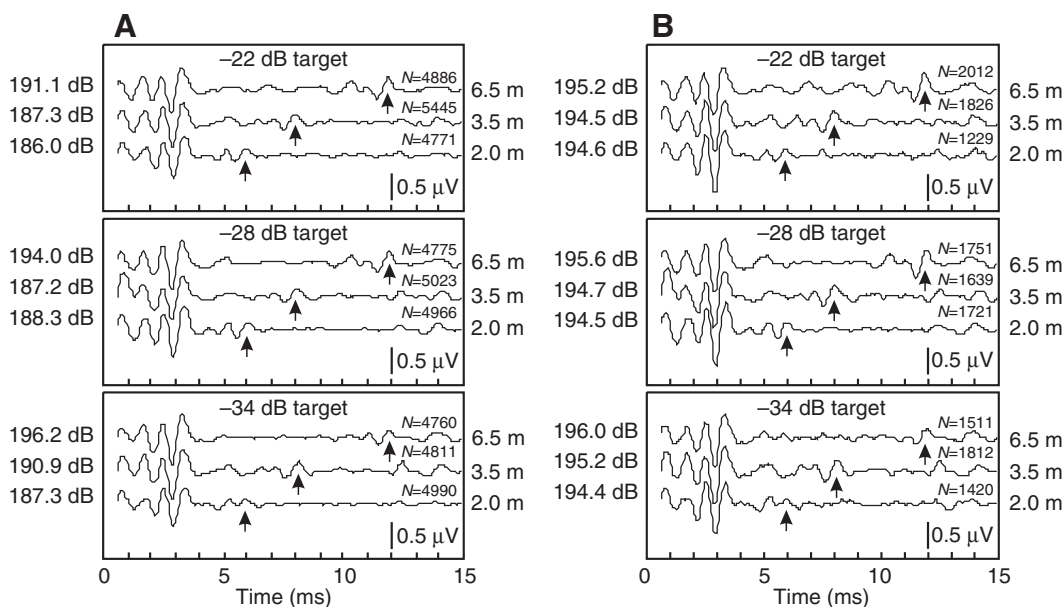

Fig. 7. AEP records during echolocation in the target-present situation (A) after averaging all of the collected AEP sweeps for each target at a distance of 2–6.5 m and (B) after averaging only the AEP sweeps with outgoing click levels between 190 and 200 dB re. 1 µPa peak-to-peak recorded by the hydrophone for each target at a distance of 2–6.5 m. Mean levels of outgoing clicks (dB re. 1 µPa peak-to-peak), number (N) of AEP sweeps used for averaging and target distance (m) are indicated for each AEP record trace. The zero point of the time scale corresponds to the time point when the hydrophone picked up the clicks and triggered the AEP recordings.
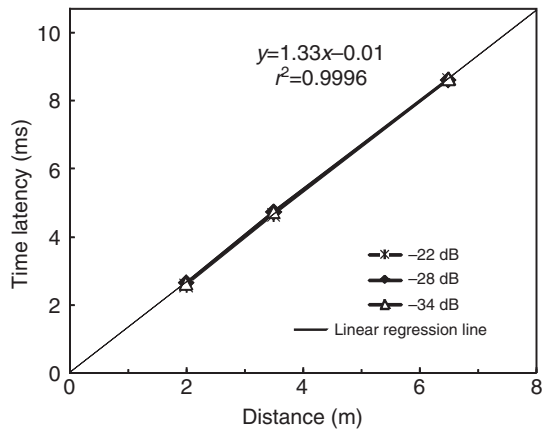
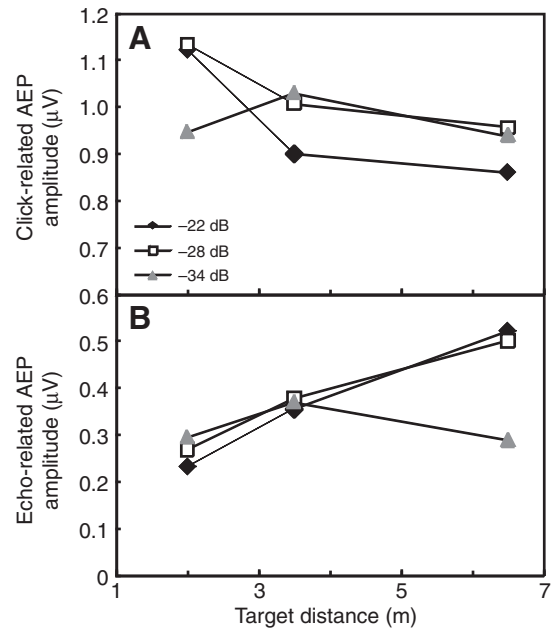Fig. 8. Dependence of the echo-related AEP time latency on target distance.

