J. comp. Physiol. 129, 223–234 (1979)

Journal
of Comparative
Physiology · A

Acoustic Evoked Activity in the Brain in Sharks

Theodore H. Bullock and Jeffrey T. Corwin

Neurobiology Unit, Scripps Institution of Oceanography and Department of Neurosciences, School of Medicine, University of California, San Diego, La Jolla, California 92093, USA

Accepted October 30, 1978

Summary. 1. Averaged evoked potentials (AEP's) were recorded in the medulla, cerebellum, mesencephalon and telencephalon of several species of carcharhinid and triakid sharks, in the anesthetized animal with microelectrodes in the exposed brain and in the unanesthetized animal with implanted electrodes.

2. A preparation is described for recording from implanted electrodes with the unanesthetized shark suspended in the water by rubber bands, subject to air- or water-borne acoustic stimuli, or electric fields or photic stimuli.

3. AEP's were found in each of the levels named above, to acoustic as well as to electric and photic stimuli. The responsive loci are discrete and small. The loci of best response are distinct for each of these three modalities. Anatomical localizations are given to within about one tenth of a neuromere but rarely to the microscopic level.

4. The form, latencies and recovery times of AEP's are given for the several levels and modalities. No interaction occurred between modalities at least with brief stimuli.

5. The best acoustic stimulus for AEP amplitude is a "click" with a resonance of a few hundred Hz. The best tone stimulus is a rapidly rising burst of about 300 Hz. This value may be a function of size of animal, species, and electrode position. The lowest sound pressure threshold observed was −8 dB re 1 μbar near the shark's head (=66 dB SPL), to a click delivered to the water surface. We do not know the velocity-wave amplitude, although it is believed to be the more relevant quantity.

6. Acoustic AEP's were markedly suppressed by background white noise or tones – best at about 100 Hz.

7. When sound was delivered very locally the largest AEP occurred if the sound source was directly over the parietal fossa in the dorsal midline of the head. When sound was delivered at a distance, from a larger speaker, experimental occlusion of the parietal fossa usually suppressed the acoustic AEP. We interpret this to support the view that the fossa is an important portal for sound.

8. In two experiments bilateral section of the VIIIth nerve twig to the macula neglecta, together with some incidental damage to the sacculus greatly reduced the acoustic AEP. This supports the view that the macula neglecta is an important concentration of acoustic receptors but does not definitely confirm that proposal. The evidence argues against any substantial role of the lateral line in these species in response to acoustic stimuli at low amplitudes.

Introduction

Until recently elasmobranchs have not been notable for their hearing. Parker (1909) struck the side of a large wooden aquarium a vigorous blow and saw a quivering of the posterior edges of the pectoral fins in *Mustelus canis*. Grading the intensity with a pendulum, he reported that after "cutting the fifth, seventh and lateral line nerves, and cocaining the pectoral regions ... the fish was found to be as sensitive to sounds as a normal fish is. This sensitiveness entirely disappeared when in addition to the operations already carried out on the fish, the eighth nerves were cut." Kritzler and Wood (1961) and Olla (1962) trained sharks to relatively strong artificial sounds. Nelson and Gruber (1963) made an important advance by introducing biologically relevant sound. Nelson and Myrberg and their coworkers (Banner, 1972; Myrberg et al., 1969, 1972, 1976; Nelson, 1967; Nelson and Johnson, 1972, 1976) have further developed the use of naturalistic sounds and added evidence

Abbreviation: AEP, Averaged evoked potential

0340-7594/79/0129/0223/$02.40

from free ranging sharks in the sea. Such animals can be attracted to a sound source from distances of more than 200 m and can rapidly turn in the correct direction toward the source. Popper and Fay (1977) provide a review. They point out that it is still unclear whether the lateral line system of mechanoreceptors is primarily responsible for this ability or the labyrinthine system and eighth nerve. These authors and especially Corwin (1977) believe that one of the non-otolithic sensory structures in the labyrinth, the macula neglecta, is particularly involved – a suggestion first made by Lowenstein and Roberts (1951) and developed by Tester et al. (1972).

An aspect of the acoustic reception that has potentially considerable interest neurologically is that the adequate stimulus for sharks is probably not the sound pressure component but the particle displacement or velocity wave component of the sound (Banner, 1967; Kelly and Nelson, 1975). At the great distances cited the aligned particle motion due to the stimulus must be exceedingly small, perhaps well below the level of some randomly directed motions of the cilia of the hair cells of the macula neglecta, including those caused by the randomly directed motions of particles inherent in the pressure component of the sound wave. Corwin (1977) suggested that hearing in sharks depends on a large number of hair cells in the macula neglecta adding their synaptic output to excite each afferent nerve fiber. The calculated mean ratio of hair cells to nerve fibers in this macula in adult gray reef sharks, *Carcharhinus menisorrah*, is about 60:1. In species with well developed maculae neglectae all the hair cells in each patch of the macula are oriented in the same direction (Corwin, 1978).

