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Investigating Auditory Brainstem Response Correlates of Basilar Membrane Nonlinearities in Dolphins

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ADMINISTRATIVE INFORMATION

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EXECUTIVE SUMMARY

Like terrestrial mammals, dolphins possess a sophisticated auditory system consisting of an outer, middle, and inner ear. The inner ear contains the cochlea, a spiral shaped, fluid-filled cavity containing the basilar membrane (BM), which supports sensitive mechanoelectric hair cells. There are two types of hair cells: The inner hair cells (IHCs) convert fluid motion in the cochlea to electrical nerve impulses which are transmitted to the brain, ultimately giving rise to the sensation of hearing. In contrast, the outer hair cells (OHCs) respond to electrochemical signals by changing their shape, which enhances and sharpens the BM response at the OHC's location. In this way, OHCs act as a nonlinear, compressive amplifier to enhance hearing sensitivity and frequency selectivity. Loss of OHC function results in a linear BM response, hearing loss, and reduced frequency selectivity. Assessment of OHC function is therefore important for identifying hearing loss and the underlying mechanisms. Proper OHC function results in high sensitivity and a nonlinear BM response; the presence of BM nonlinearities can therefore be used as an indicator of normal OHC function.

This report describes a series of experiments to test the feasibility of using auditory brainstem response (ABR) measurements to assess BM nonlinearities (and thus OHC function) in dolphins. ABRs are small voltages generated by the brain and auditory nervous system when an animal hears a sound. They can be noninvasively measured in dolphins using the same techniques utilized for newborn hearing screening. In the present study, ABRs were measured alone and in the presence of masking noise signals designed to reveal compressive nonlinearities in the BM response. Across various experiments, ABR magnitude-level functions were derived as sound stimulus features (such as frequency, amplitude, and time separation) were manipulated.

The results showed no evidence of compressive nonlinearities in any of the measurements. The most likely reasons involve the high ambient noise (for underwater testing) and the relatively high signal levels. The high ambient noise likely affected the masking paradigms, since there would be no quiet interval between the masker and hearing test signal. The high signal levels may have obscured differences between the masking conditions. High-level signals could also result in a linear, rather than compressive, BM response (i.e., signal levels above the range of compression).

Future testing should be conducted in a quiet underwater environment, such as a pool, with young animals with normal hearing. To assess whether ABR magnitude-level functions can be reliably used to directly assess OHC function, magnitude-level functions should be assessed at multiple frequencies from a larger sample of dolphins with normal hearing and hearing loss.

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ACRONYMS

ABR	auditory brainstem response
AEP	auditory evoked potential
ASSR	auditory steady-state response
BM	basilar membrane
CF	characteristic frequency
EEG	electroencephalogram
HI	hearing impaired
IHC	inner hair cell
I/O	input/output
MSC	magnitude-squared coherence
NH	normal hearing
OAE	otoacoustic emissions
OHC	outer hair cell
p-p	peak-to-peak
rms	root-mean-squared
SNR	signal-to-noise ratio
SPL	sound pressure level

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1. INTRODUCTION

1.1 DOLPHIN AUDITORY SYSTEM

Dolphins possess a sophisticated auditory system that enables them to hear directionally while underwater and process high-frequency sounds, including their echolocation pulses. Like all mammals, dolphins possess an outer, middle, and inner ear. In land mammals, the primary role of the outer and middle ears is to collect and transfer sound energy to the fluid-filled inner ear, where the sensory hair cells are located. However, the outer ear in dolphins is adapted to an aquatic environment and the middle and inner ears are specialized for high-frequency hearing. As such, the outer ear does not contain a pinna, the ear canal (external auditory meatus) is occluded, the middle ears bones do not directly contact the tympanic membrane, and the middle ear bones do not touch the skull but are suspended by ligaments and fibrous tissue (Ridgway and Au, 2009). In contrast to land mammals, where sound is primarily transmitted via the ear canal and middle ear, the primary transmission path for sound to reach the dolphin inner ear is via tissue conduction through the head, especially fatty channels in the mandible for higher frequencies.

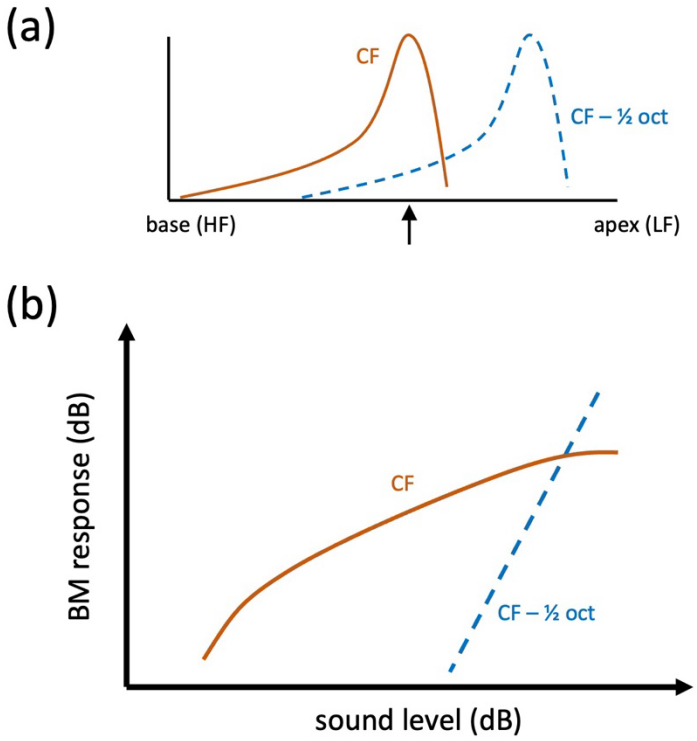
The mammalian inner ear contains the otolithic organs and three semi-circular canals of the vestibular system, as well as the cochlea, which is involved in hearing. The dolphin cochlea is analogous to that of land mammals, but with adaptations to enhance high-frequency hearing. As in terrestrial mammals, the cochlea is a spiral-shaped, conical, fluid-filled cavity divided longitudinally by the basilar membrane (BM), which supports the Organ of Corti, the receptor organ for hearing that contains the hair cells. The basilar membrane width and thickness vary in systematic fashion along the length of the cochlea. The result is a systematic change in the mechanical frequency response of the BM along its length, with high frequencies causing maximal BM displacement at the base of the cochlea (where the middle ear bones attach) and lower frequencies causing maximal displacement progressively towards the apex. In this fashion, hair cells within the Organ of Corti are "tuned" to specific frequencies based on their location along the BM, resulting in a frequency-to-place mapping within the cochlea.

Within the Organ of Corti are two types of hair cells. The inner hair cells (IHCs) transduce mechanical fluid motion in the cochlea to electrical nerve impulses that are transmitted via afferent nerve fibers to the brain, ultimately giving rise to the sensation of hearing. In contrast, the outer hair cells (OHCs) are primarily innervated by efferent nerves. OHCs respond to electrochemical changes by changing ciliary bundle length, which affects the mechanical response of the BM at the OHC's location. In this way, OHCs act as part of a feedback loop to enhance the amplitude and sharpen the frequency tuning of the BM response, acting as a nonlinear, compressive (cochlear) amplifier. Efferent stimulation of OHCs can alter cochlear amplifier gain and has been found to have a role in protecting the ear from overstimulation from noise (Le Prell et al., 2003; Fuente, 2015). Loss of OHC function results in a broadly tuned, linear BM response, resulting in decreased sensitivity (i.e., hearing loss) and reduced frequency selectivity. Assessment of OHC function is therefore important for identifying hearing loss and understanding the underlying mechanisms.

1.2 ASSESSING OUTER HAIR CELL FUNCTION

Proper OHC function can be ascertained in several ways. In land mammals, OHC function is typically assessed by measuring otoacoustic emissions (OAEs) — sounds produced by the ear either spontaneously or in response to external acoustic stimulation. Detection of cochlear microphonics — receptor potentials primarily generated by OHCs — can also be used as a sign of OHC function (Santarelli et al., 2006). Unfortunately, neither of these two methods has proven useful as a means of noninvasively examining OHC function in dolphins.

Since proper OHC function results in a nonlinear BM response, the presence of BM nonlinearities can be used as an indicator of normal OHC function. BM nonlinearities can be assessed behaviorally by comparing auditory thresholds measured in the presence of masking tones with different frequencies (Oxenham and Plack, 1997). A masker with the same frequency as the hearing test (on-frequency masker) will be affected by nonlinear BM response and show nonlinear growth of masking with masker level. A masker with frequency below the hearing test frequency (off-frequency masker) will not be affected by nonlinear BM response and will show linear growth of masking with masker level. Figure 1(a) illustrates BM traveling wave envelopes for two tones, one at the characteristic frequency (CF) of a nerve fiber located at the position of the arrow, the other with frequency 1/2-octave below the fiber's CF. Figure 1(b) illustrates the BM response at the position indicated by the arrow, in response to the two tones in Fig. 1(a). For the tone at the CF, the BM response shows a compressive nonlinearity; for the tone below the CF, the response is linear.



- Adapted from Oxenham and Bacon (2003)
- HF – high frequency, LF – low frequency

Figure 1. Illustrations of (a) BM traveling wave envelope and (b) BM response to tones at and above the CF of a nerve fiber at the arrow.

The on-/off-frequency masking paradigm has also been utilized with electrophysiological measurements, specifically measurements of auditory evoked potentials (AEPs) — small voltages produced by the brain and auditory nervous system to sound. Krishnan and Plack (2009) compared auditory brainstem response (ABR) wave V latencies measured in the presence of on- and off-frequency maskers and found evidence of a compressive nonlinearity in the growth of masking with the on-frequency masker and linear growth of masking for the off-frequency masker. Popov et al.

(2019, 2020) used ABR measurements in dolphins to compare threshold levels in the presence of on- and off-frequency maskers at a variety of hearing test (i.e., "probe") stimulus levels. Evidence of compressive nonlinearities was found at frequencies up to 90 kHz using both simultaneous (masker temporally overlaps with the probe, Popov et al., 2019) and forward masking paradigms (masker precedes the probe, Popov et al., 2020).

