MIDDLE- AND LONG-LATENCY AUDITORY EVOKED POTENTIALS IN RESPONSE TO ODDBALL STIMULI IN THE BOTTLENOSE DOLPHIN (TURSIOPS TRUNCATUS)

Erica Noelle Hernandez
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MIDDLE- AND LONG-LATENCY AUDITORY EVOKED POTENTIALS IN
RESPONSE TO ODDBALL STIMULI IN THE BOTTLENOSE DOLPHIN
(TURSIOPS TRUNCATUS)

by

Erica Noelle Hernandez

A Dissertation
Submitted to the Graduate Studies Office
of The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

Approved:

August 2007
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The long latency auditory evoked potentials (AEP) in response to repeated tones and oddball tones of a different frequency were recorded in four bottlenose dolphins (Tursiops truncatus) with differing levels of experience with this recording paradigm. The stimuli used were 100 ms pure tones, and the AEP was recorded from stimulus onset to 500 ms after stimulus onset. Difference waves that were calculated by subtracting the waveform evoked by the repeated tone from the waveform evoked by the oddball tone showed that in the latency range of 40 to 60 ms after stimulus onset showed that the response to the oddball was higher in amplitude to that of the repeated tone, especially for large differences in frequency. This trend was termed the P50 difference, and may be a useful metric of auditory discrimination in the bottlenose dolphin. Some caution should be made in interpreting these results because of the possible effects of experience on AEP recording, and because of the degree of individual differences.
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CHAPTER I

INTRODUCTION

The auditory system is arguably the most developed sense in cetaceans (Au, 2000b; Herman & Tavolga, 1980). While humans rely primarily on vision, visibility can be poor underwater due to turbidity and decreasing light levels at depth. The adaptations that cetaceans developed for underwater hearing resulted in an auditory system with different capabilities than the human auditory system. For example, humans have a maximum audible frequency range of 20 Hz to 20 kHz (Yost, 1994), compared to the bottlenose dolphin (*Tursiops truncatus*) estimated audible frequency range of 200 Hz to 160 kHz (Ketten, 2000). A dolphin's ability to forage, navigate and communicate with conspecifics using acoustic signals is crucial, given that “acoustic energy propagates underwater better than any form of energy” (Au, 2000b, p.4).

Bottlenose dolphins have three recognized types of vocalizations: (1) clicks used for echolocation, (2) wide-band burst-pulse sounds and (3) narrow-band, frequency-modulated whistles of a graded nature that are likely used in social communication (Herman & Tavolga, 1980). Echolocation is used to detect targets such as prey and obstacles by emitting and interpreting broadband clicks with frequencies up to 150 kHz. This capability is so well-developed that a dolphin can identify the presence of a 2.54 cm diameter solid steel sphere that is 73 meters away (Au, 2000a). Little research has been conducted on communication using burst-pulsed sounds such as “squawks” and “yelps”, but they have been observed in contexts of excitation and alarm (Herman & Tavolga,
Whistles can be distinguished from the other types of vocalizations by their distinctive contour when viewed on a spectrogram (a plot of frequency versus time). Many bottlenose dolphin whistles are audible to the human ear, with frequencies often ranging from 5 kHz to 20 kHz. Under ideal conditions, dolphin whistles can be heard underwater up to 25 km away from the source (Janik, 2000a). Possible functions of dolphin whistles include individual identification (Caldwell, Caldwell & Tyack, 1990), maintaining contact with group members (Janik & Slater, 1998), cueing the direction of travel in groups of dolphins (Lammers & Au, 2003), and alerting or recruiting conspecifics to a feeding event (Acevedo-Gutierrez & Stienessen, 2004, Janik 2000b).

The differences in the capabilities of the human auditory system and the dolphin auditory systems are due to marked anatomical differences. One of the most visible physical differences is that dolphins lack external ear lobes or pinnae. In humans, the pinna, or outer ear, helps with sound localization (Moore, 2004). The external auditory meatus (E.A.M.), also known as the ear canal, is apparently vestigial in bottlenose dolphins (McCormick, Wever, Palin & Ridgway, 1970). McCormick, Wever, Palin and Ridgway (1970) cut through the E.A.M. so that its path was interrupted, and observed no change in the strength of cochlear potentials in response to a 20.3 kHz vibration at the skin’s surface. They concluded that the sound conductivity of the surrounding tissue was sufficient to transmit vibrations to the auditory bullae without the E.A.M. acting as a pathway. Likewise, no change was observed in the strength of cochlear potentials in response to a 23.4 kHz vibration at the skin’s surface when the tympanic conus,
membrane and ligaments were surgically removed. When the ossicular chain was put under tension, the cochlear potentials in response to tones from 3 kHz to 100 kHz with an underwater sound source were reduced, although the removal of the malleus only had a small effect on the strength of cochlear potentials in response to the higher frequency tones. They concluded that the E.A.M., tympanic conus, membrane, and ligaments are not necessary for cetacean audition, and that the malleus is not essential to high frequency hearing in dolphins. So how do sounds reach the cochlea? McCormick and colleagues posited that the vibrations in the water are transmitted to the cochlea via bone conduction. The lower jaw of a bottlenose dolphin contains a fat channel that transmits sounds to the ear bones (more specifically the incus and stapes), which in turn stimulate the cochlea (McCormick, Wever, Palin & Ridgway, 1970).

Once sounds arrive at the cochlea, other anatomical differences help explain a dolphin's ability to hear high frequency tones. There are slightly more outer hairs on the dolphin cochlea than the human cochlea, but the most dramatic difference is the number of ganglion cells (Wever, McCormick, Palin & Ridgway, 1971). There are about three times as many ganglion cells in the dolphin, bringing the ganglion cell to hair cell ratio from 2:1 in humans to over 5:1 in dolphins. From this information, Wever and colleagues inferred that a) the dolphin requires more neural pathways to represent high frequency sounds, or b) dolphins represent more detailed information about auditory stimuli than humans, or both. These differences in the auditory systems of humans and dolphins help to explain the dolphins' superior frequency range.
A growing trend in the field of marine mammal bioacoustics is the use of electrophysiological measurements to assess auditory function in captive (Houser & Finneran, 2006b) as well as stranded animals (Cook, Manire & Mann, 2005; Nachtigall, Yuen, Mooney & Taylor, 2005). Both human and dolphin hearing thresholds have been measured using an electrophysiological measure known as the auditory evoked potential (AEP). AEP’s are readings of the change in voltages present on the scalp following an auditory stimulus. These are called “far field” recordings, because they do not directly record the neurons as they fire. Instead, they measure electrical changes read from the skin that are generated by the synchronous firing of neurons (McPherson, 1996). The voltages of interest are small, often on the order of microvolts (Luck, 2005).

Because of the small size of these voltages, the signal must be amplified. In studies of dolphin AEP’s, signals are often amplified 50,000 times or more (e.g., Finneran & Houser, 2006). AEP signals must also be averaged over a number of trials to reduce noise in the signal. Changes in skin voltage could be due to any number of processes in the brain, or gross muscle movements. Therefore, the AEP waveform is averaged together many times, so that voltage changes unrelated to the stimulus will be minimized. Theoretically, extraneous electrical activity averages out to zero, given a large enough number of trials (Luck, 2005). The advantages to using AEPs in cognitive studies are that the systems are relatively inexpensive (as compared to PET or fMRI), and they provide good temporal resolution. However, AEPs are not as good for specifying the location within the brain where a process is occurring (Luck, 2005).
There are several differences between the way that AEPs are recorded in humans and in dolphins. To record electrical activity from a dolphin’s brain, the active electrode must be placed over major muscle groups that are responsible for breathing and movement of the head. When the dolphin breathes, this will create an electrical artifact which researchers try to avoid by rejecting readings above a certain voltage (Ridgway et al., 1981). Typically, only two or three electrodes are used to record dolphin AEPs- a non-inverting electrode (+), an inverting electrode (-), and if a third electrode is used, a ground (or common) electrode. The difference between the (+) and (-) electrodes generates the one signal of interest, as opposed to human AEPs, which are capable of recording simultaneously from as many as 128 locations on the scalp (Luck, 2005).

Similarly to human data, hearing thresholds and frequency difference limens obtained from AEPs from dolphins are comparable to those obtained behaviorally (e.g. Finneran, Houser & Schlundt, 2007; Houser & Finneran, 2006a; Stueve & O’Rourke, 2003; Novitski, Tervaniemi, Houtilainen, & Näätänen, 2004; Yuen, Nachtigall, Breese & Supin, 2005). Finneran and Houser (2006) compared dolphin audiograms obtained behaviorally to AEP audiograms from the same animals. The behavioral thresholds obtained in a pool with low levels of ambient noise correlated well with AEP thresholds (r=0.98). Frequency difference limens, which are the smallest detectable changes in the frequency of a stimulus (Moore, 2004), have been obtained for bottlenose dolphins using behavioral (Thompson & Herman, 1975) as well as AEP (Supin & Popov, 2000) methods. Frequency difference detection abilities for dolphins have been
obtained using methods for frequency modulation detection limens (FMDL) rather than pure tones. FMDLs are obtained by asking the subject to report the detection of a difference between a pure tone and a frequency modulated tone with the same carrier frequency as the pure tone. FMDLs are less susceptible to variation due to the frequency being tested, and so they are considered to be more reliable than those obtained using pure tones (Moore, 2004).