Fig. 9. Dependence of (A) outgoing click-related AEP amplitude (peak-to-peak) and (B) echo-related AEP amplitude (peak-to-peak) on target distance.

mechanisms, or both. To eliminate the potential effect of click levels on AEP amplitudes, the AEPs were extracted from only those echolocation clicks with levels between 190 and 200 dB re. 1 μPa peak-to-peak (Fig. 7B). As shown in Fig. 7B, the mean levels of echolocation clicks are all approximately 195 dB for all targets and distances. However, the increased tendency of the echo-related AEP amplitudes with target distance is even more distinct for the targets with target strengths of –22 and –28 dB. Measurements of peak-to-peak AEP amplitudes of the AEP records shown in Fig. 7B are presented in Fig. 9 and indicate that: (1) the outgoing click-related AEP amplitudes decreased with target distances for the targets with target strengths of –22 and –28 dB (least-squares linear regression, r^2=0.70 and 0.82, respectively); (2) for the smallest target, with a target strength of –34 dB, the outgoing click-related AEP amplitude increased with target distance from 2 to 3.5 m, and then decreased with distance from 3.5 to 6.5 m; (3) the echo-related AEP amplitudes increased linearly with target distances for the targets with target strengths of –22 and –28 dB (least-squares linear regression, r^2=0.99 and 0.98, respectively); (4) for the smallest target, with a target strength of –34 dB, the echo-related AEP amplitude increased with distance from 2 to 3.5 m, and then decreased with distance from 3.5 to 6.5 m. These results suggest that both outgoing click-related AEP and echo-related AEP amplitudes were dependent on target distances without the potential effect of click levels, at least for the investigated targets with target strengths of –22 and –28 dB. For the –34 dB target, the relatively low outgoing click-related AEP amplitude at 2 m and echo-related AEP amplitude at 6.5 m (Fig. 9) may be alternatively explained by one of two possibilities: (1) the target strength is too small for the animal to evoke a higher AEP response or (2) the corresponding AEP complexes were actually contaminated by the background noise. The latter is perhaps more likely, as the AEP response amplitudes are usually lower than 1 μV peak-to-peak and relatively low compared with the comparable background noise (Fig. 7B).

Implications for sonar overcompensation of echo attenuation
In bat biosonar, an aerial analog of odontocete biosonar, both the sound production and auditory reception systems have been demonstrated to be highly controllable and flexible (Hartley, 1992a; Schnitzler and Henson, 1980). The perceived echo intensities were stabilized during target approach by either adjusting auditory sensitivity in the form of automatic gain control (AGC) with an increase of 11 to 12 dB per doubling of target distance (Kick and Simmons, 1984; Simmons et al., 1992) or adjusting (increasing) both sound intensity in the transmission phase by 6 dB per doubling of target distance and auditory sensitivity AGC in the receiving phase by 6–7 dB per doubling of target distance as a dual-component, symmetrical sonar control system (Hartley, 1992a; Hartley, 1992b).