1.3 OBJECTIVES

The present report describes six experiments to investigate the feasibility of using electrophysiological responses (i.e., AEPs) in dolphins to assess BM nonlinearities and thus OHC function. The ability to assess OHC function in dolphins would be important for assessing hearing loss during controlled exposure experiments (Finneran, 2015) and identifying the mechanisms underlying dolphins' ability to self-mitigate certain noise exposures (Nachtigall and Supin, 2013, 2014; Finneran, 2018; Nachtigall et al., 2018; Finneran, 2020; Finneran et al., 2023). The experiments involved measurement of ABRs, which can be measured noninvasively in dolphins and are routinely used to assess hearing in marine mammals (Houser and Finneran, 2006; Nachtigall et al., 2007; Houser et al., 2008; Strahan et al., 2020). In the present study, ABRs were measured to a probe stimulus in the presence of an on- or off-frequency masker. Both simultaneous masking and forward masking paradigms were used. Probe and/or masker level were varied across trials. Across various experiments, the delay between the masker and probe, the probe frequency, masker frequency, and masker waveform were varied. Table 1 summarizes the main features of each experiment. All experiments followed a protocol approved by the Institutional Animal Care and Use Committee at the Naval Information Warfare Center Pacific and the Navy Bureau of Medicine and Surgery, and followed all applicable U.S. Department of Defense guidelines.

Table 1. Probe and masker stimulus parameters for the six experiments.

Exp.	Dolphins	Medium	Masking Paradigm	Probe Frequency (kHz)	Probe SPL (dB re 1 µPa)	Probe-Masker Delay (ms)	Masker Frequency (kHz)	Masker SPL (dB re 1 µPa)
1	TRO	Water	Forward	90	120	2, 5, 10	45, 90	110–150
2	TRO	Water	Forward	90	115	0.25, 1, 2, 5	45, 90	105–175
3	TRO	Water	Forward	64, 90	120, 130, 140	1	45, 64, 90	110–170
4	COL OLY TRO	Air	Forward	45	115 125 125	1	32, 45	95–155
5	COM SHA	Water	Simultaneous	45	90	N/A	32, 45	90–140
6	COM SHA SPA TYH	Water	Simultaneous	45, 64	40–140	N/A	57, 81	120

- Probe and maskers were cosine enveloped tonebursts with levels expressed in peak-equivalent sound pressure level (SPL), except for Exp. 6, where probes were repetitive tonebursts specified in root-mean-squared (rms) SPL and maskers were high-pass continuous noise with SPL specified in terms of the 1/3-octave SPL.

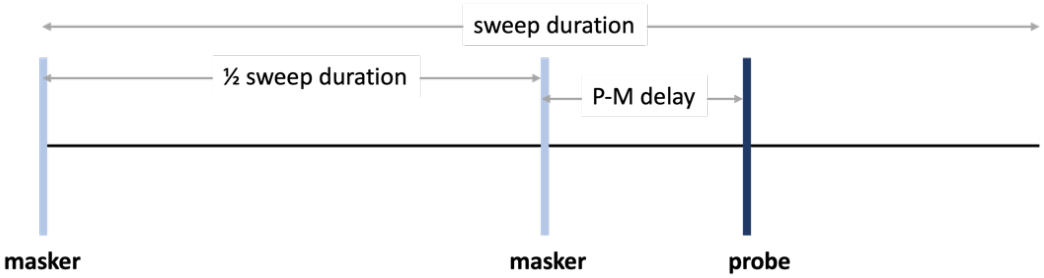
2. EXPERIMENT 1

2.1 METHODS

Experiment 1 focused on the growth of masking for on- and off-frequency maskers with different probe-masker delays. Tests were performed with a twenty-nine-year-old male dolphin (TRO) with full hearing bandwidth. Measurements were conducted in San Diego Bay, with the dolphin fully submerged and positioned with his rostrum on a plastic "biteplate" located ~1 m in front of an underwater sound projector (ITC 5446). Background ambient noise at the test site resulted from snapping shrimp, other dolphins, and occasional contributions from passing vessels and aircraft. From 20–140 kHz, median ambient noise pressure spectral density levels decreased from 63 to 50 dB re 1 $\mu Pa^2/Hz$; the mean pressure spectral density level over this frequency range was 55 dB re 1 $\mu Pa^2/Hz$.

Both the probe and masker were cosine-enveloped pure tones with a duration of eight cycles and no amplitude plateau. The probe frequency was 90 kHz and the probe SPL was 120 dB re 1 µPa (hereafter denoted "dB SPL"). Masker frequency was either 90 kHz (on-frequency) or 45 kHz (off-frequency). Masker SPL varied from 110 to 150 dB SPL.

Figure 2 illustrates the stimulus presentation order. Each stimulus epoch contained two instances of the masker, separated by 50 ms. The probe followed the second masker after a delay of 2, 5, or 10 ms. At probe-masker delays < ~8 ms, ABRs to the masker and probe overlap, preventing accurate measurement of the ABR to the probe. The first masker instance was therefore included to allow the ABR to the masker alone to be measured. The ABR to the masker alone was then subtracted from the combined ABR to the second masker and probe, to obtain the ABR to the probe alone.



- P-M (probe-masker) delay represents the time between cessation of the second masker and onset of the probe.

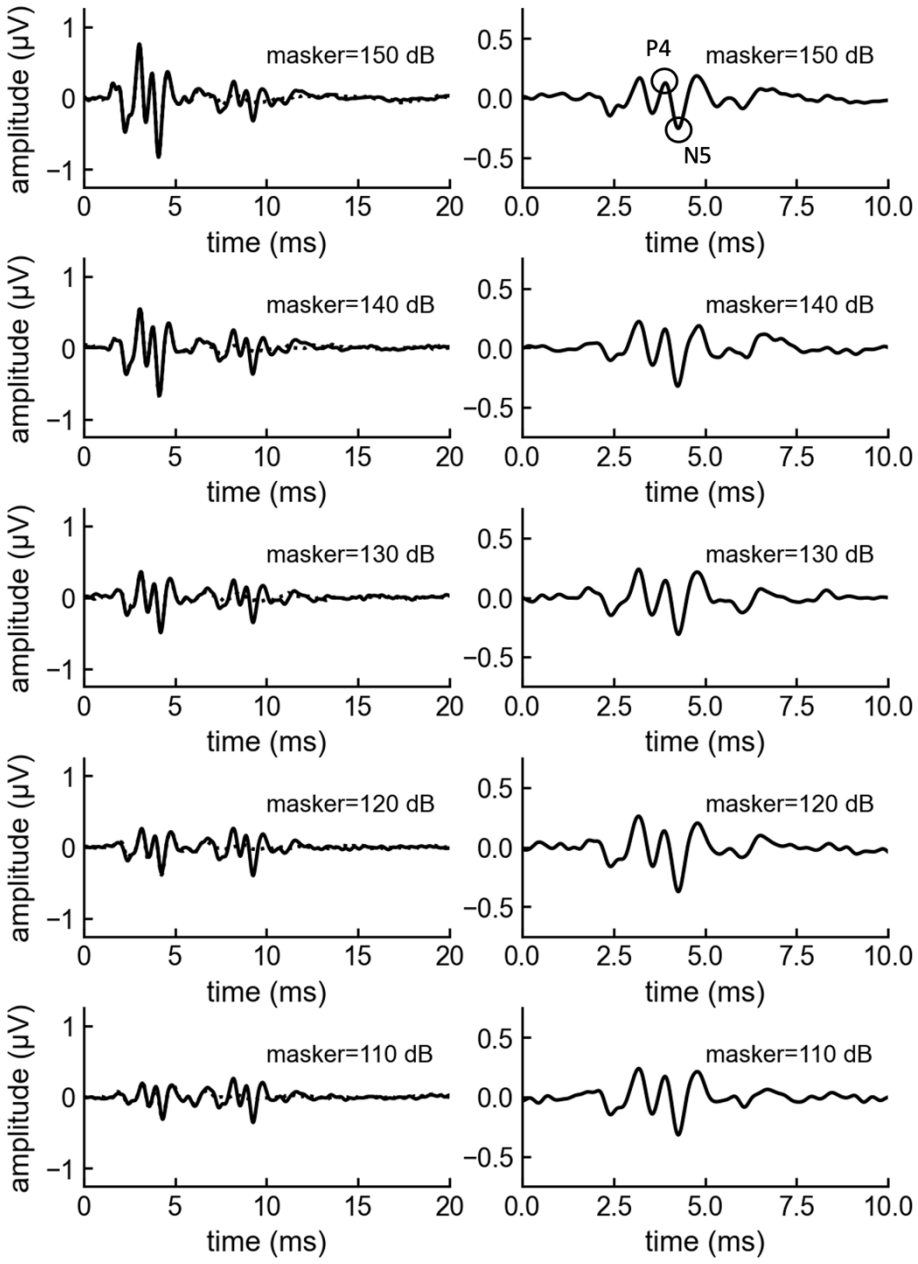
Figure 2. Sound stimulus presentation order.

Stimuli were digitally synthesized, then converted to analog at 2-MHz rate and 16-bit resolution. Analog stimuli were low-pass filtered at 150 kHz (filter), amplified (Crest CC1000), and used to drive the ITC 5446 projector. ABRs were measured using surface electrodes embedded in suction cups and placed on the dolphin's head and dorsal surface: a noninverting electrode 5-cm posterior to the blowhole, an inverting electrode just posterior to the right external auditory meatus, and a common electrode in the seawater near the dolphin. The potential difference between the noninverting and inverting electrodes was amplified (94 dB) and filtered (0.3 to 3 kHz) using a biopotential amplifier (Grass ICP511) to obtain the instantaneous electroencephalogram (EEG). The EEG was digitized at 100 kHz with 16-bit resolution (PCI-6259, National Instruments) and saved for

later analysis. Each stimulus epoch was presented 512 times and the EEG synchronously averaged using a weighted-averaging technique (Elberling and Wahlgreen, 1985) to obtain ABRs as described above. Measurements were repeated across four days to obtain four replicates (2,048 epochs of data) at each masker level and probe-masker delay combination. ABR peaks P4 and N5 were identified in the averaged ABRs (see Popov and Supin, 1990) and the peak-to-peak (p-p) amplitude between P4 and N5 measured.

2.2 RESULTS

Figure 3 shows representative examples of the averaged EEG for a probe-masker delay of 5 ms. From these data, the masker ABR (left column, dotted line) was subtracted from the masker plus probe ABR (left column, solid line) to obtain the ABR to the probe alone (right column). Amplitudes for the ABR peaks P4 and N5 were then identified and the p-p amplitude computed. Figure 4 shows the P4-N5 amplitudes for all of the experimental conditions. For the 90-kHz (on-frequency) masker, probe ABR amplitudes tended to decrease with increasing masker SPL, as expected. Although masking was more effective with the 2 ms probe-masker delay (as expected), there were no clear differences between the 5- and 10-ms probe-masker delays. Results for the 45-kHz (off-frequency) masker showed no significant change in ABR amplitude with increasing masker level from 110 to 150 dB SPL.