Behavioral FMDLs were obtained in bottlenose dolphins using an operant conditioning paradigm. Thompson and Herman (1975) trained a single bottlenose dolphin to press one paddle when it detected frequency modulated (FM) tones, and press a different paddle in response to pure tones. The smaller the modulation (as a percentage above and below the center frequency), the harder it is to discriminate the FM tones from the pure tones. A staircase method was used, and a discrimination threshold was established after 10 shifts from correct to incorrect responses were recorded from frequencies both above and below the target. For 32 kHz, the mean FMDL was 0.0037, (SD = 0.0019). This can be interpreted to mean that dolphins can correctly discriminate a constant tone at 32 kHz from a frequency modulated tone, with a modulation of 119 Hz above and below the center frequency of 32 kHz. For 45 kHz, the mean FMDL was 0.0055 (SD = 0.0030). Other than the odd point at 45 kHz, dolphin FMDLs were between 0.002 and 0.004 for tones between 2-53 kHz.

Supin and Popov (2000) also estimated FMDL's using auditory evoked potentials. They presented a continuous pure tone with 16-20 ms bursts of sinusoidal frequency modulation, with varying modulation depths around the
carrier frequency of the pure tone. For example, a 45 kHz sinusoidally frequency modulated tone with a 5% modulation depth would have a carrier frequency of 45 kHz, with variations of 1125 Hz above and below the carrier frequency. The modulation depths of these FM bursts were varied. In contrast to the current study, the subjects were placed in a small bath filled with seawater, and the test tones were projected underwater 1 m from the dolphin’s rostrum. Supin and Popov were looking for evidence of an envelope following response to the FM portion of the signal, which would indicate that the FM signal is discriminable from the pure tone. A linear interpolation was used to determine the FMDL, taking into account the modulation depths just above and below the detection of an EFR response. For the two subjects in this study, the lowest FMDLs were estimated at 0.1% of the peak to peak frequency variation, which were comparable to the behavioral estimates of the FMDL reported in Thompson and Herman (1975). The authors noted that their FMDLs were conservative estimates, since their criterion depended on detecting a physiological signal in the presence of noise. Their modulation rate used, 625 Hz, is also above the optimal modulation rates (2-3Hz) in human listeners (Kay 1982).

To estimate thresholds, Supin and Popov looked for a spectral peak magnitude of at least 70 nV at 625 Hz in the envelope following response. This number was chosen as the arbitrary level at which the authors could confidently identify a response of this magnitude as distinct from background noise. Other authors, such as Finneran, Houser and Schlundt (2007) have used the magnitude-squared coherence (MSC) as an objective measure of the presence
of an envelope following response. The MSC is an estimate of the ratio of activity due to the stimulus and the total activity (including noise) at the modulation frequency (Dobie & Wilson, 1989). Because the response of interest is an envelope following response that is time-locked to the modulation frequency of the stimulus, the MSC can be calculated using the spectral response at the modulation frequency. The spectral responses at the modulation frequency are analyzed by comparing subaverages to the grand average (for example in the current study, 20 subaverages of the total, for example 20 averages of 20 epochs each for a 400 epoch trial block). If the spectral power for the subaverages is equal to that of the grand average, then the MSC score is 1; that is, the EFR is completely due to the auditory input (Finneran, Houser & Schlundt, 2007). Conversely, when the MSC is 0, the spectral power of the grand average at the modulation frequency is 0, so the response is all noise. Critical values of the MSC have been calculated (e.g. Brillinger, 1978) to determine the probability of Type I error, and thus test the statistical significance of a given MSC score. The MSC method provides a systematic and objective way of determining whether an envelope following response was present. This is important because it provides a consistent method of detecting a response across studies, but its use is limited to studies that record periodic responses such as EFR.

How do dolphins' frequency discrimination abilities compare to those of humans? Humans cannot hear the higher frequencies that Thompson and Herman (1975) tested, but they compared underwater frequency modulation difference limens (FMDLs) for dolphins and humans at 1 kHz, 2 kHz, 4 kHz, and
8 kHz. The dolphin could not discriminate as well as humans at the 1 kHz level, with the dolphin’s mean FMDL at 0.0142, as opposed to 0.0041 for humans. However, at 4 kHz and 8 kHz the dolphins slightly outperformed humans, with FMDL values of 0.0021 (4 kHz) and 0.0022 (8 kHz) as opposed to 0.0035 (4 kHz) and 0.0056 (8 kHz) for humans. In air, humans have better frequency discrimination than in water. In a classic study (using pure tone discrimination), Shower and Biddulph (1931) found human DLFs at 0.0036 for 1 kHz, 0.0023 at 4 kHz and 0.0029 at 8 kHz in air, at 40 dB re 20 μPa above the hearing threshold. Shower and Biddulph also observed that human frequency discrimination was not as good when the sounds were introduced through bone conduction (e.g. underwater). Frequency discrimination improved as the intensity of the stimuli was increased, up to 20 to 40 dB above threshold, depending on the frequency. They also noted that frequency discrimination improved when tones were presented binaurally. As a function of percentage change, the DLFs were fairly constant from 500 Hz to 8 kHz, with DLFs increasing above 8 kHz (Shower & Biddulph, 1931).

Auditory discrimination and sensory memory have also been studied using event-related potentials and the oddball paradigm. The oddball paradigm is a method in which a stream of repeated stimuli is interrupted at unpredictable intervals by an oddball stimulus. This method is used to study whether there is a difference between middle and long latency responses to the oddball and those in response to the repeated stimuli. The advantage of using this method is that it is not limited to the use of periodic stimuli like those necessary to elicit an
envelope following response, so that many different types of auditory stimuli such as pure tones or words, may be used. One particular response that is studied using the oddball paradigm is called mismatch negativity. In humans, mismatch negativity (MMN) is measured by looking for a general negative trend in the 100-250 ms latency period on the difference wave formed by subtracting the wave evoked by the repeated stimulus in the oddball paradigm and the wave evoked by the oddball stimulus (Näätänen, 2001). MMN indicates that the subject has detected a change, whether it's a different frequency, intensity, or type of auditory stimulus. An advantage of studying MMN is that it requires neither the attention nor behavioral response of the animal, so the results do not rely on trained responses to stimuli. The MMN is enhanced when the subject is attending to the stimuli, but the response still occurs when the subject is not attending to the stream of stimuli. In fact, MMN has been recorded in response to changes in auditory stimuli in sleeping newborns, anesthetized cats, guinea pigs and rats (see review Näätänen, 2001). Because no behavioral response is required, MMN is used to test auditory function in populations where conventional testing proves difficult, such as young children and people with certain psychiatric disorders. In MMN studies, experimenters often attempt to control the stimuli by either asking subjects to count the number of deviants (e.g. Haenschel et al., 2005), or by engaging the subject in a distractor task (e.g. Novitski et al., 2004).

In addition to simply indicating a preattentive detection of a change in stimulus, MMN is thought to reflect the central sound representation (CSR), (Näätänen, 2001). The change detection process involves comparing the new
auditory stimulus to the representation of the standard stimulus, which is retained in echoic (sensory) memory. When the new stimulus is different from the representation of the previous stimulus, the mismatch negativity is observed regardless of the attention of the participant. The decrease in mismatch negativity amplitude as time elapses between stimulus onset of the standard and the subsequent deviant is thought to reflect the decay in echoic memory over a short period of time (Nääätänen, 2001). Because of this relationship, MMN has been used to probe sensory memory deficits in clinical populations such as Alzheimer’s and Parkinson’s disease patients (Pekkonen, 2000) and alcoholics (Polo, Escera, Gual, & Grau, 1999).

Although the more definitive MMN studies have used human participants, animal models have also been explored. Csepe, Karmos and Molnar (1987) described an MMN-like response in domestic cats during wakefulness and sleep. The stimuli were 1 ms 80 dB re 20 μPa pure tones with a rise and fall time of 500 μs, presented to the forehead using bone conduction. The repeated stimuli were 4 kHz tones, and oddballs were 3 kHz tones, presented at a rate of 3 Hz. The 0.23 mm diameter stainless steel electrodes with a 1 mm uninsulated tip were inserted into the cats’ primary auditory cortex, secondary auditory cortex, associational cortex and vertex. Each response was averaged 64 times, and oddball stimuli were presented at a probability of 50, 33, 20, 10 or 5%. The cats showed a MMN-like response at a latency of 30 – 70 ms while waking, and at a latency range of 50 – 200 ms while in slow wave sleep. For recording sites in the auditory cortex and at the vertex, the amplitude of the middle-latency responses
to the deviant tone increased as the probability of the oddball decreased. For both recording sites during wake, the amplitude of the response to the repeated tone was fairly constant regardless of the probability of the oddball. The authors propose that these waveform changes could be compared to the human mismatch negativity because a) there were significant differences between the response to the repeated and oddball tones, b) these responses were found in different intracranial sites but most notably in the auditory cortex, and c) the changes were still present while the cats were not attending to the stimulus (during slow waves sleep).

Although mismatch negativity has not been studied in dolphins, a similar long latency response, the P550 response, has been observed when a dolphin was presented with deviant stimuli following a sequence of standard stimuli (Woods, Ridgway, & Bullock, 1986). The P550 response to deviant whistle stimuli observed in the study was likened to the P300 response in humans. In this study, the auditory stimuli consisted of 2 different pure tones, two different dolphin whistles, and a bridge whistle tone (used to signal a reward for the animal). For each trial block, standard stimuli were presented 80% of the time, and two different types of deviant stimuli were each presented 10% of the time. The greatest amplitude of P550 response was seen when the bridge whistle tone was the deviant in test tone blocks, and when dolphin whistle 2 was a deviant in the dolphin whistle 1 blocks. These results show that the P550 response in dolphin auditory evoked potentials was greatest when signals of biological significance were interspersed with signals without biological significance.
Wood, Ridgway and Bullock posited that the P550 response in dolphins may be similar to the P300 response in humans, which is an endogenous auditory evoked potential that depends on stimulus novelty as well as the psychological state and attention of the participant (Iliadou & Iakovides, 2003). The P300 response is similar to the mismatch negativity response, but the P300 requires the participant's attention and is more pronounced for biologically significant. The role of attention in the P300 and the adjacent but not identical neuronal generators for P3am (a magnetic component of P300) and MMNm (the magnetic equivalent of MMN) suggest that the two responses may reflect two different neural processes (Alho et al., 1998).