The purpose of this paper is to report physiological evidence of shark audition by the method of recording the average evoked potential (AEP) to acoustic stimuli from various levels of the brain. We characterize the earlier and later evoked responses from medulla to forebrain using this sign of response. We also give some evidence that the locus of the peripheral receptors is probably not the lateral line, but more likely to be mainly in the macula neglecta and that the sound largely enters via the parietal fossa.

Materials and Methods

24 sharks in the Carcharhinidae and Triakidae were used in this study. The carcharhinids were the blacktip reef shark, *Carcharhinus melanopterus*, and the lemon shark, *Negaprion acutidens* captured at Enewetak Atoll, Marshall Islands. The triakids were the leopard shark, *Triakis semifasciata*, and the brown smoothhound, *Mustelus henlei*, captured in California. Specimens ranged from 330–2,300 g in weight, and from 45–80 cm in total length. The brain in a 500 g *C. melanopterus* weighs 5.2 g without the olfactory bulbs and is 12 mm wide across the middle of the mesencephalon.

The sharks were first anesthetized by a few min exposure of the gills to tricaine methane sulfonate (MS222), ca. 1:1000 or less. In one series this was followed with chloralose injected intraperitoneally (20 mg/kg). In the main series the electrodes were implanted in the brain while the animal was under MS222 and within a few min, after suspending it in the following way (Fig. 1) the animal was allowed to recover from anesthesia. Thus any damaging effect of this drug on the receptors was minimized. Fish hooks were pushed about 1 mm into the skin in the middorsal line at intervals as shown, and into the sides of the snout. Light rubber bands were stretched from these to the sides of the tank so as to suspend the shark with the dorsal surface about 1 cm below the water surface. A stream of water was directed from the front toward the mouth; the shark ventilated by opening the mouth and the gill slits with a normal rhythm and force.

When the water stream was stopped to obtain the quiet background needed for averaging responses, the animal continued to ventilate, but with obviously greater effort. The shark frequently did exhibit slight swimming movements and occasionally a few seconds of strong bending or twisting occurred. The sharks usually appeared to be in good condition for many hours but were likely to fail abruptly if held for more than 12 h.

Experimental tanks of two designs were used. The larger (inside dimensions: 155 × 45 cm wide × 35 cm of water depth), used in Enewetak, was a plywood box surrounded on four sides and underneath by 7.5 cm of sand in a larger box. When using this tank the respiratory stream was recirculated water and air bubbles were operating except during quiet periods. The smaller tank (107 × 46.5 cm wide × 17 cm of water depth), used in La Jolla, was a plywood box with plexiglas sides and rested on a 135 × 67 × 1.2 cm steel plate supported by 10 tennis balls. The larger tank was in a room without acoustic shielding and ambient noise was commonly 30 μV of output, as recorded underwater within the frequency band of 30 Hz – 3 kHz with an LC32 hydrophone, under "quit" conditions (no water flow or air bubbling). This corresponds to 13 dB re 1 μbar. The noise was not white but we cannot give a power spectrum. The smaller tank was in a commercial sound-shielded double-walled steel chamber of 1.99 × 1.93 × 1.83 m inside. Some acoustic coupling came through the fixed plastic hoses for incoming and outgoing water. The background, without flow was 8 dB re 1 μbar underwater, recorded 3 Hz–3 kHz; the highest peak was at 20 Hz and the noise power fell to a plateau between 250 and 750 Hz, then fell still lower to our upper limit of analysis at 1 kHz.

Acoustic stimuli were delivered principally in one of three ways. Wide beam air borne sound was generated by a speaker suspended 66 cm over the tank in rubber slings. Localized air borne click sounds were generated by a small speaker mounted over a 2 cm diameter heavy plastic tube 16 cm long. The end was held under about 1 cm of water, within a few mm of the skin over various parts of the shark. The third method depended on a "Polyplanar model P" speaker (Electronic Research Associates, Inc.) with a 20 × 20 cm polystyrene foam sounding board floating at the water surface. The underwater acoustic pressure was monitored by an LC32 hydrophone near the shark's head, care being taken to suspend its cable with no more than rubber bands for most of a meter before it touched any support. Under the stated conditions we make no claims about the true waveform or intensity of acoustic stimuli. Typical hydrophone recordings of the stimuli are shown in several of the figures. It must be emphasized that the values given are for sound pressure although as stated in the Introduction this may not be the relevant parameter, nor proportional to it. Sound paths are so short that reflections are part of the stimulus beginning within the first millisecond. The responses described are primarily transient responses to onset of stimuli.