- Left column: ABRs in response to masker alone (dotted line) and masker plus probe (solid line). Time-zero for each trace is the acoustic masker onset.
- Right column: ABRs in response to the probe. Time-zero for the probe ABR is the acoustic arrival time of the probe at the dolphin.
- Masker SPL (in dB re 1 μPa) is indicated in each panel.
- ABR peaks P4 and N5 are circled in the top right panel.

Figure 3. Representative ABR waveforms measured during Exp. 1 with a probe-masker delay of 5 ms.

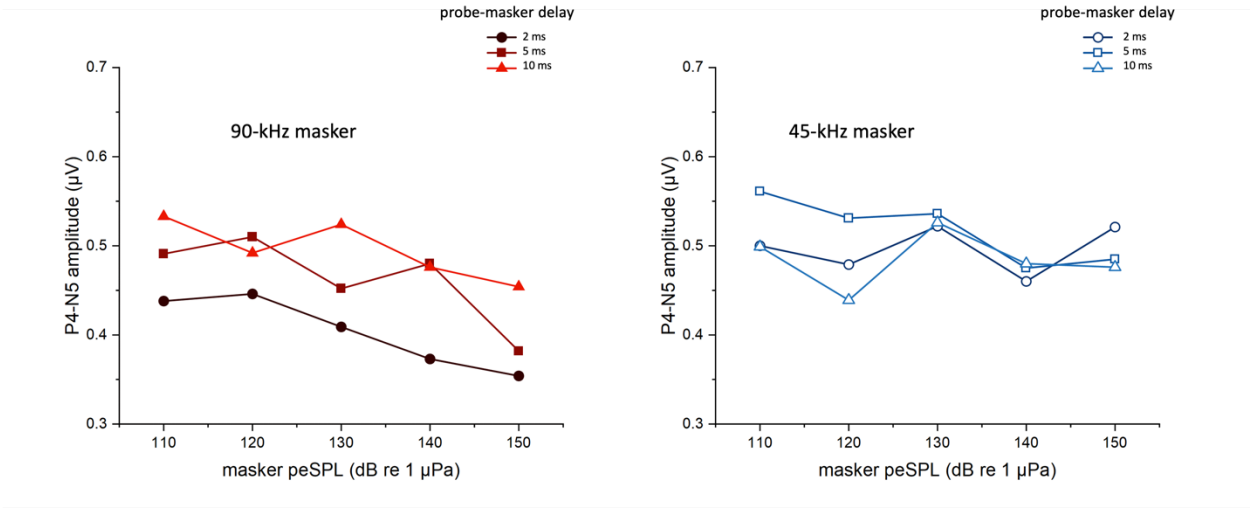


Figure 4. Probe ABR P4-N5 amplitude as a function of masker SPL for the on-frequency (90 kHz, left) and off-frequency (45 kHz, right) maskers during Exp. 1.

3. EXPERIMENT 2

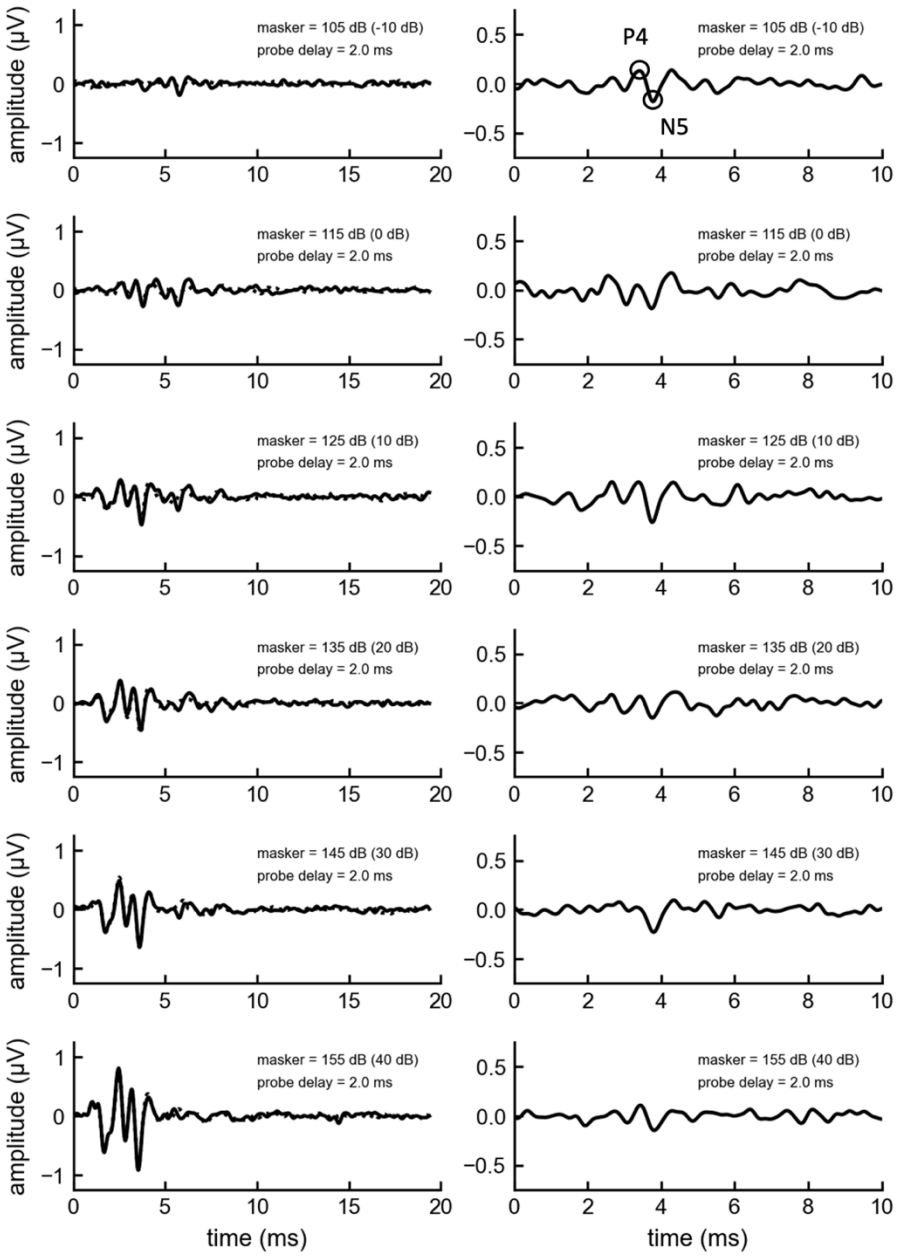
3.1 METHODS

Experiment 2 was similar to Exp. 1, with a focus on the growth of masking for on- and off-frequency maskers with different probe-masker delays. The primary differences from Exp. 1 were the probe SPL (lowered to 115 dB SPL), probe-masker delays (added 0.25 and 0.5 ms), masker SPLs (increased up to 175 dB SPL), and delay between the first and second masker stimuli (increased to 62.5 ms). The changes were made to increase the potential for masking after Exp. 1 showed only limited masking effects, especially for the off-frequency masker. The test subject, environment, and methods were otherwise identical to Exp. 1.

3.2 RESULTS

Figure 5 shows representative examples of the averaged EEG for a probe-masker delay of 2 ms. Figure 6 shows the P4-N5 amplitudes for the various experimental conditions. For the 90 kHz (on-frequency) masker, probe ABR amplitudes tended to decrease with increasing masker SPL (upper left panel) and increase with increasing probe-masker delay (lower left panel). Comparison of the 45-kHz (off-frequency) and 90-kHz (on-frequency) maskers showed only small differences in masking patterns with a probe-masker delay of 0.5 ms.

Overall, the data exhibited a large amount of variability, making interpretation of these results in terms of a compressive nonlinearity impossible.



- Left column: ABRs in response to masker alone (dotted line) and masker plus probe (solid line). Time-zero for each trace is the acoustic masker onset.
- Right column: ABRs in response to the probe. Time-zero for the probe ABR is the acoustic arrival time of the probe at the dolphin.
- Masker SPL (in dB re 1 μPa) is indicated in each panel, along with the masker level relative to the probe (in parentheses).
- ABR peaks P4 and N5 are circled in the top right panel.

Figure 5. Representative ABR waveforms measured during Exp. 2 with a probe-masker delay of 2 ms.

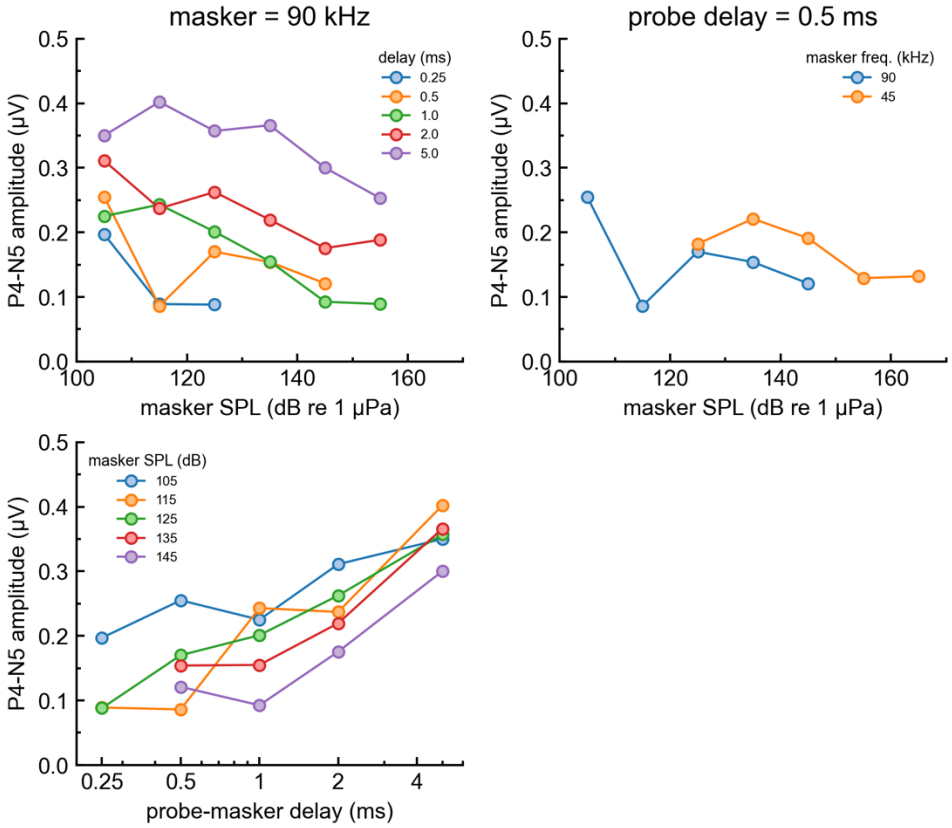


Figure 6. Probe ABR P4-N5 amplitude as a function of masker SPL and probe-masker delay during Exp. 2.