The focus of the current study was to identify general long-latency components of the AEP in the bottlenose dolphin (*Tursiops truncatus*) and to investigate the presence of a consistent pattern in the long-latency response when presented with oddballs that differed in frequency from the repeated tones, similar to the MMN response in humans. Frequency modulation difference limens (FMDLs) have been established for bottlenose dolphins using a behavioral method (Thompson & Herman, 1975), providing a useful guide to which discriminations the dolphins should and should not be able to make. In addition, Supin and Popov (2000) reported a method of measuring FMDLs using the envelope following response to frequency-modulated tones. According to existing FMDLs for bottlenose dolphins, the 0.1% FM bandwidth and higher should be distinguishable from a pure tone (subject to individual differences). For the EFR experiment in the current study, it was hypothesized that an EFR
will be recorded for the FM tones with greater than a 0.1% modulation depth. For the oddball experiments of the current study, it was hypothesized that the peak amplitudes of the difference waves for the control condition (standard and oddball of 40 kHz pure tones) would not be significantly different from zero. It was hypothesized that oddball tones would elicit a different pattern of responses than the repeated tones, as evidenced by a difference wave that was significantly different from zero. It was also hypothesized that the oddball tone would elicit a greater response compared to the repeated tone when the oddball decreased in occurrence.

The bottlenose dolphin auditory system is markedly different from the human auditory system, an adaptation to the aquatic environment where visibility is limited. Auditory evoked potentials such as the EFR that are used to assess auditory function in humans (Luck, 2005) are now used to rapidly assess hearing capabilities of both captive (Houser & Finneran, 2006) and stranded marine mammals (Cook, Manire & Mann, 2005; Nachtigall, Yuen, Mooney & Taylor, 2005). However, long latency components of the auditory evoked potential such as mismatch negativity have not been extensively studied in marine mammals. Long-latency AEPs that are not restricted to the use of periodic stimuli (required to produce the EFR) could be a useful tool to assess a dolphin’s ability to discriminate differences in a wide array of stimuli. The current study used oddball tones that were a different frequency than repeated tones to investigate

Training an animal on a behavioral measure of auditory discrimination can take months. If an AEP measure of discrimination is established, it could significantly
decrease the time that it would take to conduct studies of auditory discrimination, and it would open up the potential subject pool from specially trained animals to any animal that can remain stationary during testing.
CHAPTER II

METHODS

Subjects

Four bottlenose dolphins (*Tursiops truncatus*) housed at the Space and Naval Warfare System Center, San Diego participated in this experiment. The dolphin BLU was a 41 year old female bottlenose dolphin that weighed 425 pounds at the time of testing. The dolphin BOB was a 28 year old male bottlenose dolphin that weighed 525 pounds at the time of testing. The dolphin SAY was a 27 year old female bottlenose dolphin that weighed 540 pounds at the time of testing. The dolphin WEN was a 23 year old male bottlenose dolphin that weighed 420 pounds at the time of testing.

The four animals had different levels of experience with the experimental setup of this study. BLU and WEN had extensive experience with in-air hearing tests using the same equipment used in the current study and with spending time out of the water in the mats as was also used in the current study. BOB and SAY had little previous experience with in-air hearing tests and were not accustomed to laying still on the testing mat while out of the water. However, SAY had extensive experience participating in other experiments that required behavioral responses. The dolphins BOB and SAY both moved around in the mat, causing a problem with excessive myogenic artifacts during recording.

Hearing sensitivity in the frequency range used in the current study was tested for all four animals in air using the AEP method described by Houser & Finneran (2006b). Figure 1 shows an audiogram for the dolphins BLU, SAY,
WEN and BOB, and Table 1 shows the thresholds in dB re 1 μPa at each frequency tested. The thresholds presented in Figure 1 and Table 1 were calculated using a regression to 0 volts based on the input/output function.

Figure 1. Audiograms for BLU, SAY, WEN, and BOB, measured in air using the AEP methods described in Houser & Finneran (2006b). The thresholds presented for BLU and WEN are an average threshold from both ears, while SAY and BOB shows the left ear thresholds only. Error bars represent the standard deviation of the threshold across measurement sessions. Error bars are absent in the dolphins BOB and SAY when there were not multiple measurements at that frequency.

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Table 1.

Hearing sensitivity (dB re 1 μPa) of the dolphins BLU, SAY, BOB and WEN measured in air using AEP method described in Houser & Finneran (2006b)

<table>
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<tr>
<th>frequency (kHz)</th>
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<th>BLU right</th>
<th>SAY left</th>
<th>SAY right</th>
<th>BOB left</th>
<th>BOB right</th>
<th>WEN left</th>
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<td>-</td>
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<td>135.5</td>
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</table>

Materials

A custom-designed portable evoked potential recording system was used to measure AEPs in this study. The system consisted of an amplifier for the outgoing stimuli, a bioamplifier for the incoming AEP readings, high and low pass filters, and a PC system running custom software (Finneran, Houser, & Schlundt, 2007) to acquire data and present stimuli. Auditory evoked potentials were measured using 10-mm gold cup electrodes (Grass FH-E6G series) embedded in 25-mm diameter silicon (Rhodia V-1065) suction cups. The stimuli were generated in the custom software, sent to the outgoing amplifier, and presented to the dolphin using a jawphone (Brill, Moore & Dankiewicz, 2001). The jawphone consisted of a B&K 8105 spherical hydrophone embedded in degassed silicone rubber, tipped with a suction cup to attach to the jaw. The
jawphone was moistened with water and attached using the suction cup to the pan bone of the dolphin, a pathway that is important to sound transduction in the bottlenose dolphin (Bullock et al., 1968). The intensity of outgoing stimuli was estimated by measuring the underwater rms sound pressure levels (SPLs) 15 cm from the center of the jawphone, as previously described (Finneran & Houser, 2006; Houser & Finneran, 2006). The 15-cm distance between jawphone and calibration hydrophone was based on the distance between the jawphone attachment point and the auditory bullae as estimated via computed tomography (Houser et al., 2004). The present study emphasizes the comparison of AEP recordings in response to frequent and infrequent stimuli, so the use of the jawphone to present tones is acceptable.

Testing Electrode Contact

Prior to recording, the electrode contact was tested by looking for an EFR response to a 25 ms 40 kHz sinusoidally amplitude modulated (SAM) tone at 130 dB re 1 \( \mu \)Pa. The modulation depth was 100% and the modulation frequency was 1 kHz. The outgoing stimuli were filtered from 1 to 150 kHz. Each recorded epoch was 35 ms in duration, with an analysis window from 5 to 27 ms after stimulus onset. The sampling rate was 10 kHz. A 300 Hz to 3 kHz bandpass filter was applied to the incoming data, and the incoming signals were amplified by a factor of \( 10^5 \). According to previously obtained audiograms, the dolphins being tested could all hear a 40 kHz SAM tone at 130 dB re 1 \( \mu \)Pa. If the EFR response was detected, as confirmed by a significant MSC at the 0.01 level, the amplitude of the response at the modulation frequency of 1 kHz was used as a
metric to decide whether electrode contact was considered acceptable. The signal level criteria were based on previously observed response levels that yielded acceptable results, and the criteria were established independently for each animal. The signal level criteria were as follows: BLU - 500 nV, BOB - 100 nV, SAY 50 - nV, WEN - 100 nV.

Data acquisition settings for long-latency AEP recording

For the oddball experiments, the bandpass filter was changed to 1 Hz - 100 Hz, and the incoming signals were amplified with $2 \times 10^4$ gain. Before recording, an oscilloscope was used to monitor electrical activity to look for the presence of an EKG artifact. If an EKG signal was identified, the reject level was set so that any epochs with an EKG in any part of the 500 ms epoch should have been rejected. However, some accepted epochs likely contained EKG artifacts because of the imprecise nature of this precaution. For the oddball experiments, the recording epoch was 500 ms, and the 100 ms stimuli were presented at a rate of 2 per second. The sampling rate was 500 Hz. The stimuli for all long-latency trials were 100 ms pure tones with 10-ms linear rise/fall times. This stimulus length and epoch duration were based on those used by Novitski et al. (2004). The frequency of the tones varied by condition, but they were all presented at 130 dB re 1 μPa, as indicated by the jawphone calibration discussed previously.

Experiment 1: Mapping

A series of recording sites were tested to determine the best electrode placement for measuring long-latency auditory evoked potentials in dolphins.
AEPs were measured with the noninverting (⁺) electrode at each of the following sites: along the midline at 4 cm, 5 cm, 6 cm and 7 cm posterior to the blowhole, and 4 cm lateral to the blowhole, contralateral to the ear being tested, at 4 cm, 5 cm and 6 cm posterior to the blowhole. The inverting (⁻) electrode was placed just behind the external auditory meatus contralateral to the ear being tested. The ground electrode was placed along the midline either approximately 15 centimeters anterior to the dorsal fin or on the dorsal fin itself.