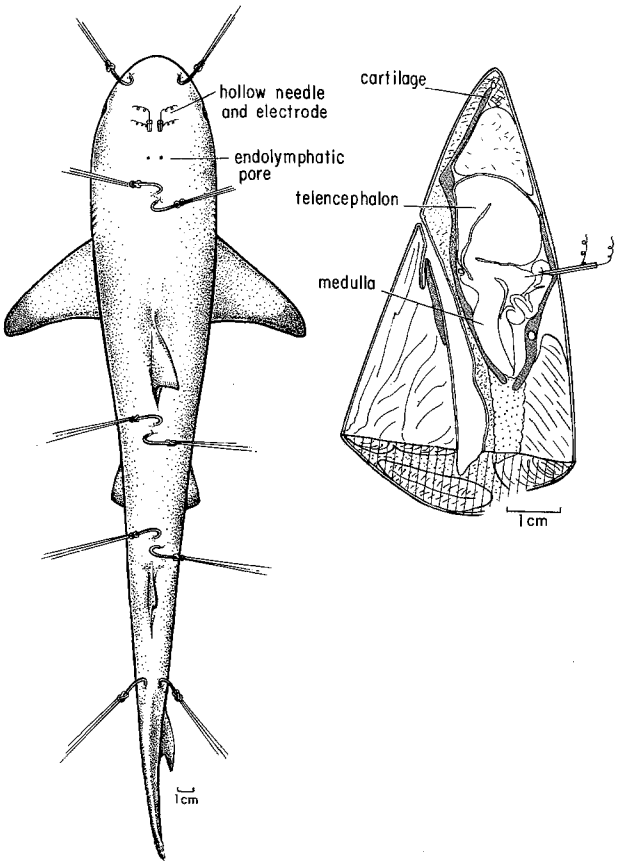

Fig. 1. Blacktip reef shark, *Carcharhinus melanopterus,* showing the method of suspending by rubber bands and implanting electrodes through shortened hypodermic needles. Inset shows a parasagittal section with electrode tip in optic tectum. Other figures come from this species and preparation

Two forms of non-acoustic stimulation were applied during the recording from each locus. Diffuse light in the form of brief flashes from a Grass model PS-2 photostimulator was delivered singly and in pairs at varied interflash intervals. Electric current was delivered in the form of square pulses 0.1–100 ms in duration, singly or in pairs or trains at rates up to 80/s, or as sine waves via electrodes across the tank that generated a large dipole, not a homogeneous field on the scale of the shark. High series resistance (200 kOhm) maintained a virtually constant current and minimized the effects of electrode polarization. Electric field intensity was directly measured as voltage gradient between microelectrodes a few cm apart in the position of the shark's head, after the animal was removed from the tank.

Microelectrodes were tungsten with baked insulation and about 1–15 MOhm resistance at 1 kHz. They were introduced in either of two ways. In anesthetized preparations, with the dorsal surface of the cranium removed, electrodes were inserted under visual control to avoid excessive dimpling when penetrating the meninx. In the implanted sharks 1 cm long tips of hypodermic needles had been inserted under anesthetic and were held by the cranium and skin; they acted as guide tubes for the microelectrodes. Leads to the electrodes were 25 μm wire, permitting movements of the shark without artifacts in the recording. Insertion, as to angle and depth, was by reckoning from measurements of frozen hemisectioned heads of similar size. Advancement of the electrode to recording depths was by hand in steps of about 0.5 mm. Lesions were made for marking selected loci by passing 1 mA for 10 s, using a 5 Hz square wave. The reference was either a remote electrode or the concentric, uninsulated, hypodermic needle which stopped short of the active electrode tip by 2–15 mm. Recording was done with conventional a–c, differential amplifiers usually 3 Hz–3kHz; averaging was done with a Nicolet model 1070.

In one series of animals a wide exposure of the brain was made under anesthetic, the shark was maintained under Tubocurarine chloride (5 mg/kg, intravenously in the tail) or chloralose and a glass micropipette broken off to a diameter of ca. 5–10 μm was used for the active electrode. The pipette contained a dye, Chicago blue (5%), which could be iontophoretically deposited to mark a recording locus. This technique rarely succeeded under our conditions.

Results

Observations will be presented for each of several major brain regions – primarily medullary, mesencephalic and telencephalic. The main result is that responses to acoustic stimuli are found in each of these regions in restricted loci that are distinct from the best loci for photic and electric responses.

A. Medulla

Evoked potentials time locked to suitable acoustic stimuli were encountered in circumscribed loci. The resolution of electrode placement and depth control did not permit a systematic comparison among the sites though we gained the impression that several distinct "hot spots" were encountered in different specimens. This seemed more likely to be due to different electrode positions as described in Section 5, below, than to different conditions of the preparations.