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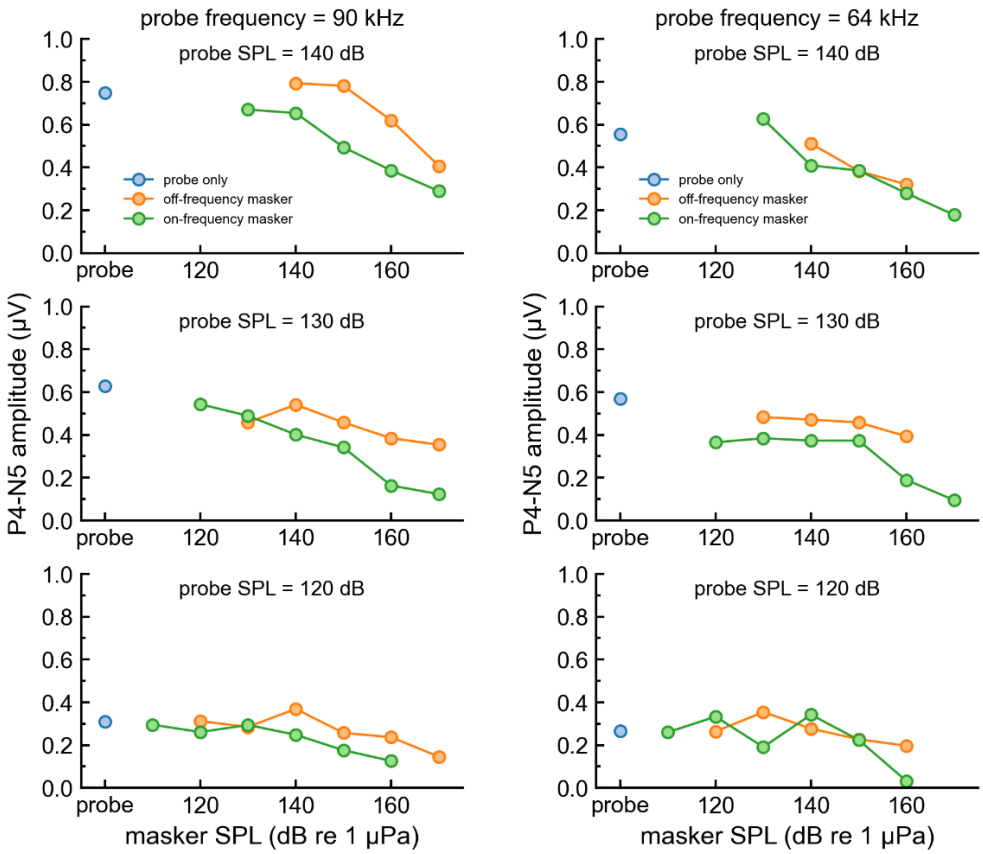
4. EXPERIMENT 3

4.1 METHODS

Experiment 3 was focused on the growth of masking for on- and off-frequency maskers with different probe frequencies and levels. Based on the results from Exps. 1 and 2, the probe-masker delay was fixed at 1 ms and the off-frequency maskers were moved closer to the probe frequencies (1/2-octave below as opposed to 1-octave). The test subject (TRO), environment, and methods were otherwise identical to Exps 1 and 2.

4.2 RESULTS

Figure 7 shows P4-N5 amplitudes as functions of masker SPL for each combination of probe frequency, probe SPL, and masker frequency. At low masker SPLs, probe ABR amplitudes were similar to the probe-only condition, as expected. As masker level increased, P4-N5 amplitudes eventually decreased, with less masking (higher P4-N5 amplitude) typically seen with the off-frequency masker. For the 90-kHz probe, the on-/off-frequency masking patterns at 140 dB SPL showed the expected behavior: more shallow growth of masking for the on-frequency masker, but similar masker SPLs at the probe threshold. However, at lower probe SPLs, the on-frequency masker SPL that would be required to fully mask the probe (P4-N5 amplitude of zero) was relatively high and similar to that of the off-frequency condition, which did not fit the expected compressive behavior. For the 64-kHz probe, masking curves for the on- and off-frequency maskers were similar, which also does not fit the compressive pattern.



- Each row shows results with a different probe SPL (140, 130, 120 dB SPL, from top to bottom).
- The three series in each panel show results for the probe only (i.e., no masker), the on-frequency masker (same frequency as the probe), and the off-frequency masker (1/2-octave below the probe frequency).

Figure 7. Probe ABR P4-N5 amplitude as a function of masker SPL and probe-masker delay during Exp. 3.

5. DISCUSSION OF EXPERIMENTS 1–3

Experiments 1–3 showed similar results: the data exhibited a large amount of variability and the ABR amplitude's dependence on masker level and probe-masker delay did not conform to expected patterns based on existing terrestrial and marine mammal data. Specifically, the off-frequency maskers had a limited effect on the probe amplitude, and the effects of the on- and off-frequency maskers were often similar. The reasons for these observations are not known. Ambient noise underwater at the test site was relatively high. It is possible that the high ambient noise levels affected the forward masking paradigm (i.e., there was no truly quiet interval between the masker and probe). The relatively high probe and masker levels necessitated by the ambient noise may also have affected the results: at high levels, auditory filter bandwidths increase and cochlear place specificity decreases. This would result in blurring the distinction between the on- and off-frequency maskers.

To attempt to address some of these issues, Exp. 4 was conducted with the dolphins in air. This avoided the high ambient noise levels in San Diego Bay and allowed lower stimulus levels to be used. Additional replications were also conducted to help reduce variability.

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6. EXPERIMENT 4

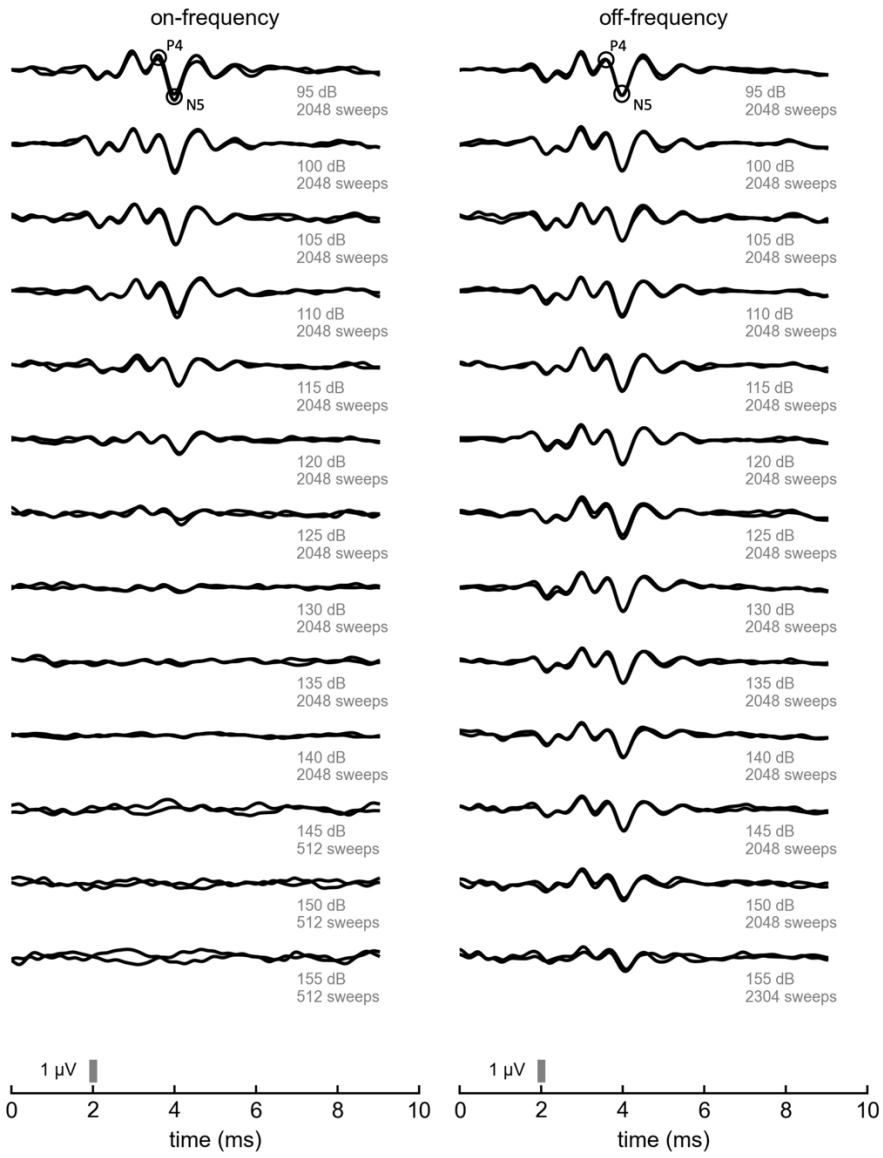
6.1 METHODS

Experiment 4 focused on the growth of masking for on- and off-frequency maskers with different levels. Unlike Exps. 1–3, Exp. 4 was conducted with the dolphins beached out of the water and resting on a foam mat. Sounds were delivered using a sound projector embedded in a suction cup and placed on the sound reception area of the mandible. Out-of-water testing was performed to avoid the relatively high ambient noise levels in San Diego Bay. Three male dolphins were tested: COL, OLY, and TRO, with upper hearing limits of 80, 54, and 134 kHz, respectively. The dolphins COL and OLY were therefore considered to be "hearing impaired" (HI) and TRO considered to have "normal hearing" (NH). The probe frequency was lowered to 45 kHz to accommodate the high-frequency hearing loss in COL and OLY. The probe SPL was set approximately 25 dB above threshold, and thus varied by dolphin: 115 dB SPL for COL and 125 dB SPL for OLY and TRO. Masker frequencies were 45 kHz (on-frequency) and 1/2-octave below at 32 kHz (off-frequency). The probe-masker delay was fixed at 1 ms. Each stimulus epoch was presented 512 times and the EEG synchronously averaged using a weighted-averaging technique to obtain ABRs as described above. ABR measurements were conducted as in the previous experiments. Measurements were repeated over multiple days to obtain at least 2,048 epochs for conditions where ABRs were visible. ABR peaks P4 and N5 were identified in the averaged probe ABRs and the p-p amplitude between P4 and N5 and the latency of peak N5 measured.