In odontocetes, data from field experiments have shown that they do possess a transmission AGC mechanism, i.e. intensity compensation in the transmission phase of a sonar cycle (Au and Benoit-Bird, 2003). The intensity of their echolocation clicks increased roughly at a rate of 6 dB per doubling of target distance (Au and Benoit-Bird, 2003; Au and Herzing, 2003; Au and Würsig, 2004; Au et al., 2004; Jensen et al., 2009; Li et al., 2006; Rasmussen et al., 2002). The intensity increase of 6 dB per doubling of target distance may compensate for the echo attenuation to some extent. However, this compensation may be not complete because the echo attenuation of 6–12 dB per doubling of target distance should occur as the animal approaches a target, depending on the target size. Recently, an AEP study during echolocation in a false killer whale demonstrated that the echo-related AEP amplitudes were independent of target distance (Nachtigall and Supin, 2008; Supin et al., 2004; Supin et al., 2005; Supin et al., 2009). These results indicated that compensation mechanism in auditory sensitivity to stabilize perceived echo intensities existed in the false killer whale, possibly as a consequence of forward masking of the weak returning echo by the powerful outgoing click (Supin et al., 2008; Supin et al., 2009). However, whether the odontocetes control both sound transmission and reception synchronously in their biosonar cycle, as observed in bats, has yet to be documented.

In the present study, the echo-related AEP amplitudes of the dolphin increased linearly with target distance for the targets with target strengths of –22 and –28 dB, demonstrating that the perceived echo intensities were not only stabilized but increasing with increasing target distance, and the echo attenuation with target distance was not only compensated but overcompensated. This

overcompensation of echo attenuation could even be accomplished by auditory sensitivity compensation alone without the potential effect of outgoing click levels on AEP amplitudes (Fig. 7B, Fig. 9B). The motivation and mechanisms are still open to investigation. However, it should be noted that the outgoing click-related AEP amplitudes tend to decrease with target distance (Fig. 9A). For the target with a target strength of -34 dB, the echo-related AEP amplitude decreased with an increase in target distance from 3.5 to 6.5 m. A possible explanation for this is that the overcompensation mechanism of echo attenuation could be restrainable by determinate target strength and target distance. Alternatively, the low echo-related AEP amplitude for the -34 dB target at 6.5 m might have been contaminated by the background noise.

Considering that the click levels also increased with target distance by a factor of approximately 10 to $17.5 \log R$ (i.e. 3–5 dB increase per doubling of target distance, slightly lower than previous data from wild animals) depending on target strength, the subject in the present study demonstrated a dual-component sonar control system formed by intensity compensation behavior in both the transmission and receiving phases of a sonar cycle at the investigated target distances. This dual-component sonar control system as a whole functions not only to stabilize the perceived echo intensity as observed in bat biosonar, but also to increase the perceived echo intensity with target distance, at least for the present targets with target strengths of -22 and -28 dB.

CONCLUSIONS

The results presented in this paper demonstrated that another species (*T. truncatus*) besides the false killer whale (*P. crassidens*) responds to both outgoing clicks and their echoes with two definite AEP complexes during echolocation. The echo-related AEP amplitudes of the dolphin were obviously lower than the outgoing click-related AEP amplitudes. However, for the targets with target strengths of -22 and -28 dB, the peak-to-peak amplitudes of the echo-related AEPs were dependent on the target distance. The echo-related AEP response amplitudes increased with distance, demonstrating sonar overcompensation of echo attenuation with target distance. This overcompensation of echo attenuation could even be accomplished by auditory sensitivity compensation alone without the potential effect of outgoing click levels on AEP amplitudes. These results appear different than those reported previously in a false killer whale, in which a distance-independent invariant and comparable echo-related AEP amplitude relative to the outgoing click-related AEP amplitude was documented (Supin et al., 2003; Supin et al., 2004; Supin et al., 2005; Supin et al., 2009). The differences might be explained as interspecific differences or a difference in the individual animals' echolocation behavior. The present subject, an Atlantic bottlenose dolphin, is different or potentially different than the false killer whale with respect to click characteristics, sound production behavior, anatomic shape of the head and hearing capability, etc. We assumed that at least some of these differences would account for the different observations of the AEP response during echolocation between the two animals. Alternatively, because only a single individual of each species was investigated in both cases, individual differences may not be rejected.