1. Form of Response and Times of Peaks

Figure 2A shows a representative averaged evoked potential (AEP) following a click stimulus delivered to the floating speaker. The hydrophone record of the acoustic event near the shark's head appears below. The microelectrode in the rostral lateral medulla sees a series of waves with a rough periodicity that appears to be equal to that of the stimulus. Evidently the single pulse of current in the speaker elicited a damped resonant oscillation as its acoustic output with further stimulus changes in the water tank, and this in turn a still less damped oscillation in the acoustic nervous system, with a pronounced latency. Since the hydrophone is only centimeters from any part of the shark, only a fraction of a millisecond at most can be attributed to sound propagation time in the

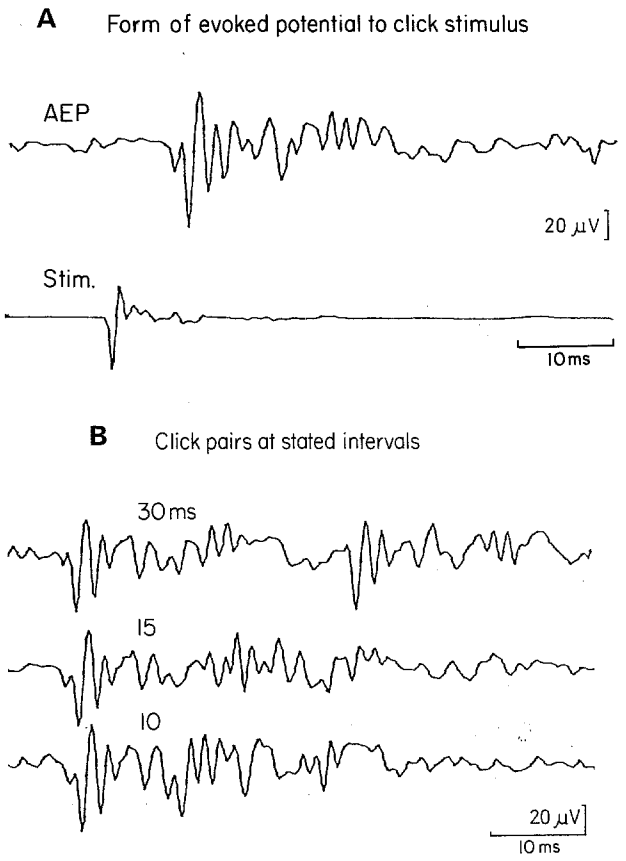

Fig. 2A and B. Averaged evoked potentials (AEP) to single clicks (**A**) and pairs of clicks (**B**) at several intervals, recorded in the anteromedial medulla. Each AEP in this and subsequent figures is the computer average of 16 responses, unless otherwise stated. Positivity of the electrode in the brain causes an upwards deflection

water. The latency can best be given from the first stimulus peak to the first AEP peak and in this instance may be taken as 6.0 or 7.5 ms depending on whether the first small AEP negativity or the first large negative peak is chosen. In other experiments loci also in the medulla, but not necessarily in the same part of the medulla, responded with first peak latencies of 5 to 15 ms. Later peaks are not always periodic (Fig. 5A) but typically there are at least 3 or 4 peaks out to 45 ms. There is sometimes a spikey component in the response that is not perfectly synchronized, and is therefore smoothed out by the averaging process; note that the waves in Fig. 2A and 2B are about 2 ms in period, suggesting well synchronized impulses in a pool of neurons.

2. Response After Prior Stimulation

Pairs and trains of clicks at various intervals evoke responses with little or no sign of facilitation, only a refractoriness and gradual recovery. The dynamics of recovery differ markedly between experiments, presumably with locus. For example, a lateral site only about 500 μm below the dorsal surface of the medulla showed complete recovery at 30 ms between clicks, about 50% recovery of AEP amplitude at 15 ms, possibly even more at 10 ms (Fig. 2B). An anteromedial and deeper locus in another shark showed virtually no recovery at 50 ms, about 25% at 70, 50% at 100 and 75% at 200 ms. Commonly, depression grows progressively if stimuli are repeated at 2/s.

3. Best Stimuli; Sensitivity

Clicks are the most effective acoustic events for evoking responses by this method. However, the clicks under our conditions are in fact an uncontrolled form of resonant transient. Tone bursts were used with two forms of repetition: phase congruent and phase non-congruent. In the latter successive bursts are passed by a tone-shaping gate that opens and closes without regard to the phase of the oscillator generating the tone. Since the computer sums AEP's that are time-locked to the gate, we obtain a slow wave nearly free of any frequency-following potential (Fig. 3). The first peak latency is difficult to specify precisely but is about 20–40 ms or less. There are only a few peaks, for example, one positive and one negative. The response is a transient or onset response; occasionally there is also a tone-off response. Tone burst duration therefore has little effect except upon the recovery. Rise time of a shaped tone burst is important; it is maximally effective at 5–10 ms.