6.2 RESULTS

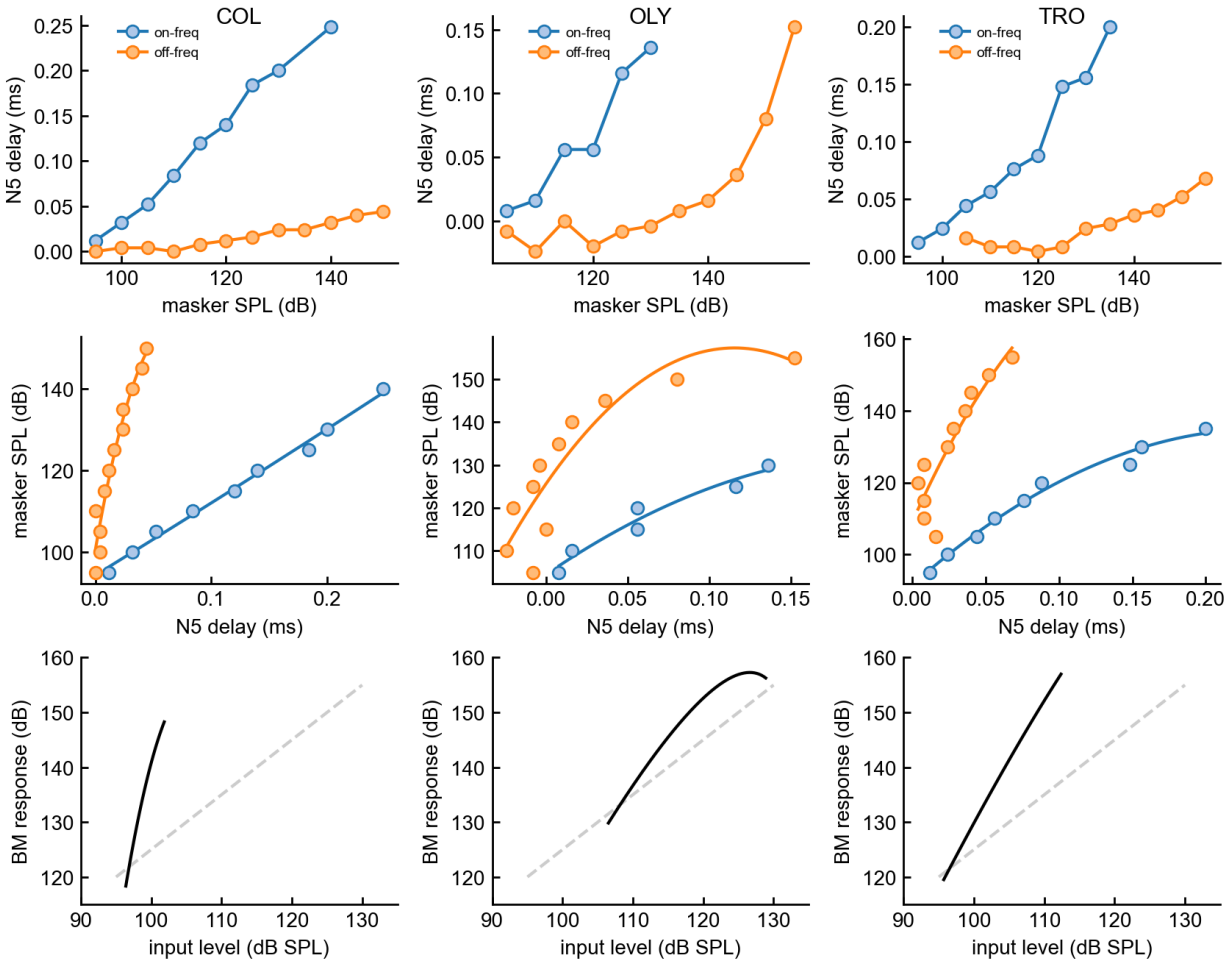
Figure 8 shows representative ABR waveforms for the dolphin COL. Signal quality was generally good at lower masker SPLs but decreased as masker SPL increased. At the higher masker SPLs, signal-to-noise ratio (SNR) was low and ABR peaks could not always be visually identified, especially with the on-frequency masker.

Using the P4 and N5 peak data, BM response as a function of input sound level was estimated following the method of Krishnan and Plack (2009): First, N5 delay was calculated by normalizing the N5 latencies for each dolphin using the latency measured for the probe alone (i.e., no masker). N5 delays were then plotted versus masker SPL for each dolphin (Fig. 9, top row). Next, the graph axes were reversed, and masker level data as a function of N5 delay were fit with a third-order polynomial (Fig. 9, middle row). Interpolation was then used to determine masker levels for the on- and off-frequency masker that resulted in the same N5 delay. The level of the off-frequency masker represents the BM response (in dB). The level of the on-frequency masker represents the input SPL (in dB SPL). The process was then repeated at a number of N5 delays to construct a curve of BM response vs. input level (Fig. 9, bottom row). The analysis was also performed using P4-N5 amplitude instead of N5 latency, without normalizing the amplitudes to the probe-alone condition. (Fig. 10).



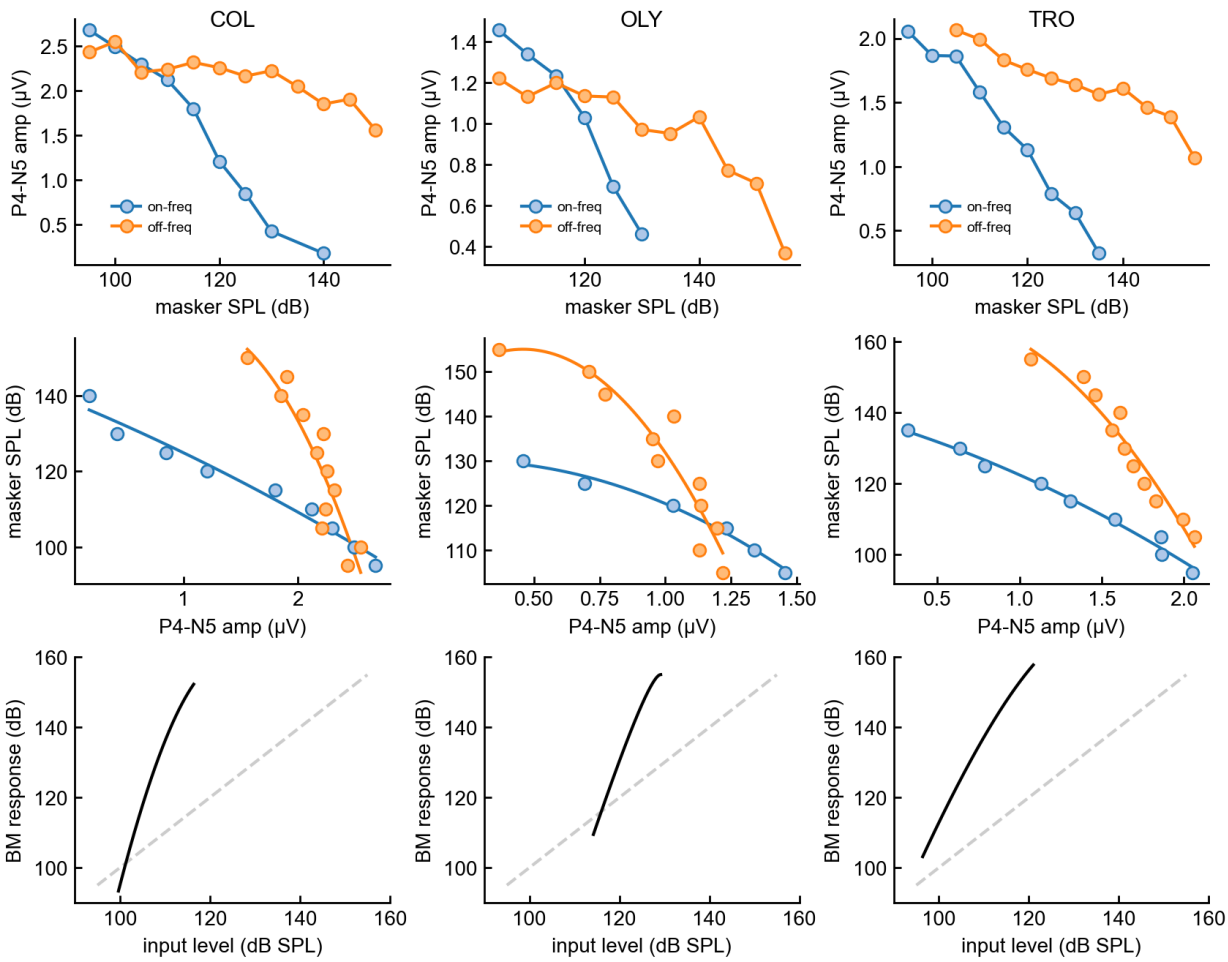
- Time-zero is the acoustic arrival time of the probe at the dolphin.
- Each trace represents the averaged EEG based on 1/2 the total epochs of data (to allow variability to be assessed).
- Peaks P4 and N5 are circled in the top traces.
- The text near each trace indicates the masker SPL (in dB SPL) and total number of EEG sweeps (epochs).

Figure 8. Example probe ABR waveforms measured in the dolphin COL with on-frequency (left column) and off-frequency (right column) forward maskers.



- Top row: N5 delays (N5 latency relative to latency of probe-only ABR) versus masker level.
- Middle row: data from top row with axes reversed and cubic curve-fit applied.
- Bottom row: BM response versus input SPL (thick line), derived using the method described in the text (see Krishnan and Plack, 2009).
- The dashed lines have a slope of 1 and are included for comparison of the BM response slope.

Figure 9. Derivation of BM response for the three dolphins (COL, OLY, TRO) from N5 latency measurements.



- Top row: P4-N5 amplitude versus masker SPL.
- Middle row: data from top row with axes reversed and cubic curve-fit applied.
- Bottom row: BM response versus input SPL (thick line), derived using the method described in the text (see Krishnan and Plack, 2009).
- The dashed lines have a slope of 1 and are included for comparison of the BM response slope.

Figure 10. Derivation of BM response estimate for the three dolphins (COL, OLY, TRO) from P4-N5 amplitude measurements.