The stimuli used in the mapping experiment were 876 ms 40 kHz pure tones at an SPL of 130 dB re 1 μPa. The outgoing stimuli were filtered from 1 to 150 kHz. This stimulus was the same used by Woods, Ridgway and Bullock (1986) when recording intracranial long-latency AEPs in a bottlenose dolphin. AEPs were recorded using each electrode configuration listed above with the dolphin BLU. The combination of recording sites with the most clear and consistent response was chosen for the electrode placement in Experiment 3.

Experiment 2: EFR to FM stimuli

To test the frequency discrimination abilities of two of the test animals (BLU and BOB), a discrimination experiment similar to Supin and Popov (2000) was conducted to test for an envelope following response to sinusoidally frequency-modulated tones. The same equipment was used as Experiment 1. The bandpass filter for Experiment 2 was set to 300 Hz – 3 kHz, with 10⁵ gain. The stimuli were 25 ms frequency modulated tones with a 1 ms linear rise/fall at a carrier frequency of 40 kHz. The modulation frequency for these tones was
625 Hz, as suggested in Supin’s article. The following FM bandwidths were tested: 10%, 6%, 5%, 4%, 3%, 2%, 1%. For each condition, 500 averages of the evoked response were recorded. The epoch duration was 35.3 ms with an analysis window from 5 to 27.4 ms. The analysis window length of 22.4 ms was chosen because the FM signal had a modulation period of 1.6 ms, and an integral multiple of the modulation period was necessary to produce a spectral value at the modulation frequency, thus avoiding spectral leakage. Epochs with a peak voltage greater than 20 μV were excluded from analysis. MSC was used to determine whether a response was detected (Finneran & Houser, 2006) at the modulation frequency. The MSC was calculated over 20 sub-averages, and an alpha level of 0.01 was used to determine statistical significance.

The dolphin BLU participated in an addendum to Experiment 2 where the stimuli were 48 ms FM tones with a carrier frequency of 40 kHz and modulation depths of 10%, 5%, 4%, 3%, 2%, 1%, 0.8%, 0.5%, 0.1%, 0.05%, and 0.01%. For this experiment, the analysis window was 5-44.8 ms with an epoch length of 65 ms. The reject level for both parts of Experiment 2 was 20 μV.

Experiment 3: Oddball paradigm

The purpose of Experiment 3 was to identify areas of the AEP waveform where differences exist between responses to the repeated and oddball tones. The electrode placement that provided the best signal according to Experiment 1 was used. For Experiment 3, the bandpass filter was set to 1-100Hz and 2 x 10^4 gain was applied. The repeated stimuli were 100 ms tones with a frequency of
40 kHz. Oddball tones varied in frequency depending on the trial, but there was never more than one type of oddball tone tested in the same trial block. In most cases, approximately 50 oddballs and 200 non-rejected repeated tones were collected in each trial block, but this depended on the reinforcement schedule of the animal being tested. Usually five to six trial blocks were presented to the animal in one testing session. Each block of trials contained repeated tones and one type of oddball tone, which was presented at a probability of 10%, 20%, or 30% depending on the condition. The first 15 stimuli in each trial block were the repeated tone, so that the pattern of repetition could be established. The oddball tones that followed were pseudo-randomized so that two oddball stimuli were never presented consecutively and at least two repeated tones would precede each oddball. Both repeated and oddball tones were presented at 130 dB re 1 μPa, at a rate of 2 tones per second. All outgoing stimuli were filtered from 1 to 150 kHz.

**Differential Responses to rare vs. common oddballs**

To test the response to the oddball depending on the probability of oddball presentation, SAY and BLU participated in a condition where 40 kHz was the repeated tone, and 30 kHz oddball tones were presented at probabilities of 10%, 20%, and 30% in separate trial blocks.

**Control condition: Switching the frequency of the repeated and oddball**

To test whether the responses remained the same if the repeated tone and oddball were reversed, BLU participated in a condition where 30 kHz was the repeated tone and 40 kHz was the oddball.
Control condition: Identical repeated and “oddball” tones

The control condition consisted of identical 40 kHz “repeated” and “oddball” tones, to identify any artificial differences between the standard and oddball waveforms. BLU, SAY, WEN and BOB all participated in the control condition.

Control condition: Shorter 10 ms stimulus

To test whether the observed long latency responses were merely a product of the length of the stimulus, the dolphin SAY participated in a trial where the 40 kHz repeated tone and 30 kHz oddball tone were both 10 ms in duration, with either a 1 ms rise and fall time or no rise/fall. If for example the P150 was an “off” response occurring approximately 50 ms after the stimulus ends, the P150 response would not be present for a 10 ms stimulus.

Responses to oddballs depending on the percent of frequency deviance

To test the responses to oddball tones as they grew more similar to the repeated tones, 40 kHz tones served as the repeated and the following frequencies were used as oddballs, occurring in 20% of trials: 30 kHz, 39 kHz, 39.25 kHz, 39.5 kHz, 39.75 kHz, 39.875 kHz, and 39.938 kHz. These oddballs represent the following percentages of frequency differences to the repeated tones: 25%, 2.5%, 1.875%, 1.25%, 0.625%, 0.3125%, and 0.155%. Due to differences in the access to each animal, not all subjects participated in all of these conditions. BLU and SAY both participated in all of the above oddball conditions. WEN participated in the 39.5 kHz, 39.75 kHz, and 39.875 kHz oddball conditions. BOB participated in the 30 kHz, 39 kHz and 39.5 kHz
conditions.

**Oddball-only condition**

An “oddball-only” condition was performed to compare responses to different tones used as the oddball tone in other trials, but without intervening repeated tones of a different frequency. The oddball-only condition “oddballs” were presented in 20% of trials, but there were no intervening repeated tones. BLU participated in “oddball-only” trials with “oddballs” of 30 kHz, 39 kHz, and 39.5 kHz. SAY participated in “oddball-only” trials with “oddballs” of 30 kHz and 39.5 kHz. WEN participated in an “oddball-only” trial with 39.5 kHz “oddballs”, and BOB participated in “oddball-only” trials with 30 kHz and 39 kHz “oddballs”.

**Data analysis**

In all conditions, the waves evoked by the repeated and oddball stimuli were averaged separately during data collection. A data analysis program was customized using MATLAB software. To standardize data analysis, all epochs containing peaks with amplitudes greater than 50 µV were rejected from analysis. This reject level was based on the highest amplitude P50 responses recorded during the oddball-only condition. The reject level was based on a review of individual epochs rather than the grand average waveform. After all epochs for identical conditions were compiled separately for each subject, difference waves were calculated by subtracting the grand average wave evoked by the repeated tone from the grand average wave evoked by the oddball tone. By this method of calculation, a positive value for the difference wave indicated that the oddball tone evoked a higher amplitude response than the repeated tone. After visually
inspecting the difference waveforms, one-tailed independent t tests were used to
determine whether areas with apparent differences were statistically significant.
The independent t test rather than a repeated-measures t test was chosen
because there was no meaningful way to pair individual epochs from the oddball
and repeated tone conditions as required for a repeated-measures t test. The
Satterthwaite’s approximation of degrees of freedom was used because the
sample came from a population with unknown and possible unequal variance
(Ames & Webster, 1991). The alpha level used for these t tests was 0.05.

For the P50 response, the latency at which the largest difference in
amplitudes between the grand averaged wave for the repeated tone and the
grand average wave for the oddball wave occurred between 40 ms and 60 ms
was used as the basis for the t test. One-tailed independent t tests were used to
compare, the amplitudes for each individual epoch in response to the repeated
tone to those in response to the oddball tone at the latency (in the 40 ms to 60
ms window) with the greatest amplitude difference. A t test was performed on
the amplitude of the response at the determined latency for all accepted epochs
of the specific oddball condition being tested. The same method was used for
the N75 response, using the largest negative amplitude of the mean difference
wave between 64 ms and 86 ms. This method of statistical analysis was
modeled after those used by Novitski et al. (2004). These measurements were
done separately for each animal and each oddball condition.

All graphs in the following section are presented with positive values “up”-
that is, positive amplitudes are on the upper portion of the x axis. While human
event-related potential research often presents graphs as “negative up”, this convention has not been historically used in the marine mammal evoked potential literature.
CHAPTER III

RESULTS

Mapping

The most clear and consistent AEP waveforms were measured with the noninverting (+) electrode 4 cm posterior to the blowhole, and 4 cm lateral to the midline on the opposite side of the ear being tested. The inverting (-) electrode remained just behind the external auditory meatus, contralateral to the ear being tested. The ground electrode was placed on the dolphin’s body along the midline about 10 cm anterior to the dorsal fin.

EFR response to FM tones

For the 25 ms stimuli, the 1% modulation depth was the lowest depth with a significant MSC for the dolphin BLU. The MSC value, used to test the significance of the evoked response, was 0.6205 for the 1% modulation condition, well above the critical value of 0.2152. For the 48 ms stimulus, BLU’s lowest significant modulation depth was also 1%, with an MSC value of 0.3611. This corresponds to a successful discrimination of a tone which featured frequency modulation of 200 Hz above and 200 Hz below the carrier frequency of 40 kHz. Figure 2 shows the evoked response waveforms resulting from exposure to the frequency modulated tones, in decreasing modulation depths for the dolphin BLU. Figure 3 shows the response spectra from the 25 ms FM tones at 5%, 4%, 3%, 2% and 1% modulation depths. Note the peaks at the modulation frequency of 625 Hz. All of the responses in Figures 2 and 3 were significant. Figures 4 and 5 show the waveforms and spectras, respectively, of the EFR
responses to the 48 ms FM tone with a 40 kHz carrier and modulation depths of 5%, 4%, 3%, 2%, 1%, 0.8%, 0.5%, 0.1%, 0.05%, 0.01%, with the largest modulation depths starting at the top of the graphs.