Using a slow rise time to avoid click effects and varying the tone frequency at a constant sound pressure, the largest non-congruent AEP was found at about 300 Hz in several blacktip reef sharks. Usually no response could be detected at 50 Hz or at 800 Hz even at higher sound pressures. In the leopard shark the best frequency appeared to be lower – 200 Hz, but it would be difficult to determine whether this is a real species difference.

Congruent tone bursts reveal a frequency-following component at twice the stimulus frequency (Fig. 4). This frequency doubling appears for stimuli up to at least 300 Hz. Unlike the slow onset response of the preceding paragraph this component is maintained throughout a long tone burst.

Sensitivity is not satisfactorily measurable under our conditions, in a form readily compared with other conditions of measurement. With the considerable background noise in our larger tank, the most sensitive loci gave responses to clicks, if we averaged 128 evoked potentials, when the LC32 hydrophone moni-

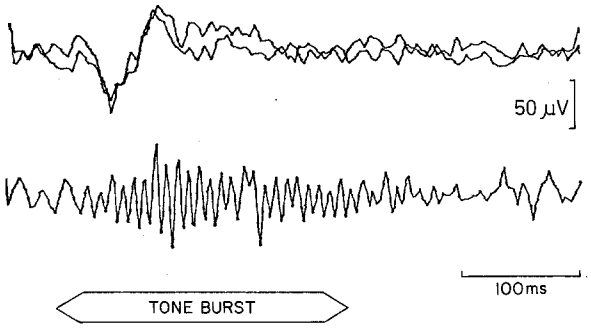

Fig. 3. Two AEP's to tone bursts, in anteromedial medulla. The 32 successive stimuli at 350 Hz whose responses were averaged were not congruent, that is the tone burst did not begin at a fixed phase of the tone frequency. The lower record is a hydrophone output from a single tone burst at 100 Hz and high amplitude. It is not averaged because the average of a series of non-congruent tones is nearly zero. It is high in intensity because at physiological intensity a single tone burst does not stand out from the background noise. The low frequency is used for clarity in reproduction

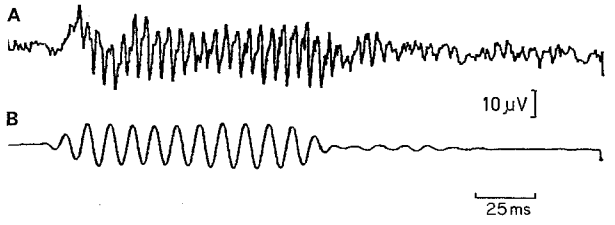

Fig. 4. A AEP to congruent tone bursts, recorded from the rostral, medial medulla. **B** The hydrophone record of the stimulus at 315 Hz. Both records are averages of 32 sweeps. Note frequency doubling in the brain

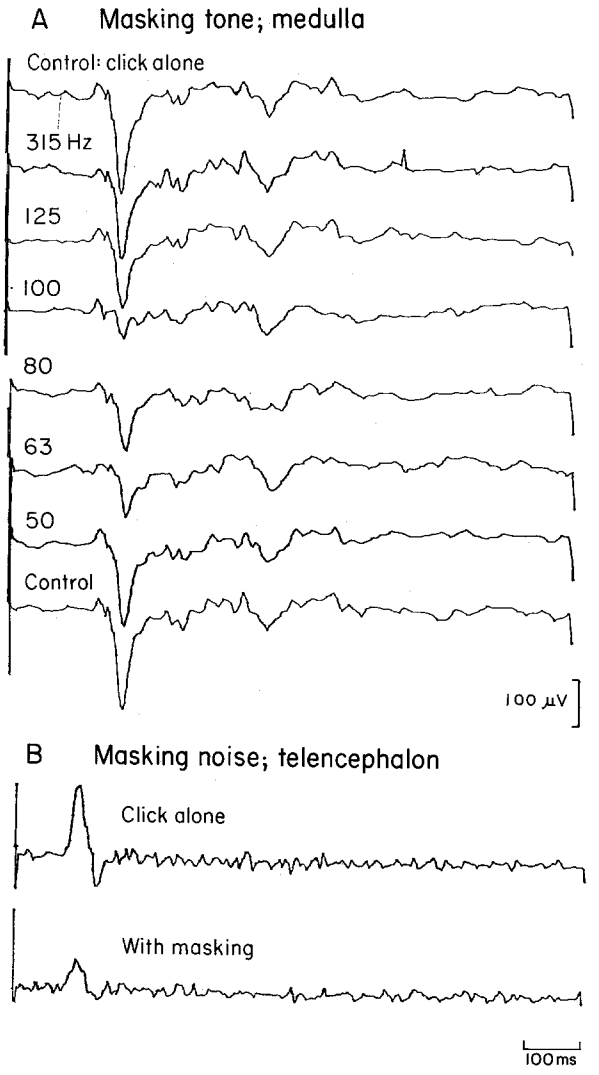

Fig. 5 A and B. Suppression of AEP to a test click by a masking background. **A** Masking with a tone of 27 dB re 1 μbar at various frequencies; **B** Masking with a white noise added to the prevailing background; the addition did not cause a measurable increase in peak to peak hydrophone output over the background. The test clicks are submaximal but not calibrated