6.3 DISCUSSION

For a normal cochlea, plots of BM response vs. input level should have a slope of ~1 at low input levels and < 1 (~0.2) at higher levels; at even higher levels it may become linear (slope = 1) again (Krishnan and Plack, 2009). In contrast to this expected behavior, Figs. 9 and 10 show BM response slopes > 1 over most of the input level range and no substantial compression at the higher levels. The reason for the differences from expected behavior, as seen in human data, are not known. It is possible that the stimulus sound levels required to complete the test matrix were still too high to avoid spread of excitation within the cochlea and/or the frequency separation between on- and off-frequency maskers was not large enough. Two of the three dolphins also had pre-existing high-frequency hearing loss; it is possible that they may not show BM compression in the same fashion as an NH animal. Testing in air with dolphins is logistically challenging, limits the available dolphins

and session durations, and makes calibration of the sound stimuli difficult. Given these issues and the failure to obtain clear evidence of BM nonlinearities, further testing was continued in water rather than in air.

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7. EXPERIMENT 5

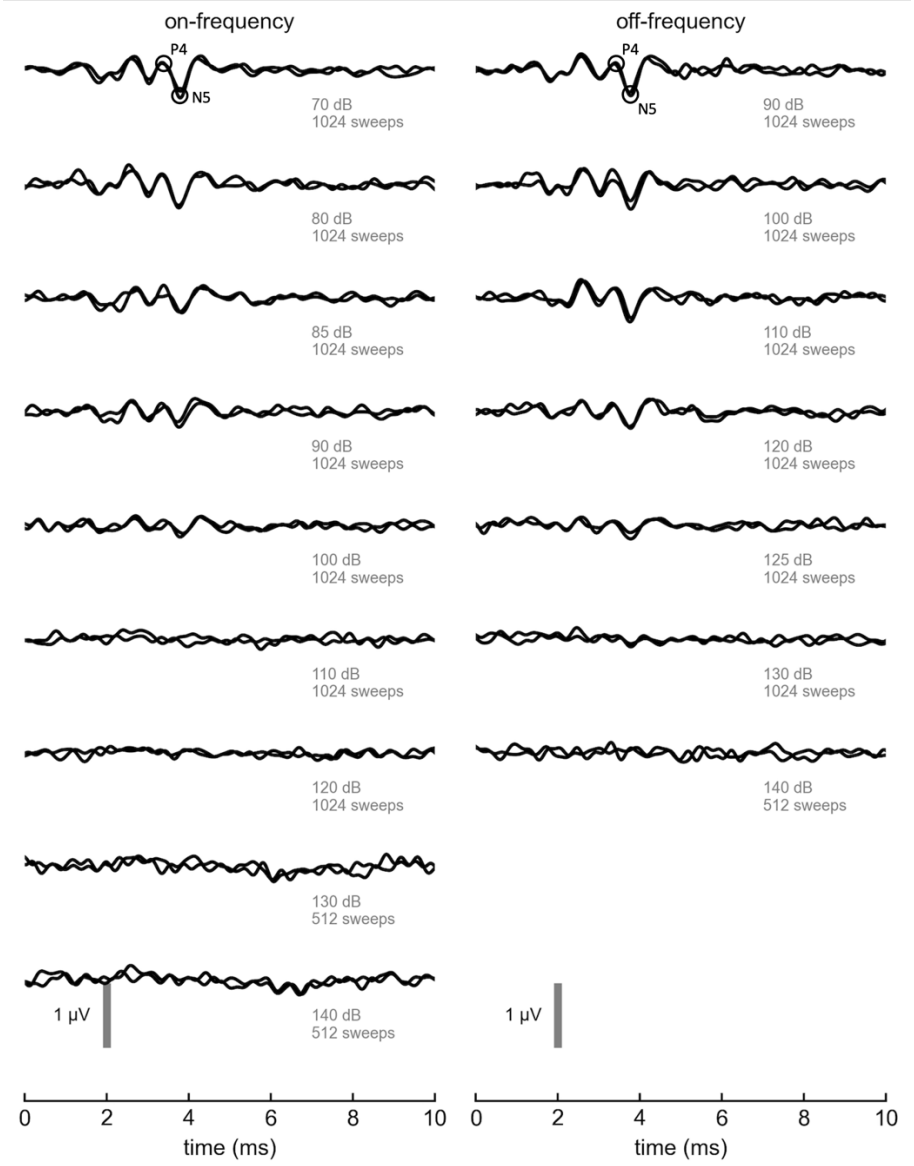
7.1 METHODS

Experiment 5 focused on the growth of masking for on- and off-frequency maskers with different levels, using a *simultaneous* masking paradigm rather than the forward masking paradigm used in Exps. 1–4. Measurements were conducted in San Diego Bay, with the dolphin fully submerged and positioned on a plastic "biteplate" located ~1 m in front of an underwater sound projector (ITC 5446). Two dolphins with full hearing bandwidth were tested (dolphins SHA and COM). The probe was an 8-cycle, cosine enveloped toneburst with frequency of 45 kHz and SPL of 101 dB SPL. The masker was a 20-ms tone, centered on the probe stimulus, with the same rise/fall envelope as the probe. Masker frequencies were 45 kHz (on-frequency) and 32 kHz (off-frequency). ABR measurements were conducted as in the previous experiments. Each stimulus epoch was presented 256 times and the EEG synchronously averaged using a weighted-averaging technique to obtain ABRs as described above. Measurements were repeated four times, over the course of two sessions, to obtain 1,024 sweeps at experimental conditions except the higher masker levels (where probe ABRs were not measurable). ABR peaks P4 and N5 were identified in the averaged probe ABRs and the p-p amplitude between P4 and N5 and the latency of peak N5 measured.

7.2 RESULTS

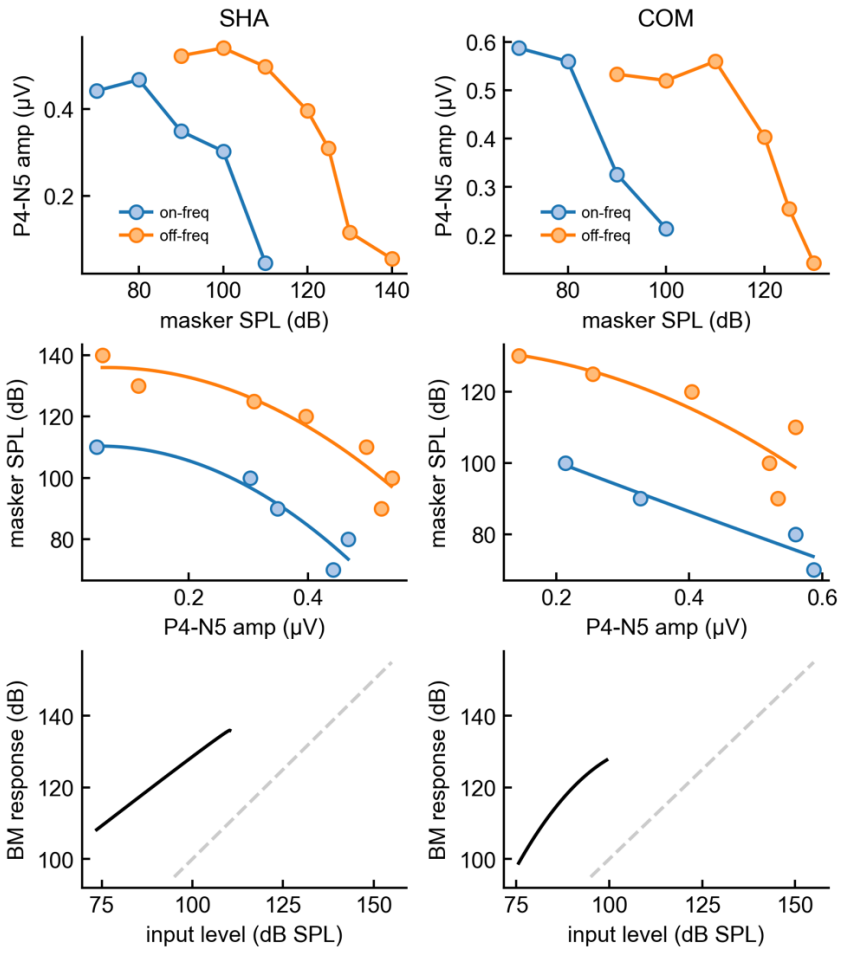
Figure 11 shows representative ABR waveforms for the dolphin COM. Signal quality was generally good at lower masker SPLs but decreased as masker SPL increased. At the higher masker SPLs, SNR was low and ABR peaks could not always be identified, especially with the on-frequency masker.

Using the P4-N5 p-p amplitudes, BM response as a function of input sound level was estimated (Fig. 12) following the method of Krishnan and Plack (2009) and as described above in Exp. 4. N5 latencies showed little change with masker SPL and thus did not support estimates of a compressive BM response.



- Left column: on-frequency (45 kHz) masker
- Right column: off-frequnecy (32 kHz) masker
- Time-zero is the acoustic arrival time of the probe at the dolphin.
- Each trace represents the averaged EEG based on 1/2 the total epochs of data (to allow variability to be assessed).
- Peaks P4 and N5 are circled in the top traces.
- The text near each trace indicates the masker SPL (in dB SPL) and number of EEG sweeps (epochs).

Figure 11. Example probe ABR waveforms measured in the dolphin COM during Exp. 5.



- Top row: P4-N5 amplitude versus masker SPL.
- Middle row: data from top row with axes reversed and cubic curve-fit applied.
- Bottom row: BM response versus input SPL (thick line), derived using the method described in the text (see Krishnan and Plack, 2009).
- The dashed lines have a slope of 1 and are included for comparison of the BM response slope.

Figure 12. Derivation of BM response estimate for the dolphins SHA and COM from P4-N5 amplitude measurements.

7.3 DISCUSSION

BM response slopes for SHA and COM were close to 1 dB/dB and no substantial compression was seen. The reasons for the lack of obvious compression in the estimated BM responses are not known. It is possible that the stimulus sound levels were too high to avoid spread of excitation within the cochlea and/or the frequency separation between on- and off-frequency maskers was not large enough.

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8. EXPERIMENT 6

8.1 METHODS

In Exp. 6, AEP amplitudes were measured as a function of probe level. The goal was to compare slopes of the AEP amplitude versus level functions (input/output (I/O) functions) to identify any systematic differences between animals with normal hearing and those with high-frequency hearing loss. The specific question was whether animals with normal hearing would have shallower I/O functions compared to animals with hearing loss (see Encina-Llamas et al., 2021). Increasing stimulus level causes basal spread of excitation within the cochlea (i.e., towards higher frequencies), which can result in an additional increase in AEP amplitude. Therefore, measurements were conducted with and without high-pass masking noise. The noise cutoff frequency was chosen to mask frequencies just above the probe and thus limit the basal spread of excitation.