Figure 2. Evoked response waveforms for 40 kHz, 25 ms frequency modulated tones with modulation depths of 5%, 4%, 3%, 2%, and 1% for the dolphin BLU.
Figure 3. Evoked response frequency spectra for 40 kHz, 25 ms frequency modulated tones with modulation depths of 5%, 4%, 3%, 2%, and 1% for the dolphin BLU. Note the peaks at the modulation frequency of 625 Hz.

Figure 4. Evoked response waveforms for 40 kHz, 48 ms frequency modulated tones with modulation depths of 5%, 4%, 3%, 2%, 1%, 0.8%, 0.5%, 0.1%, 0.05%, 0.01% for the dolphin BLU.
Figure 5. Evoked response spectra for 40 kHz, 48 ms frequency modulated tones with modulation depths of 5%, 4%, 3%, 2%, 1%, 0.8%, 0.5%, 0.1%, 0.05%, 0.01% for the dolphin BLU.

The lowest detected envelope following response to a 25 ms frequency modulated tone for the dolphin BOB was to the 4% modulation depth, with an MSC value of 0.5076, which is greater than the critical value of 0.2152. This corresponds to a successful discrimination of a tone with frequency modulation 800 Hz above and 800 Hz below the carrier frequency of 40 kHz. However, BOB's recording had a lot of noise in the signal, which may have been due to excessive movement during recording, or difficulty in placing electrodes optimally, as BOB had a larger head circumference than some of the other animals. Of the four animals tested, BOB had the least experience with this testing setup.

*Differential Responses to rare vs. common oddballs*

Figures 6 and 7 show the difference waves produced by subtracting the waveform evoked by the 40 kHz repeated tone from the waveform evoked by the
30 kHz oddball tone with probabilities of 30%, 20% and 10% in the dolphins BLU (Figure 6) and SAY (Figure 7). For BLU, at the P50 site of the difference wave, the greatest amplitude of the difference wave occurred for the 10% probability oddball (\(M = 14.69, \text{SD}_0 = 11.44, \text{SD}_r = 11.31, t(401.95) = 12.98, p < 0.001\)), followed by the 20% probability oddball (\(M = 7.00, \text{SD}_0 = 13.06, \text{SD}_r = 12.05, t(800.89) = 7.92, p < 0.001\)) and finally the 30% probability oddball (\(M = 4.37, \text{SD}_0 = 10.87, \text{SD}_r = 10.87, t(658) = 5.16, p < 0.001\)). For SAY, the P50 site on the difference waves for the 10% probability oddball (\(M = 10.30, \text{SD}_0 = 12.82, \text{SD}_r = 11.74, t(478.32) = 9.22, p < 0.001\)) and 20% probability oddball (\(M = 11.28, \text{SD}_0 = 12.43, \text{SD}_r = 12.10, t(427.68) = 9.53, p < 0.001\)) had similar amplitudes at the P50 site, but the 30% probability oddball (\(M = 4.58, \text{SD}_0 = 13.33, \text{SD}_r = 10.06, t(414.8) = 4.10, p < 0.001\)) produced a difference wave with a much smaller P50.

The difference waves for both BLU and SAY showed a significant N75 deflection on all of the different oddball probabilities except for BLU at 30% probability (\(M = -0.30, \text{SD}_0 = 9.22, \text{SD}_r = 10.87, t(657.8) = -0.41, p = 0.340\)). There was no consistent pattern of N75 difference wave amplitudes varying with rarity of the oddball (see Table 2). The t-test results for the P50 and N75 responses to 10%, 20% and 30% probabilities of oddballs for the dolphins BLU and SAY are shown in Table 2.

Figure 8 is the difference waveform for the dolphin BOB in response to 40 kHz repeated tones and 30 kHz oddballs at 20% probability. This difference wave shows a similar pattern, but with the positive deflection occurring at a greater latency. The P50 difference was not significant (\(M = 1.56, \text{SD}_0 = 12.07, t(658) = \)).
SDr = 14.09, t (437.52) = 1.23, p = 0.109). At a latency of 60 ms, the greatest latency used for the P50 analysis, the mean difference was only 1.56 ms. The N75 difference was significant, also at the latest latency used for the analysis, 86 ms (M = -2.92, SD₀ = 12.43, SD𝑟 = 14.09, t (434.23) = -2.42, p = 0.008). However, the recordings for the dolphin BOB were erratic due to myogenic artifacts.
Figure 6. Difference waves for BLU when the repeated was a 40 kHz pure tone and the oddball was a 30 kHz tone with either 30%, 20% or 10% probability.

Figure 7. Difference waves for SAY when the repeated was a 40 kHz pure tone and the oddball was a 30 kHz tone with either 30%, 20% or 10% probability.

Figure 8. Difference wave for BOB when the repeated was a 40 kHz pure tone and the oddball was a 30 kHz tone with a 20% probability of occurrence.
Table 2
P50 and N75 Auditory Evoked Responses to 40 kHz repeated tones and 30 kHz oddball tones presented at rates of 10%, 20% and 30% for the dolphins BLU and SAY.

<table>
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<th>Subject</th>
<th>Frequency of the repeated tone (kHz) / Frequency of the oddball tone (kHz)</th>
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<th>Mean amplitude difference between responses to the repeated and oddball tones (µV)</th>
<th>SD, oddball</th>
<th>SD, repeated</th>
<th>t statistic</th>
<th>Degrees of freedom</th>
<th>p value</th>
<th>Number of averages</th>
<th>latency (ms)</th>
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</table>
Control conditions

The experiments shown in Figures 9, 10 and 11 all served as types of control conditions. Figure 9 shows the difference waves for the dolphin BLU when the frequencies of the repeated and oddball tones are reversed— in one condition the repeated tone is 40 kHz and the oddball is 30 kHz, in the other the repeated is 30 kHz and the oddball is 40 kHz. When the repeated tone was 40 kHz and the oddball tone was 30 kHz, there was a significant difference at the P50 (M = 7.00, SD₀ = 13.06, SDᵣ = 12.05, t(800.89) = 7.92, p < 0.001) and N75 (M = -2.96, SD₀ = 10.75, SDᵣ = 12.05, t(802.45) = -3.79, p < 0.001) sites. When the conditions were reversed and the repeated was 30 kHz and the oddball was 40 kHz, there were also significant differences at both the P50 site (M = 9.42, SD₀ = 11.24, SDᵣ = 9.47, t(384.84) = 9.04, p < 0.001) and the N75 site (M = -3.58, SD₀ = 10.78, SDᵣ = 9.47, t(388.31) = -3.54, p < 0.001). Figure 9 demonstrates that for the dolphin BLU, the same pattern of a P50 and an N75 deflection occurs when the oddballs are reversed, so the response is not simply due to different responses to those specific frequencies.

In Figure 10, the difference waves for the dolphin SAY for a 40 kHz repeated tone and 30 kHz oddball are shown with different stimulus durations: 10 ms with 1 ms rise/fall, 10 ms with no rise/fall, and 100 ms with 10 ms rise/fall. For the short stimulus condition with a 1 ms rise and fall time, the difference wave showed significant response at P50 (M = 5.27, SD₀ = 11.26, SDᵣ = 12.22, t(508.59) = 5.09, p < 0.001), but not N75 (M = -1.30, SD₀ = 12.03, SDᵣ = 12.22, t(511.83) = -1.24, p = 0.108). When the stimuli were 10 ms with no rise/fall, the
P50 difference was significant (M = 4.35, SD\textsubscript{o} = 10.75, SD\textsubscript{r} = 12.56, t(607.5) = 4.65, p < .001) and the N75 difference was significant as well (M = -1.93, SD\textsubscript{o} = 11.65, SD\textsubscript{r} = 12.56, t(620.52) = -2.02, p = 0.022). Note that the amplitude of the difference at P50 is larger for the 100 ms stimulus, but that the P50 difference persists when the stimulus is only 10 ms in length, so it is not caused solely by stimulus length.

Figure 11 shows the difference waves for all four animals when the repeated and oddball tone are identical 40 kHz 100 ms tones. Table 3 shows the t-test results at the P50 and N75 sites for each animal. Several of the responses had p values below 0.05 for the control condition (for example the N75 responses for the dolphins BLU and SAY), indicating a significant difference in waveforms evoked by identical stimuli. Only BOB showed a significant P50 difference for this control condition (M = 1.98, SD\textsubscript{o} = 12.04, SD\textsubscript{r} = 12.45, t(469.47) = 1.75, p = 0.040). Note that BOB’s waveform was somewhat erratic, mirroring data acquisition difficulties that were present in Experiment 2. Both BLU (M = -1.18, SD\textsubscript{o} = 9.40, SD\textsubscript{r} = 9.59, t(797.87) = -1.79, p = 0.037) and SAY (M = -3.29, SD\textsubscript{o} = 14.34, SD\textsubscript{r} = 13.51, t(366.62) = -2.14, p = 0.017) showed significant N75 differences when the stimuli were identical.
Figure 9. Difference waves for the dolphin BLU when the repeated and oddball tone are reversed— in one condition the 40 kHz tone was the repeated tone and 30 kHz was oddball, and in the other condition the oddball was 40 kHz.

Figure 10. Difference waves for the dolphin SAY when the 30 kHz oddball tone was either 10 ms in (with 0 ms or 1 ms rise and fall) or 100 ms in duration.