The present results also demonstrated that the dolphin controlled both sound transmission and reception synchronously in a biosonar cycle within the investigated target distance, to not only compensate but overcompensate the echo attenuation with target distance for the targets with certain target strengths.

REFERENCES

Au, W. W. L. (1993). *The Sonar of Dolphins*. New York: Springer-Verlag.

Au, W. W. L. and Benoit-Bird, K. J. (2003). Automatic gain control in the echolocation system of dolphins. *Nature* **423**, 861-863.

Au, W. W. L. and Herzing, D. L. (2003). Echolocation signals of wild Atlantic spotted dolphin (*Stenella frontalis*). *J. Acoust. Soc. Am.* **113**, 598-604.

Au, W. W. L. and Würsig, B. (2004). Echolocation signals of dusky dolphins (*Lagenorhynchus obscurus*) in Kaikoura, New Zealand. *J. Acoust. Soc. Am.* **115**, 2307-2313.

Au, W. W. L., Popper, A. N. and Fay, R. (2000). *Hearing by Whales and Dolphins*. New York: Springer-Verlag.

Au, W. W. L., Ford, J. K. B., Horne, J. K. and Allman, K. A. N. (2004). Echolocation signals of free-ranging killer whales (*Orcinus orca*) and modelling of foraging for chinook salmon (*Oncorhynchus tshawytscha*). *J. Acoust. Soc. Am.* **115**, 901-909.

Bullock, T. H. and Ridgway, S. H. (1972). Evoked potentials in the central auditory system of alert porpoises to their own and artificial sounds. *J. Neurobiol.* **3**, 79-99.

Hartley, D. J. (1992a). Stabilization of perceived echo amplitudes in echolocating bats. II. The acoustic behavior of the big brown bat, *Eptesicus fuscus*, when tracking moving prey. *J. Acoust. Soc. Am.* **91**, 1133-1149.

Hartley, D. J. (1992b). Stabilization of perceived echo amplitudes in echolocating bats. I. Echo detection and automatic gain control in the big brown bat, *Eptesicus fuscus*, and the fishing bat, *Noctilio leporinus*. *J. Acoust. Soc. Am.* **91**, 1120-1132.

Ibsen, S., Au, W., Nachtigall, P. and Breese, M. (2009). Functional bandwidth of an echolocating Atlantic bottlenose dolphin (*Tursiops truncatus*). *J. Acoust. Soc. Am.* **125**, 1214-1221.

Jensen, F. H., Bejder, L., Wahlberg, M. and Madsen, P. T. (2009). Biosonar adjustments to target range of echolocating bottlenose dolphins (*Tursiops* sp.) in the wild. *J. Exp. Biol.* **212**, 1078-1086.

Kick, S. A. and Simmons, J. A. (1984). Automatic gain control in the bat's sonar receiver and the neuroethology of echolocation. *J. Neurosci.* **4**, 2725-2737.

Møhl, B., Au, W. W. L., Pawloski, J. and Nachtigall, P. E. (1999). Dolphin hearing: relative sensitivity as a function of point of application of a contact sound source in the jaw and head region. *J. Acoust. Soc. Am.* **105**, 3421-3424.

Nachtigall, P. E. and Moore, P. W. B. (1988). *Animal Sonar: Processes and Performance*. New York: Plenum.

Nachtigall, P. E. and Supin, A. Y. (2008). A false killer whale adjusts its hearing when it echolocates. *J. Exp. Biol.* **211**, 1714-1718.