Measurements were conducted with two NH dolphins (COM, SHA) and two HI dolphins (SPA, TYH). Upper hearing limits for COM, SHA, SPA, and TYH were 136, 124, 92, and 76 kHz, respectively.

The probe stimulus was a sequence of 16 tonebursts presented at a rate of 1000 s^{-1} (i.e., toneburst onsets were spaced 1-ms apart). Tonebursts presented to dolphins at such high rates produce a complex AEP called the auditory steady-state response (ASSR). The ASSR consists of a series of overlapping ABRs and has a fundamental frequency equal to the toneburst repetition rate (i.e., 1 kHz). Probe frequencies were 45 and 64 kHz. Each toneburst had a duration of 16 cycles with a cosine envelope and no amplitude plateau. The masker was broadband noise with a lower cutoff 1/3-octave above the probe (57 or 81 kHz) and an upper cutoff at 160 kHz. Within this bandwidth, the noise was digitally compensated to be spectrally pink (Finneran et al., 1999). The noise was continuously presented during measurements.

Probe stimuli were digitally synthesized, then converted to analog at 1 MHz rate and 16-bit resolution. Probe stimuli were low-pass filtered at 150 kHz (filter) and input to a mixer (SM5, Tucker-Davis). Masking noise was digitally synthesized, then converted to analog at 500 kHz rate and 16-bit resolution and input to the second mixer channel. The mixer output was then amplified (Crest CC1000) and used to drive an ITC 5446 underwater sound projector. Sound levels were calibrated using a miniature hydrophone placed at the midpoint of the dolphin's lower jaws when on the biteplate (without the dolphin present). Probe stimuli were calibrated in terms of the rms SPL over the 16-ms duration of the toneburst sequence. The masker level was calibrated in terms of the SPL in 1/3-octave bands.

ABRs were measured using surface electrodes embedded in suction cups and attached to the dolphin's head and dorsal surface: a noninverting electrode 5-cm posterior to the blowhole, an inverting electrode just posterior to the right external auditory meatus, and a common electrode in the seawater near the dolphin. The potential difference between the noninverting and inverting electrodes was amplified (94 dB) and filtered (0.3 to 3 kHz) using a biopotential amplifier (Grass ICP511) to obtain the instantaneous EEG. The EEG was digitized at 100 kHz with 16-bit resolution (PCI-6259, National Instruments). Each stimulus epoch was presented 512 times and the EEG synchronously averaged using a weighted-averaging technique to obtain ASSR. The averaged ASSR was then Fourier transformed and the complex spectral value at the repetition rate (1 kHz) extracted. The mean ASSR amplitude was calculated via coherent averaging and the magnitude-squared coherence (MSC) at 1 kHz was calculated using 16 subaverages (Dobie and Wilson, 1989). The MSC was used to test each ASSR measurement for significance relative to the residual background noise in the EEG, at a significance level of $\alpha = 0.01$. Only statistically significant ASSR values are reported.

8.2 RESULTS

Figure 13 shows representative ASSR waveforms (left) and corresponding spectra (right) measured from COM with the 45-kHz probe without masking noise.

Figure 14 shows ASSR amplitude in linear units as a function of probe level for all experimental conditions. For the NH dolphins with the probe alone, ASSR amplitudes increased linearly at low SPLs, often exhibited a plateau from ~100–120 dB SPL, then increased sharply as SPL increased above ~120 dB SPL. This matches previous reports of ASSR magnitude-level functions in dolphins (Supin et al., 2001). In the HI dolphins, the steep rise at high SPLs was not observed, and ASSRs tended to increase linearly with SPL. For SPA at 45 kHz, ASSR amplitudes plateaued above 120 dB SPL. When masking noise was added, ASSR amplitudes in the NH dolphins were smaller and the steep rise at high SPLs was not present. This reveals significant upward (basal) spread of excitation in the NH dolphins, extending above the frequency content of the probe and at least 1/3-octave above the probe center frequency (see Fig. 14 inset graphs). Since the steep increase in ASSR amplitude was not present with the masker, it is clear that the increase in ASSR amplitude at high SPLs was due to this upward spread of excitation in the cochlea (see Supin et al., 2001). For the HI dolphins, the masker had little effect, indicating no significant upward spread of excitation in the HI dolphins.

Figure 15 shows the same data from Fig. 14, but with ASSR amplitudes in dB rather than linear units. The use of a dB-amplitude scale allows the degree of compression to be assessed and the slopes to be compared with those reported for humans. Best-fit slopes ranged from 0.36 to 0.54 dB/dB and all data showed compressive growth of ASSR amplitude with probe SPL (i.e., slopes were all < 1 dB/dB). However, there were no systematic differences in slopes between the NH and HI impaired animals, despite large differences in absolute thresholds (up to 30 dB) and between upper cutoff frequencies. The data from the NH animals with and without masking noise were fit well using the same slope. This suggests that basal spread of excitation did not significantly affect the rate of compressive growth.

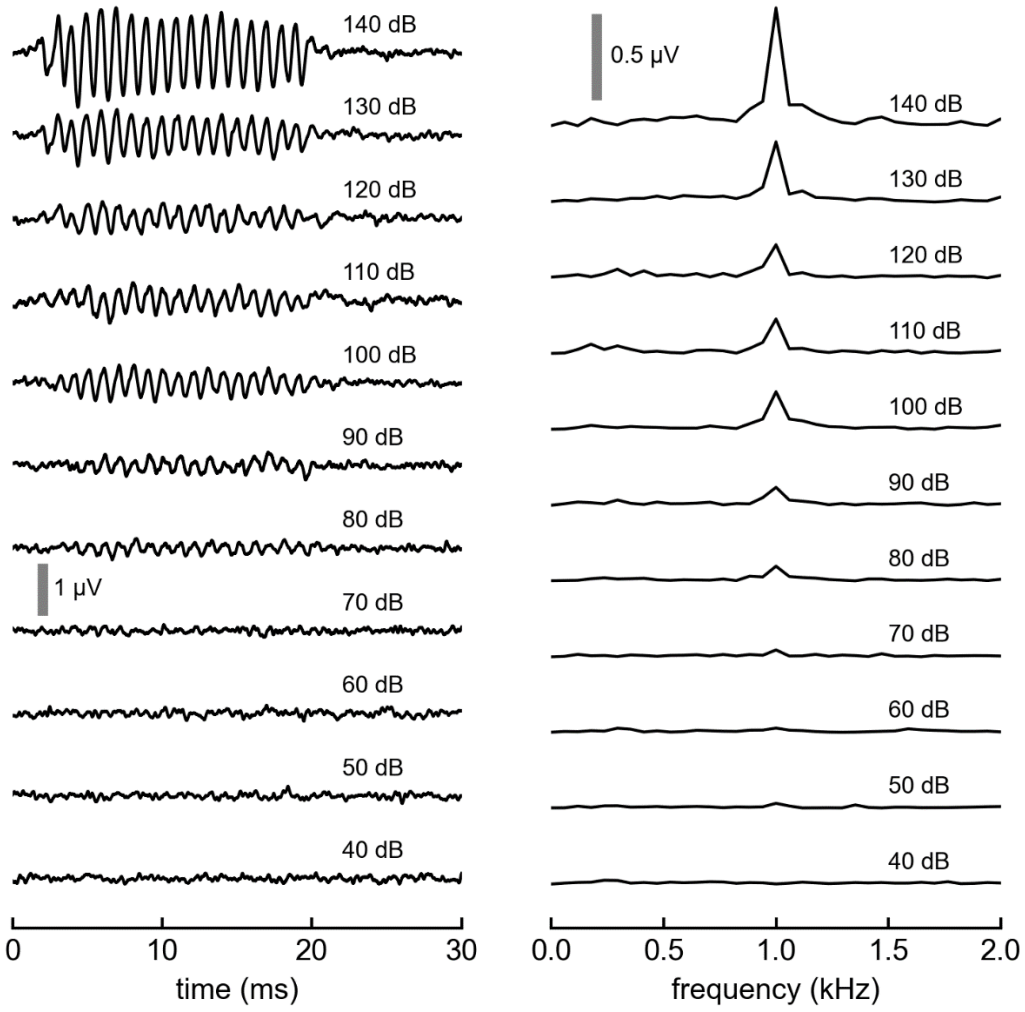
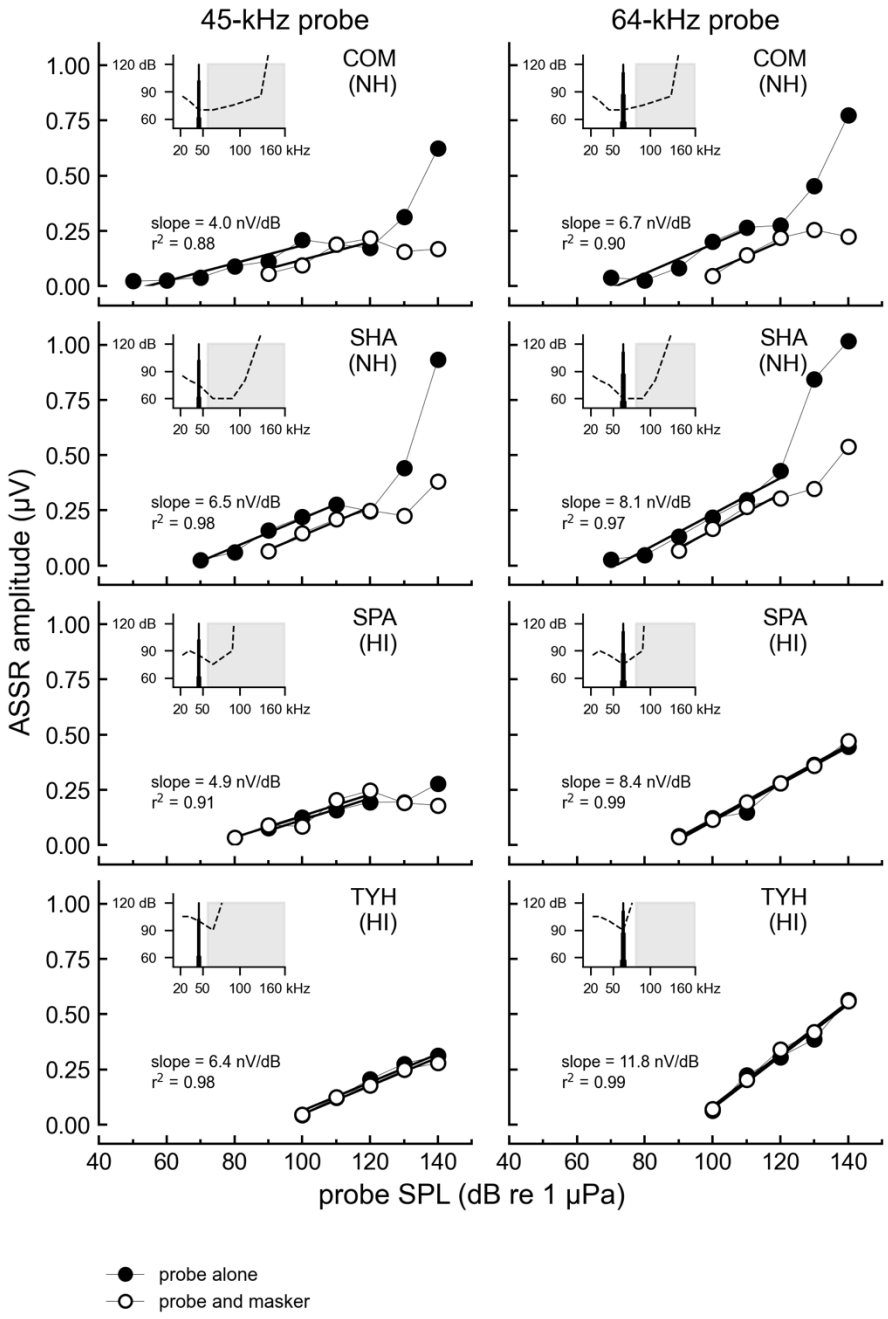
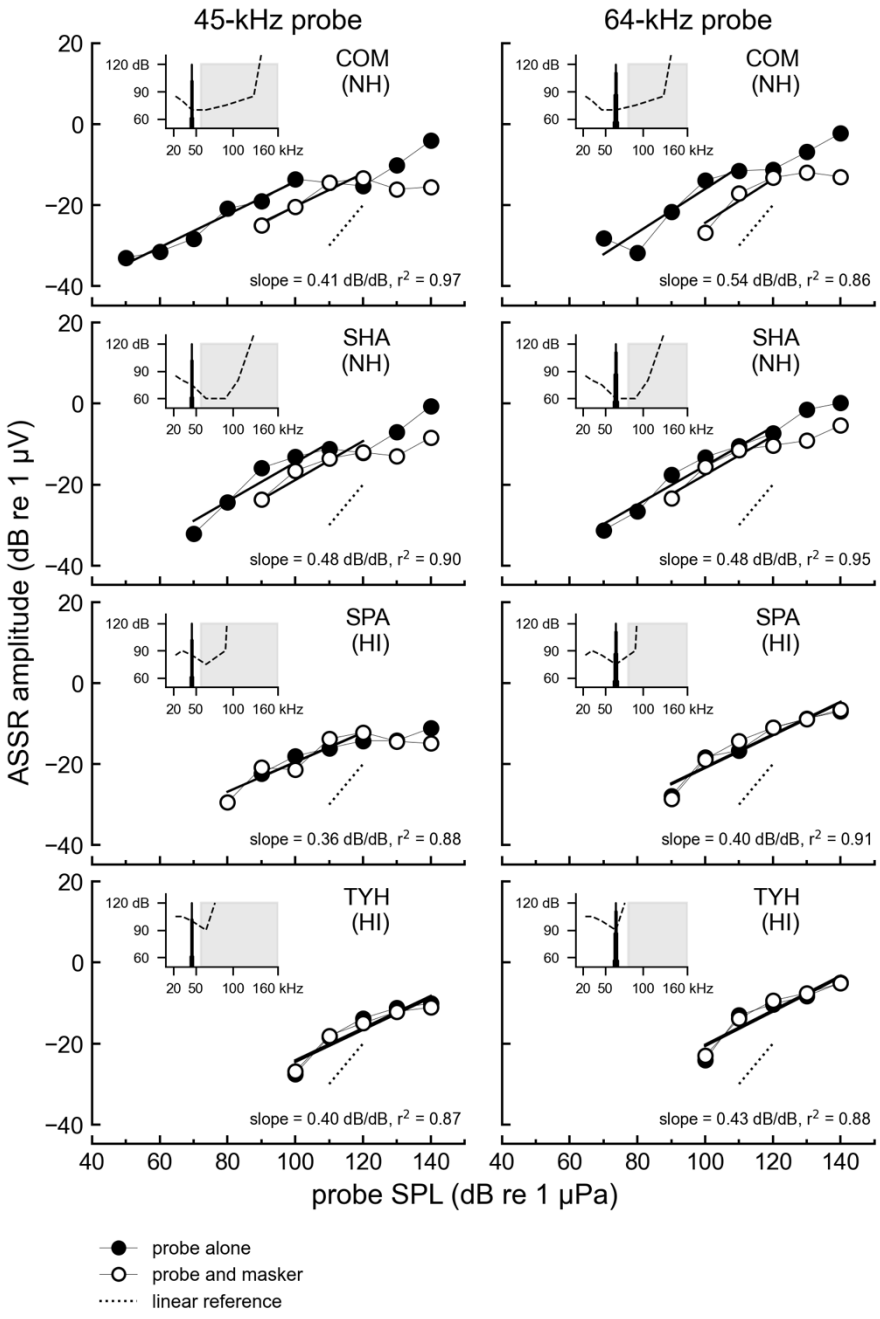