tor output was 3 μV peak to peak (filtered to pass 30 Hz–3 kHz). This is equal to −8 dB re 1 μbar. The background noise during the experiment, recorded through the same filters, was broad-band noise of 50 μV = 17 dB re 1 μbar. Standing waves and nodes in the tank that are established in the first 3–5 ms are part of the stimulus and render such numbers only approximations of the effective stimulus. We could not place the hydrophone in the most relevant place in the tank to represent the shark's portal of sound entry because that place is not yet well enough specified.

4. Suppression

The AEP to a click or tone is markedly depressed by a background of approximately white noise, or by water running, or by a pure tone (Fig. 5). In one series a standard click AEP was most depressed by a masking tone of 100 Hz; higher and lower tones adjusted to give the same peak to peak hydrophone voltage output were less effective (Fig. 5A). We did not make a parametric study of the relative intensities of masking and test sounds.

5. Anatomical Localization

In most specimens we do not have microscopic verification of exact electrode location, for technical reasons. Gross localization was sometimes verified by fixing or freezing the specimen with electrodes in place and subsequently hand sectioning in the plane of and close to the electrode. Two general regions of the

medulla were active. One is anterolateral and dorsal, therefore in the acousticolateralis area. The other is anteromedial and deep, about midtegmental.

6. Relation to Potentials Evoked by Non-Acoustic Stimuli

AEP's to light flash stimulation are present in the medulla but are small and late. The latency of the first wave is more than 50 ms. On the available evidence we cannot decide whether this activity is actually in the tectum and recorded by current spread or is local activity due to optic projections. It is not sharply localized as the acoustic and electric AEP's are.

General cutaneous stimulation was not systematically studied. Stroking the skin, especially of the head, with a soft object elicits "hash" – a shower of spikey activity in many units. Vibration by gentle tapping of the body or a fin does the same. In neither case can we decide without cutting nerves whether the effective stimulus is partly acoustic via water or tissue conduction, as it may well be. Even stroking probably causes strong acoustic stimuli because of the rough texture of the skin. It can mask click AEP's.

AEP's to weak electric current in the sea water are of special interest because specialized receptors are known, the ampullae of Lorenzini (Kalmijn, 1974; Murray, 1974; Obara and Bennett, 1972; Andrianov et al., 1974), central responses are known (Bullock, 1978) and the electric and acoustic apparatus are both parts of the acousticolateralis system. Hence they are homologous and the issue is pertinent: are their central projections intermingled or distinct?

We found medullary potentials evoked by electric fields in the tank. They are complex, with several peaks. The first peak is usually at 12 ms if the polarity of the stimulating current is the more effective one; it may be up to 25 ms in the less effective polarity. A long series of waves and wavelets, often with spikes, lasts for more than 100 ms. The AEP is primarily a transient response; on and off responses are usually clear and of different amplitude, according to the polarity of the stimulus. Brief pulses (5 ms) are effective and still shorter pulses (0.1 ms) simply require an increase in voltage. The best stimulus for AEP is a 15–30 Hz square wave but a sine wave is almost equally effective. A very clear AEP has been recorded in several sharks with a stimulus as weak as 0.015 μV/cm, averaging 512 responses. Recovery following a moderately strong stimulus is about 75% at 50 ms and nearly complete at 100 ms.

The best loci for responses to electric fields are distinct from the best loci for acoustic stimuli. Generally, there is a visible electric AEP wherever there is an acoustic response but the contrary is not true. Our material does not permit definite statements about the relative extent of these two response modalities. They are both rather widespread mediolaterally, rostrocaudally and dorsoventrally.

The responses to these different forms of stimuli add algebraically, without interaction, when they are elicited at the same time, or at various intervals relative to each other.

B. Cerebellum

Many loci are silent to both acoustic and electric stimuli of the kinds used in the foregoing. No attempts have been made to search with more natural or with moving stimuli. Some loci respond to both clicks and electric pulses with spike bursts. The main burst to a click reaches a negative going peak at about 16 ms, (therefore called N16), and is followed by positive waves P27, P53 and several more. The only localization that can be described from the material at hand is that such responsive loci have been found in the posterior third of the corpus cerebelli, midway between the median line and the lateral border, about 2.5 mm deep to the cerebellar surface. We have not mapped the unresponsive loci.