Figure 11. Difference waves for the dolphins WEN, SAY, BOB, and BLU when the repeated and "oddball" tones were both 40 kHz (no difference).
Table 3
Auditory Evoked Responses to the control condition, where the repeated and "oddball" tone were identical 40 kHz 100 ms tones, for the dolphins BLU, SAY, WEN, and BOB

<table>
<thead>
<tr>
<th>Subject</th>
<th>Frequency of the repeated tone (kHz) / Frequency of the oddball tone (kHz)</th>
<th>Response</th>
<th>Mean amplitude difference between responses to the repeated and oddball tones (pV)</th>
<th>SD, oddball</th>
<th>SD, repeated</th>
<th>t statistic</th>
<th>Degrees of freedom</th>
<th>p value</th>
<th>number of averages</th>
<th>latency (ms)</th>
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<tbody>
<tr>
<td>BLU</td>
<td>40/40</td>
<td>P50</td>
<td>0.89</td>
<td>11.38</td>
<td>9.59</td>
<td>1.20</td>
<td>775.62</td>
<td>0.115</td>
<td>400</td>
<td>56</td>
</tr>
<tr>
<td>SAY</td>
<td>40/40</td>
<td>P50</td>
<td>1.31</td>
<td>14.91</td>
<td>13.51</td>
<td>0.89</td>
<td>364.49</td>
<td>0.188</td>
<td>185</td>
<td>60</td>
</tr>
<tr>
<td>WEN</td>
<td>40/40</td>
<td>P50</td>
<td>1.39</td>
<td>7.72</td>
<td>7.57</td>
<td>1.58</td>
<td>299.89</td>
<td>0.058</td>
<td>151</td>
<td>48</td>
</tr>
<tr>
<td>BOB</td>
<td>40/40</td>
<td>P50</td>
<td>1.98</td>
<td>12.04</td>
<td>12.45</td>
<td>1.75</td>
<td>469.47</td>
<td>0.040</td>
<td>236</td>
<td>60</td>
</tr>
<tr>
<td>BLU</td>
<td>40/40</td>
<td>N75</td>
<td>-1.18</td>
<td>9.40</td>
<td>9.59</td>
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<td>797.87</td>
<td>0.037</td>
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<td>SAY</td>
<td>40/40</td>
<td>N75</td>
<td>-3.29</td>
<td>14.34</td>
<td>13.51</td>
<td>-2.14</td>
<td>366.62</td>
<td>0.017</td>
<td>185</td>
<td>70</td>
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<tr>
<td>WEN</td>
<td>40/40</td>
<td>N75</td>
<td>-0.78</td>
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<td>7.57</td>
<td>-0.88</td>
<td>291.41</td>
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<td>72</td>
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<tr>
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<td>N75</td>
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<td>12.45</td>
<td>-0.32</td>
<td>460.68</td>
<td>0.377</td>
<td>236</td>
<td>74</td>
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</tbody>
</table>
Responses to oddballs depending on the percent of frequency deviance

Figure 12 shows the difference waves for BLU when the repeated tone was 40 kHz and the oddball was 30 kHz, 39 kHz, or 39.5 kHz. The P50 differences for BLU were significant for all three oddballs tested: 30 kHz (M = 7.00, SD_o = 13.06, SD_r = 12.05, t(800.89) = 7.92, p < 0.001 ), 39 kHz (M = 6.48, SD_o = 12.19, SD_r = 11.75, t(379.48) = 5.29, p < 0.001 ), and 39.5 kHz (M = 6.58, SD_o = 9.54, SD_r = 8.64, t(728.87) = 9.82, p < 0.001 ). The N75 response was significant when the oddball was 30 kHz (M = -2.96, SD_o = 10.75, SD_r = 12.05, t(800.45) = -3.79, p < 0.001 ) and 39.5 kHz (M = -1.87, SD_o = 8.19, SD_r = 8.64, t(736) = -3.09, p = 0.001 ) but not 39 kHz (M = -1.37, SD_o = 11.23, SD_r = 11.75, t(374.81) = -1.26, p = 0.104). For a summary of the mean differences and t test results depending on the carrier frequency of the oddball, see Table 4 for the P50 difference and Table 5 for the N75 difference.

Figure 13 shows the difference waves for SAY in response to 40 kHz repeated tones and either 30 kHz, 39 kHz or 39.5 kHz oddballs. The P50 difference was significant for all three oddball tested: 30 kHz (M = 11.28, SD_o = 12.43, SD_r = 12.10, t(427.68) = 9.53, p < 0.001 ), 39 kHz (M = 3.29, SD_o = 13.82, SD_r = 13.39, t(365.64) = 2.32, p = 0.010), and 39.5 kHz (M = 3.05, SD_o = 11.49, SD_r = 11.30, t(359.9) = 2.55, p = 0.006). The N75 difference was significant when the oddball was 30 kHz (M = -2.12, SD_o = 12.20, SD_r = 12.10, t(424.98) = -1.72, p = 0.042) but not 39 kHz (M = -2.18, SD_o = 12.92, SD_r = 13.39, t(365.39) = -1.59, p = 0.057) or 39.5 kHz (M = -0.76, SD_o = 10.52, SD_r = 11.30, t(359.96) = -0.69, p = 0.246).
Figure 14 shows the differences waves for the dolphin BOB when the repeated tone was 40 kHz and the oddball tone was 30 kHz, 39 kHz or 39.5 kHz. BOB did not show a significant P50 difference for any of the oddballs tested- 30 kHz (M = 1.59, SD₀ = 13.07, SDᵣ = 14.09, t(437.52) = 1.23, p = 0.109), 39 kHz (M = 1.41, SD₀ = 11.84, SDᵣ = 10.56, t(499.47) = 1.41, p = 0.079) or 39.5 kHz (M = 1.94, SD₀ = 11.79, SDᵣ = 12.78, t(341.8) = 1.47, p = 0.072). BOB did show a significant N75 difference when the oddball was 30 kHz (M = -2.92, SD₀ = 13.43, SDᵣ = 14.09, t(434.23) = -2.42, p = 0.008) but not 39 kHz (M = -0.94, SD₀ = 10.73, SDᵣ = 10.56, t(502.09) = -1.03, p = 0.151) or 39.5 kHz (M = -0.82, SD₀ = 11.49, SDᵣ = 12.78, t(342.11) = -0.64, p = 0.26).
Figure 12. Difference waves for the dolphin BLU when the repeated tone was 40 kHz, and the oddball was 30, 39, or 39.5 kHz.

Figure 13. Difference waves for the dolphin SAY when the repeated tone was 40 kHz, and the oddball was 30, 39, or 39.5 kHz.

Figure 14. Difference waves for the dolphin BOB when the repeated tone was 40 kHz, and the oddball was 30, 39, or 39.5 kHz.
Figure 15 shows the difference waves for the dolphin BLU when the repeated tone was 40 kHz and the oddball tone was 39.5 kHz, 39.75 kHz or 39.875 kHz. The scale on the x axis of Figures 14, 15, 16 has been changed to show only the responses in the first 100 ms, to illustrate subtle deflections in the P50 and N75 areas when the oddballs are very close in frequency to the repeated tones. To facilitate comparisons of difference waves between the dolphins BLU, SAY and WEN, the response to the 39.5 kHz oddball, shown previously in Figures 12 and 13 for BLU and SAY respectively, are reproduced in Figures 15 and 16. BLU shows a significant P50 response when the oddball is 39.5 kHz, 39.75 kHz (M = 4.26, SD₀ = 10.93, SDᵣ = 9.37, t(473.02) = 4.61, p < 0.001) and 39.875 kHz (M = 2.95, SD₀ = 11.91, SDᵣ = 12.30, t(365.62) = 2.34, p = 0.010). BLU showed a significant N75 difference when the oddball was 39.5 kHz and 39.75 kHz (M = -2.20, SD₀ = 10.06, SDᵣ = 9.37, t(469.75) = -2.61, p = 0.005) but not 39.875 kHz (M = -1.84, SD₀ = 12.38, SDᵣ = 12.30, t(365.93) = -2.61, p = 0.005).

Figure 16 shows the difference waves for the dolphin SAY when the repeated tone is 40 kHz and the oddball tones are 39.5 kHz, 39.75 kHz or 39.875 kHz. SAY showed a significant P50 response when the oddball tone was 39.5 kHz, and 39.875 kHz (M = 2.87, SD₀ = 10.81, SDᵣ = 11.13, t(351.71) = 2.46, p = 0.007) but 39.75 kHz (M = 0.49, SD₀ = 12.30, SDᵣ = 13.16, t(467.84) = 0.42, p = 0.338). There was no significant N75 difference for the dolphin SAY when the oddball was 39.5 kHz, 39.75 kHz (M = -1.71, SD₀ = 12.67, SDᵣ = 13.16, t(467.43) = -1.41, p = 0.079) or 39.875 kHz (M = -0.84, SD₀ = 11.69, SDᵣ = 11.13, t(351.98)
= -0.67, p = 0.250).