Figure 13. Waveforms (left) and frequency spectra (right) measured in the dolphin COM during Exp. 6 with the 45-kHz probe and no masking noise.



- The left and right columns show results with 45 and 64 kHz probes, respectively.
- Each row shows results for a different dolphin (COM, SHA, SPA, TYH).
- Filled symbols represent measurements with the probe alone.
- Open symbols show results when probe was presented with continuous high-pass masking noise with cutoff 1/3-octave above the probe frequency.
- Solid lines show linear regressions to the lower-amplitude data, with the slope of the regressions shared within each panel.
- The inset graphs show the frequency content of the probe (solid black lines), the dolphin's audiogram (dashed lines, hearing threshold vs. frequency), and the masker frequency content (shaded region).

Figure 14. ASSR amplitude (in linear units of µV) versus probe SPL measured during Exp. 6.



- The left and right columns show results with 45- and 64-kHz probes, respectively.
- Each row shows results for a different dolphin (COM, SHA, SPA, TYH).
- Filled symbols represent measurements with the probe alone.
- Open symbols show results when probe was presented with continuous high-pass masking noise with cutoff 1/3-octave above the probe frequency.
- Solid lines show linear regressions to the lower-amplitude data, with the slope of the regressions shared within each panel.
- The dotted line has slope of 1 dB/dB (a linear reference).
- The inset graphs show the frequency content of the probe (solid black lines), the dolphin's audiogram (dashed lines, hearing threshold vs. frequency), and the masker frequency content (shaded region).

Figure 15. ASSR amplitude (in dB) versus probe SPL measured during Exp. 6.

8.3 DISCUSSION

Previous work in humans examined the potential for ASSR magnitude-level slopes to be used in assessing peripheral level compression in the auditory system (Encina-Llamas et al., 2021). This previous study found that slopes of ASSR magnitude-level functions were smaller (more compression) in NH subjects compared to HI subjects, and slope values were similar to those obtained with distortion-product OAEs and basilar membrane compression values measured invasively from animals; however, mathematical modeling of the auditory nerve suggested that compression estimates could be highly influenced by basal spread of excitation (Encina-Llamas et al., 2021).

Results from Exp. 6 of the present study showed clear basal spread of excitation with increasing SPL in the NH dolphins; however, at lower SPLs this basal spread did not appear to affect the rates of compressive growth of the ASSR with increasing level. This does not match previous predictions based on a mathematical model of the auditory nerve (in humans) used to simulate ASSRs (Encina-Llamas et al., 2021). Slopes of the ASSR magnitude-level functions ranged from ~0.4 to 0.5 dB/dB, higher than the range of ~0.2–0.5 dB/dB reported by Encina-Llamas et al. (2021) for humans and the range of 0.2–0.4 dB/dB reported by Popov et al. (2020) for dolphins. The present study showed no systematic differences between NH and HI animals. This was unexpected given the relatively large threshold differences between subjects. It remains unclear whether ASSR magnitude-level functions — obtained using repetitive tonebursts at the frequencies and levels employed here — can reliably assess peripheral (basilar membrane) compression in dolphins.

9. CONCLUSIONS

A consistent result across all experiments was a failure to show clear evidence of compressive nonlinearities. The most likely reasons involve the high ambient noise (for underwater testing) and the relatively high signal levels. The high ambient noise likely affected the off-frequency masking paradigm, since there would be no truly quiet interval between the masker and probe. The high signal levels may have resulted in too great a spread of excitation within the cochlea and obscured differences between the on- and off-frequency masking conditions. High-level probes could also result in linear BM response (i.e., probe levels above the range of compression) rather than compressive.

To avoid these issues in future testing, compressive nonlinearity measurements using masking paradigms should be conducted in a quiet underwater environment, such as a pool, with young animals with normal hearing. Although Exp. 6 showed little difference in I/O function slopes between NH and HI animals, the small number of dolphins and limited data at the lower levels of the I/O functions make interpretation difficult. A review of similar data, collected from a large number of animals at a number of frequencies may resolve if AEP magnitude-level functions can reliably assess BM compression in dolphins.

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14. ABSTRACT

Assessment of outer hair cell function within the mammalian auditory system is important for identifying hearing loss and understanding the underlying mechanisms.This report describes a series of experiments to test the feasibility of using noninvasive auditory brainstem response measurements to assess basilar membrane nonlinearities (and thus outer hair cell function) in the bottlenose dolphin. Auditory brainstem responses were measured to probe sound stimuli with and without additional masking noise. Both simultaneous masking and forward masking paradigms were used. Probe and/or masker level were varied across trials. Across various experiments, the delay between the masker and probe, the probe frequency, masker frequency, and masker waveforms were varied. The results showed no evidence of compressive nonlinearities in any of the measurements. The most likely reasons involve the relatively high ambient noise in the test environment and the relatively high signal levels. Future testing should be conducted in a quiet underwater environment, such as a pool, with young animals with normal hearing. To assess whether ABR magnitude-level functions can be reliably used to directly assess outer hair cell function, magnitude-level functions should be assessed at multiple frequencies from a larger sample of dolphins with normal hearing and hearing loss.

15. SUBJECT TERMS

dolphin; hearing; auditory evoked potential; auditory brainstem response; basilar membrane, compression, nonlinearity

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