C. Mesencephalon

Many loci have been responsive to sound and many loci have not. Many of the characteristics of the AEP responses are essentially like those from the medulla, so the following does not repeat details given above.

Click stimuli cause a complex series of waves and spike bursts. The exact form differs between loci as well as with intensity and quality of the click. First wave latencies to moderately high intensity but not maximal clicks can be as short as 6 ms but usually fall in the range of 8–18 ms. Recovery varies but is usually nearly complete by 50 ms. In some sites a period of facilitation occurs such that an AEP about 120% of control is seen when the interval between clicks is 20 ms. Since acoustic interaction due to reflections rather than neural interaction is possible, this observation can not be given much weight. The best stimuli are the same as for the medulla. A frequency-following response at twice the stimulus frequency is still seen at this level with congruent tone burst stimuli.

Localization is apparently sharper and more restricted than it is in the medulla. Moving the electrode a few tenths of a mm can dramatically change

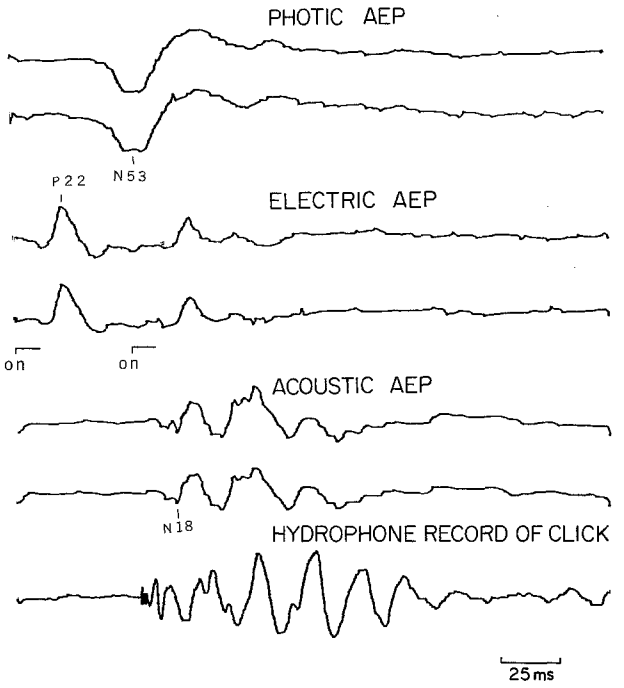

Fig. 6. AEP's from the same site in the mesencephalon to 3 forms of stimuli. The photic response followed a brief flash of diffuse light, delivered at the moment the sweep started; the electric stimulus was two 10 ms pulses of weak current (3 μV/cm at the shark's head) 50 ms apart, as shown by the two bars; the acoustic stimulus was a resonant "click" delayed 50 ms from the sweep start and shown on the hydrophone record

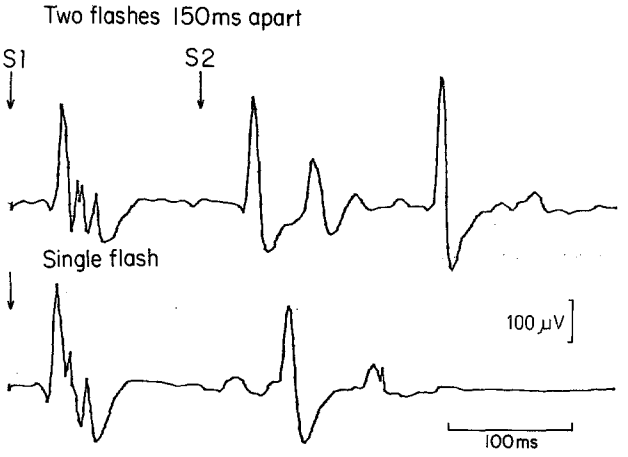

Fig. 7. Typical AEP's to light flashes – single (below) or paired (above) as recorded in the optic tectum. Note the discrete, late, slow waves. The main upwards peak to the first flash (S1) is 236 μV; 32 responses were averaged

an acoustic AEP. In different loci we have had good photic response without acoustic or electric responses, good photic and electric, good photic, electric, and acoustic (Fig. 6), electric and acoustic almost without photic, and good acoustic and photic without electric response.

The photic response at its best is very much larger than either of the others (Fig. 7) and the best electric response is somewhat larger than the best acoustic response. The order of latencies is acoustic (8–18 ms), electric (12–22 ms), and photic (45–60 ms). Photic responses are best superficially in the tectum. Electric responses are best quite deep and acoustic in between in midtegmentum. We do not have enough information to order these modalities mediolaterally. The torus semicircularis is too poorly defined in elasmobranchs to say whether it embraces the loci of acoustic and electric responses. Both acoustic and electric responsivity continue back into the large medullary region for these modalities.