Nachtigall, P. E., Mooney, T. A., Taylor, K. A. and Yuen, M. M. L. (2007). Hearing and auditory evoked potential methods applied to odontocete cetaceans. *Aquat. Mamm.* **33**, 6-13.

Norris, K. S. (1968). The echolocation of marine mammals. In *The Biology of Marine Mammals* (ed. H. T. Andersen), pp. 391-423. New York: Academic Press.

Johnson, S. C. (1967). Sound detection thresholds in marine mammals. In *Marine Bio-acoustics* (ed. W. Talvoga), pp. 247-260. New York: Pergamon Press.

Li, S., Wang, D., Wang, K. and Akamatsu, T. (2006). Sonar gain control in echolocating finless porpoises (*Neophocaena phocaenoides*) in an open water. *J. Acoust. Soc. Am.* **120**, 1803-1806.

Rasmussen, M. H., Miller, L. A. and Au, W. W. L. (2002). Source levels of clicks from free-ranging white beaked dolphins (*Lagenorhinchus albirostris* Gray 1846) recorded in Icelandic waters. *J. Acoust. Soc. Am.* **111**, 1122-1125.

Richardson, W. J., Greene, C. R., Jr, Malme, C. I. and Thomson, D. H. (1995). *Marine Mammals and Noise*. San Diego, CA: Academic Press.

Ridgway, S. H., Bullock, T. H., Carder, D. A., Seeley, R. L., Woods, D. and Galambos, R. (1981). Auditory brainstem response in dolphins. *Proc. Natl. Acad. Sci. USA* **78**, 1943-1947.

Schnitzler, H. U. and Henson, O. W., Jr (1980). Performance of airborne animal sonar systems. In *Animal Sonar Systems* (ed. R. G. Busnel and J. F. Fish), pp. 109-181. New York: Plenum Press.

Schusterman, R. J. (1980). Behavioral methodology in echolocation by marine mammals. In *Animal Sonar Systems* (ed. R. G. Busnel and J. F. Fish), pp. 11-41. New York: Plenum Press.

Simmons, J. A., Moffat, A. J. M. and Masters, W. M. (1992). Sonar gain control and echo detection thresholds in the echolocating bat, *Eptesicus fuscus*. *J. Acoust. Soc. Am.* **91**, 1150-1163.

Supin, A. Y., Popov, V. V. and Mass, A. M. (2001). *The Sensory Physiology of Aquatic Mammals*. Boston, MA: Kluwer.

Supin, A. Y., Nachtigall, P. E., Pawloski, J. and Au, W. W. L. (2003). Evoked potential recording during echolocation in a false killer whale *Pseudorca crassidens*. *J. Acoust. Soc. Am.* **113**, 2408-2411.

Supin, A. Y., Nachtigall, P. E., Au, W. W. L. and Breese, M. (2004). The interaction of outgoing echolocation pulses and echoes in the false killer whale's auditory system: evoked-potential study. *J. Acoust. Soc. Am.* **115**, 3218-3225.

Supin, A. Y., Nachtigall, P. E., Au, W. W. L. and Breese, M. (2005). Invariance of evoked-potential echo-responses to target strength and distance in an echolocating false killer whale. *J. Acoust. Soc. Am.* **117**, 3928-3935.

Supin, A. Y., Nachtigall, P. E. and Breese, M. (2008). Forward masking as a mechanism of automatic gain control in odontocete biosonar: a psychophysical study. *J. Acoust. Soc. Am.* **124**, 648-656.

Supin, A. Y., Nachtigall, P. E. and Breese, M. (2009). Forward-masking based gain control in odontocete biosonar: an evoked-potential study. *J. Acoust. Soc. Am.* **125**, 2432-2442.

Supin, A. Y., Nachtigall, P. E. and Breese, M. (2010). Target distance-dependent variation of hearing sensitivity during echolocation in a false killer whale. *J. Acoust. Soc. Am.* **127**, 3830-3836.