Figure 17 shows the difference waves for the dolphin WEN when the repeated tone was a 40 kHz tone and the oddballs were 39.5 kHz, 39.75 kHz or 39.875 kHz tones. The P50 differences were significant for the dolphin WEN for the 39.75 kHz oddball at a latency of 50 ms (M = 1.54, SD₀ = 8.93, SDᵣ = 7.75, t(366.81) = 1.78, p = 0.038) and the 39.875 kHz oddball at a latency of 46 ms (M = 1.11, SD₀ = 6.79, SDᵣ = 7.37, t(447.05) = 1.67, p = 0.048) but not for the 39.5 kHz oddball (M = 0.85, SD₀ = 7.98, SDᵣ = 8.93, t(471.99) = 1.10, p = 0.136). The P50 difference amplitudes that were significant for the dolphin WEN were lower in magnitude than those of the other dolphins tested because of the lower amount of variance present in the grand average waveforms for WEN compared to the other animals tested. The N75 differences were not significant for any of the oddballs tested with the dolphin WEN – 39.5 kHz (M = -0.48, SD₀ = 7.46, SDᵣ = 8.93, t(470.53) = -0.66, p = 0.256), 39.75 kHz (M = 0.33, SD₀ = 7.28, SDᵣ = 7.75, t(373.62) = 0.43, p = 0.665), or 39.875 kHz (M = 0.78, SD₀ = 6.81, SDᵣ = 7.37, t(438.81) = 1.12, p = 0.868).
Figure 15. Difference waves for the dolphin BLU when the repeated tone was 40 kHz and the oddball was 39.5, 39.75 or 39.875 kHz.

Figure 16. Difference waves for the dolphin SAY when the repeated tone was 40 kHz and the oddball was 39.5, 39.75 or 39.875 kHz.

Figure 17. Difference waves for the dolphin WEN when the repeated tone was 40 kHz and the oddball was 39.5, 39.75, or 39.875 kHz.
Table 4

**P50 Auditory Evoked Responses to frequent tones vs. oddball tones presented during 20% of trials, for the dolphins BLU, SAY, WEN, and BOB.**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Frequency of the repeated tone (kHz)</th>
<th>Frequency of the oddball tone (kHz)</th>
<th>Mean amplitude difference between responses to the repeated and oddball tones (µV)</th>
<th>SD, oddball</th>
<th>SD, repeated</th>
<th>t statistic</th>
<th>Degrees of freedom</th>
<th>p value</th>
<th>number of averages</th>
<th>latency (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLU</td>
<td>40 / 30</td>
<td>7.00</td>
<td>13.06</td>
<td>12.05</td>
<td>7.92</td>
<td>800.89</td>
<td>&lt; 0.001*</td>
<td>404</td>
<td>50</td>
<td>50</td>
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<tr>
<td>SAY</td>
<td>40 / 30</td>
<td>11.28</td>
<td>12.43</td>
<td>12.10</td>
<td>9.53</td>
<td>478.32</td>
<td>&lt; 0.001*</td>
<td>215</td>
<td>48</td>
<td>48</td>
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<tr>
<td>BOB</td>
<td>40 / 30</td>
<td>1.59</td>
<td>13.07</td>
<td>14.09</td>
<td>1.23</td>
<td>437.52</td>
<td>0.109</td>
<td>221</td>
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<td>60</td>
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<tr>
<td>BLU</td>
<td>40 / 39</td>
<td>6.48</td>
<td>12.19</td>
<td>11.75</td>
<td>5.29</td>
<td>379.48</td>
<td>&lt; 0.001*</td>
<td>191</td>
<td>52</td>
<td>52</td>
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<tr>
<td>SAY</td>
<td>40 / 39</td>
<td>3.29</td>
<td>13.82</td>
<td>13.39</td>
<td>2.32</td>
<td>365.64</td>
<td>0.010*</td>
<td>184</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>BOB</td>
<td>40 / 39</td>
<td>1.41</td>
<td>11.84</td>
<td>10.56</td>
<td>1.41</td>
<td>499.47</td>
<td>0.079</td>
<td>254</td>
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<tr>
<td>BLU</td>
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<td>8.30</td>
<td>3.18</td>
<td>151.4</td>
<td>&lt; 0.001*</td>
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<td>54</td>
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<td>1.10</td>
<td>471.99</td>
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Table 4 continued

<table>
<thead>
<tr>
<th>Subject</th>
<th>Frequency of the repeated tone (kHz) / Frequency of the oddball tone (kHz)</th>
<th>Mean amplitude difference between responses to the repeated and oddball tones (µV)</th>
<th>SD, oddball</th>
<th>SD, repeated</th>
<th>t statistic</th>
<th>Degrees of freedom</th>
<th>p value</th>
<th>number of averages</th>
<th>latency (ms)</th>
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<tbody>
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<td>10.93</td>
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<td>13.16</td>
<td>0.42</td>
<td>467.84</td>
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<td>366.81</td>
<td>0.038*</td>
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<tr>
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<td>40 / 39.875</td>
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<td>10.81</td>
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<td>2.46</td>
<td>351.71</td>
<td>0.008*</td>
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<tr>
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<td>-0.47</td>
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<td>384.84</td>
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### Table 5

**N75 Auditory Evoked Responses to frequent tones vs. oddball tones presented during 20% of trials, for the dolphins BLU, SAY, WEN, and BOB.**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Frequency of the repeated tone (kHz) / Frequency of the oddball tone (kHz)</th>
<th>Mean amplitude difference between responses to the repeated and oddball tones (µV)</th>
<th>SD, oddball</th>
<th>SD, repeated</th>
<th>t statistic</th>
<th>Degrees of freedom</th>
<th>p value</th>
<th>number of averages</th>
<th>latency (ms)</th>
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<td>BLU</td>
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<td>10.75</td>
<td>12.05</td>
<td>3.79</td>
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<td>&lt; 0.001*</td>
<td>404</td>
<td>78</td>
</tr>
<tr>
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<td>12.20</td>
<td>12.10</td>
<td>-1.72</td>
<td>424.98</td>
<td>0.043*</td>
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<td>70</td>
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<td>BOB</td>
<td>40 / 30</td>
<td>-2.92</td>
<td>13.43</td>
<td>14.09</td>
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<tr>
<td>BLU</td>
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<td>-1.37</td>
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<td>11.75</td>
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<td>-2.18</td>
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<td>-1.59</td>
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<td>-1.41</td>
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<td>10.78</td>
<td>10.14</td>
<td>-0.16</td>
<td>466.47</td>
<td>0.438</td>
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<tr>
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<td>8.64</td>
<td>-3.09</td>
<td>736</td>
<td>0.001*</td>
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<td>0.256</td>
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<td>Subject</td>
<td>Frequency of the repeated tone (kHz) / Frequency of the oddball tone (kHz)</td>
<td>Mean amplitude difference between responses to the repeated and oddball tones (μV)</td>
<td>SD, oddball</td>
<td>SD, repeated</td>
<td>t statistic</td>
<td>Degrees of freedom</td>
<td>p value</td>
<td>number of averages</td>
<td>latency (ms)</td>
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<tr>
<td>---------</td>
<td>---------------------------------</td>
<td>---------------------------------</td>
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<td>0.077</td>
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<tr>
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<td>11.13</td>
<td>-0.67</td>
<td>351.98</td>
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<tr>
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<td>-2.06</td>
<td>426.94</td>
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<td>388.31</td>
<td>&lt; 0.001*</td>
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Figures 18 and 19 show the magnitude of amplitude differences between waveforms evoked by the repeated stimulus compared to those evoked by the oddball stimulus as they are related to the percentage of frequency difference between the repeated and oddball tones for the P50 and N75 sites, respectively. The mean amplitude differences plotted in these figures can be found in Tables 4 and 5. In Figure 18, there is a pattern of a P50 difference amplitude plateau for the dolphin BLU for frequency differences of 25% to 1.25%, then declining steadily from 1.25% to 0.155%. The asterisks represent points where the P50 difference was significant at the 0.05 level. All of the P50 difference amplitudes were significant for the dolphin BLU except for the lowest frequency difference tested, 0.155%. SAY did not show as stable of a pattern of P50 difference amplitudes related to frequency difference magnitude. The P50 difference amplitude was much higher (11.28 μV) for the 25% frequency difference than for the next lowest frequency difference of 2.5% (3.28 μV). From 2.5% to 1.25% frequency differences for the dolphin SAY, the P50 difference amplitudes remained stable, but there was not a steady decline from differences smaller than 2.5%, as observed with BLU’s responses. The N75 difference amplitudes remained similar in magnitude regardless of the frequency difference tested, and did not show a pattern of significant differences depending on frequency difference of the oddball.
Figure 18. A plot of the amplitude of the P50 portion of the difference wave for the dolphins BLU and SAY, by the percentage of frequency difference between the repeated and oddball tones.

Figure 19. A plot of the amplitude of the N75 portion of the difference wave for the dolphins BLU and SAY, by the percentage of frequency difference between the repeated and oddball tones.
Figures 20 and 21 compare the grand average waveforms of the responses to 30 kHz oddball tones with presentation rates of 20% (thick line), 40 kHz repeated tones with a presentation rate of 80% (thin line) and 30 kHz “oddball-only” tones presented without intervening repeated tones (dashed line). Note that the P50 response to the oddball tone when it is presented during the repeated tone / oddball trials is similar in amplitude to the P50 response for the oddball-only trial. This pattern can be observed for the responses of both BLU and SAY.

Figure 20. Grand average waveforms for the dolphin BLU when the repeated tone was 40 kHz and the oddball tone was 30 kHz. The oddball-only condition had only the 30 kHz “oddball” presented with the same rules but with no intervening standards.
Figure 21. Grand average waveforms for the dolphin SAY when the repeated tone was 40 kHz and the oddball tone was 30 kHz. The oddball-only condition had only the 30 kHz "oddball" presented with the same rules but with no intervening standards.
CHAPTER IV
DISCUSSION

This set of experiments was an exploratory study to describe the general long-latency AEP waveform recorded non-invasively in the bottlenose dolphin (*Tursiops truncatus*) and to ascertain whether there are reliable differences between waveforms evoked by repeated and oddball stimuli. For the general long latency response in the dolphins BLU, SAY, and WEN, there was usually a positive peak at a latency of about 50 ms, a negative peak at a latency of about 75 ms, and a positive peak at a latency of about 150 ms. However, the 150 ms peak was less visible when a shorter 10 ms stimulus was used, so this was likely a response to stimulus offset in the 100 ms stimulus. The P50 and N75 deflections in the grand average waveforms were replicated in the dolphin SAY with the shorter stimulus of 10 ms.

For the oddball paradigm, there was a general pattern of significant differences between responses to the repeated and oddball tones - the response to the oddball had a significantly higher amplitude at about 50 ms in latency. The amplitude of this P50 difference was related to the magnitude of difference between the frequencies of the repeated and oddball tones in the dolphin BLU and to a limited extent in the dolphin SAY. The P50 oddball response on the grand average waveform to the 30 kHz oddballs (a 25% frequency difference) showed amplitudes similar to the P50 response when there was no intervening 40 kHz repeated tone (oddball-only trial). Thus the response to the 30 kHz oddball in this condition was not attenuated by exposure to the 40 kHz repeated
tones, but may also indicate the P50 response to the oddball is somehow related to the temporal properties of the oddball presentation. In the dolphins BLU and SAY, after an initial plateau, as the oddball and repeated tones grew closer in frequency from 2.5% to 0.155%, differences in response amplitudes at the P50 site became smaller (with the exception of the 0.3125% difference). There was no apparent relationship between frequency difference and amplitude differences of the N75 response for repeated and oddball tones.

The amplitude of the P50 response was inversely proportional to the probability of oddball occurrence. Both dolphins showed higher amplitude responses in general for oddballs presented 10% or 20% of the time compared to those presented 30% of the time. The difference in responses to 10% and 20% oddballs did not seem great enough to justify the increase in time that it would take to acquire data when oddballs were present in only 10% of trials. However, an oddball presented in 30% of trials may not elicit differences in P50 amplitude that would prove reliable enough for future studies.

The results did not show a clear correlate to mismatch negativity in the bottlenose dolphins that were tested. The fact that the difference between responses to repeated and oddball tones in the bottlenose dolphin resulted in amplitudes that were more positive for oddball stimuli (rather than negative) does not necessarily disqualify it from being an example of mismatch negativity. The placement of the electrodes can influence the polarity of the response, and no attempt was made in this study to map the optimal electrode placement to measure the P50 difference. In the other animal models of mismatch negativity,
there were multiple active electrodes to compare the amplitude of the proposed mismatch negativity at different sites (such as vertex and auditory cortex). This study included only one active electrode, placed approximately at the vertex. However, the P50 difference observed in this study did share some characteristics in common with MMN as it is seen in humans. The difference between response amplitudes to the repeated and oddball tones at the P50 site increased as the oddball probability decreased, especially in the animal BLU. Also, the difference between response amplitudes for the repeated and oddball tones was fairly stable for the larger differences in frequency between the two tones and decreased steadily as the frequency difference decreased below 2.5%. There were marked individual differences in the P50 difference amplitudes between animals, and only the animal BLU showed a reasonably consistent pattern of P50 amplitude differences related to oddball probability and magnitude of deviance from the repeated tone. These results introduce a potentially useful metric for studying auditory discrimination in the bottlenose dolphin, but referring to the P50 difference as MMN is premature. The possibility that the P50 is a function of stimulus onset should be investigated in future studies by increasing the rise and fall time of the stimulus, making the onset more gradual.

Using a significant difference in the P50 response amplitudes in response to repeated and oddball tones as a metric for determining frequency discrimination corresponded fairly well to the previously established method of looking for an envelope following response to the frequency modulation of a tone. For the dolphin BLU, data were collected for both the EFR method of determining
frequency modulated difference limens (FMDLs), and the P50 method of determining difference limens for pure tones. BLU was also the subject with the most experience with AEP experiments, and had the cleanest waveforms, so was an ideal subject for the comparison. According the FMDL, the dolphin BLU was able to discriminate a 1% peak to peak frequency modulation of 200 Hz above and below the carrier frequency of 40 kHz. With the P50 method, the lowest difference between pure tone frequencies that was statistically significant was a 0.3125% frequency difference of 125 Hz below the carrier frequency of 40 kHz. The FMDLs attempted using a frequency difference of 0.4% and 0.25% below the carrier frequency of 40 kHz did not elicit an EFR. In humans, frequency discrimination limens obtained using frequency modulated tones rather than pure tones have been found to be more stable when comparing across frequencies. Both of these AEP methods depend on detecting an electrical signal in the presence of background noise, and are probably conservative estimates of the discrimination ability of the subjects. Pairing these tests with behavioral discrimination of these stimuli would be a useful extension of this study, so that the agreement between behavioral and AEP methods of measuring auditory discrimination can be assessed. It should be noted that the statistical analyses used to determine a significant response are different for the EFR method and the P50 method. The MSC that is used for the EFR method is calculated specifically at the modulation frequency, whereas the P50 response is tested at the latency within a 20 ms range with the largest difference.

Improved methods of analyzing the difference in responses to oddballs
and repeated tones should also be investigated. If there are ways to improve the signal to noise ratio for the recordings, perhaps successful discriminations can be identified in smaller frequency differences than those tested in this study. When identical 40 kHz tones were used as both repeated and "oddball" tones, the dolphin BOB showed a significant difference at the 0.05 level. In addition, the difference waves for BOB seem to be an inverted version of those seen in the other dolphins in this study. The P50 false positive and different waveform patterns may reflect the difficulty in recording clean signals from this inexperienced animal. This problem may be a common one for animals that have not had previous experience lying out of the water on a mat while being tested. The dolphins BLU and SAY showed significant N75 differences when the oddball and repeated stimuli were identical, further illustrating that the N75 difference in response amplitudes was not a reliable indicator of change detection in this study. It is possible that the N75 amplitude is greatly influenced by attention, and that the variability in the animals' attention across testing sessions affected the results of the t test at this site.

The amount of previous experience participating in AEP experiments seems to affect the noise level in the AEP recordings, so this should be taken into consideration. The dolphins BLU and WEN had extensive experience with this paradigm before participating in this set of experiments, and the AEP grand average waveforms were much smoother and less noisy than those of BOB and SAY. However, the dolphin SAY had extensive experience participating in other research tasks, and eventually became a good participant (minimal movement
artifacts) in this experiment.

The amplitude and latency of the P50 difference may also have been affected by cognitive factors such as the animal's attention and the biological significance of the stimuli. The experimental task did not include any attempt to direct the subjects' attention to the oddball stimuli or to ask the subject to ignore stimuli. Previous oddball paradigm research with humans has often included instructions to the participant to either ignore the stimuli or to make a behavioral indication (such as pressing a button) that the oddball was detected (Novitski et al., 2004). The amplitude of the P50 difference in particular may increase if the dolphins were trained on a simultaneous behavioral discrimination task where they are asked to identify oddballs as they occur or to count the number of oddballs and report a total at the end of a trial block. Future studies should incorporate the factor of the subject's attention, so that the responses to oddball stimuli can be compared when the animal is or is not paying attention. Previous studies of the P300 response in humans (Iliadou & Iakovides, 2003) and P550 response in bottlenose dolphins (Woods, Ridgway & Bullock, 1986) have noted that the greatest differences in response amplitudes for oddball versus repeated tones are recorded when the oddball stimuli have biological significance. Future studies should incorporate oddballs with biological significance, rather than the pure tones used in this study.

Future experiments should provide more measures to control for individual differences in response amplitudes. For example, perhaps a system of electrode placements should be developed to account for individual differences of the
amount of tissue between the skull and skin surface, and relative placement depending on the circumference of the head. A similar system called the 10-20 system exists for humans so that electrodes are placed in positions so that the same brain region is recorded by each electrode regardless of the size and shape of each participant’s head. Such a system may have improved measurements for the dolphin BOB, whose AEPs were very different from the other three animals tested. In addition, the stimulus intensity should be calibrated for each animal so that it is the same level above each animal’s threshold at the test frequency. Future experiments should also incorporate a standard reject level at the time of data acquisition, so that an equal number of averages for each condition and each animal can be obtained. One problem with the current study was that once a standard reject level was established, data had already been collected. Perhaps the data would have been more consistent if the same number of averages were used to calculate responses for each condition. Alternately, the Kalman filtering technique could be used to weight individual averages so that epochs with less noise are given more weight in the grand average (Hall, 2007).

Now that long-latency responses to frequent and oddball stimuli recorded non-invasively have been reported, future studies can focus on different types of oddballs. For example, a dolphin’s ability to distinguish between two different types of conspecific whistles before and after noise exposure could be tested using the oddball paradigm. Decrements in this ability could have meaningful consequences for marine mammals, especially if the undetected changes in
conspecific whistles indicated that another dolphin had found prey items or
detected a predator. These experiments would benefit greatly by accompanying
behavioral studies to determine the amount of agreement between the two
methods.
REFERENCES


of central auditory mechanisms in cetaceans. *Journal of Comparative Physiology*, 59, 117-156.


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Näätänen, R. (2001). The perception of speech sounds by the human brain as reflected by the mismatch negativity (MMN) and its magnetic equivalent (MMNm). *Psychophysiology, 38*, 1-21.


techniques. Paper presented at the 149th meeting of the Acoustical Society of America, Vancouver, BC.


the dolphin, *Tursiops truncatus*: hair cells and ganglion cells.

*Proceedings of the National Academy of the Sciences, 68*(12), 2908-2912.