As in the medulla, no interaction is seen when AEP's for these several modalities are elicited at the same time or one leading the other. Acoustic background noise masks acoustic click responses, but does not influence the size of the electric AEP.

D. Telencephalon

In very circumscribed sites in the cerebrum good responses are found to acoustic stimuli. The form of the AEP is shown in Figs. 8 and 9 – a complex series of waves including reproducible components as short as 3 ms in duration. The first large peak can be as early as 9 ms and the series of waves can last 100 ms. With weaker stimuli or in less sensitive loci the first clear deflection may crest at 20 or even 50 ms. Recovery can be very slow, in one locus after a strong response it was only slight at 200 ms. The best stimuli are the same as for lower levels. To tone bursts AEP's in a typical locus are distinct from 100 to 630 Hz, best at about 300 Hz (Fig. 9) and not discernible at 40 or at 1,000 Hz. Masking of a test response by a white noise background is as obvious as at lower levels.

Localization of acoustic sensitivity in the forebrain is rather sharp and restricted. Though loci are usually multimodal, the best responses to acoustic stimuli are in distinctly different sites from those for the best photic and even those for the best electric stimuli. The best acoustic area is in the middle third, rostrocaudally, the medial third, mediolaterally, and the lower half, dorsoventrally.

E. Evidence of the Peripheral Path

1. Role of the Parietal Fossa

Tester et al. (1972), Fay et al. (1974), Popper and Fay (1977) and Corwin (1977) have proposed that the main port of entry of acoustic stimuli is the parietal

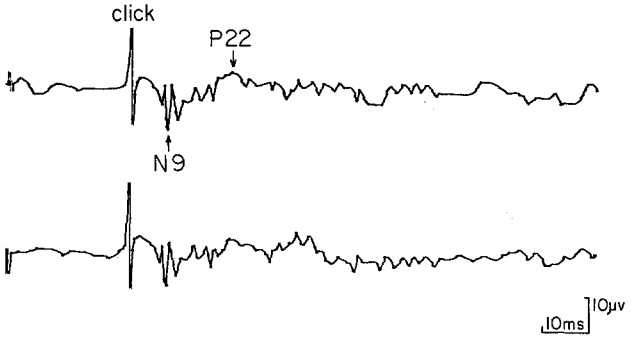

Fig. 8. Acoustic AEP's in the telencephalon to click stimuli. Replicate averages to show that significant waves occur for at least 60 ms

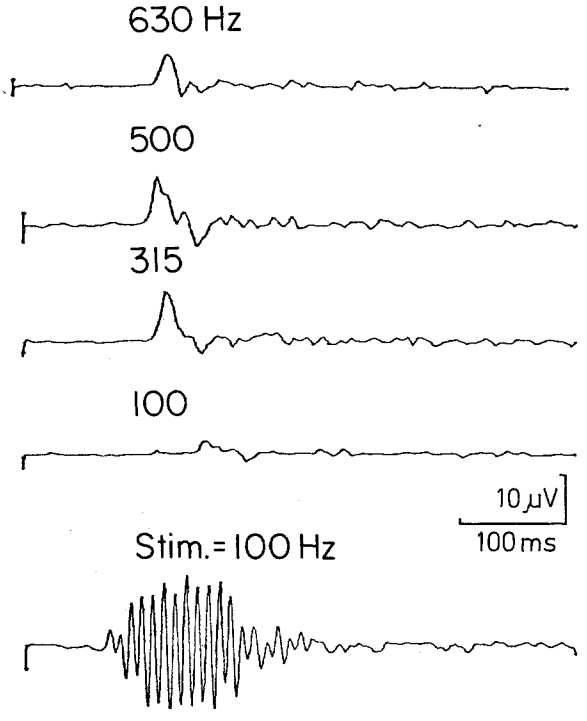

Fig. 9. Acoustic AEP's in the telencephalon to noncongruent tone burst stimuli, of which one sample is shown below

fossa – an area on top of the head where the cranium is depressed ventrally with a jelly-like tissue filling the space between the skin and the cranium, making this region feel soft to palpation. The endolymphatic ducts open to the outside in the caudal half of the fossa. If this is an important region for the entry of sounds that elicit our form of AEP in the brain, experimental interference with the parietal fossa should disturb these responses.

We have recorded AEP's in sensitive loci before and after obstructing the fossa. Obstructors have been small objects such as a 20 mm diameter disc of sponge rubber carrying a 47 g disc of lead, a rubber cylinder filled with modelling clay, and a glass tube fitted to the head by clay and closed at the top, filled with air, so that its effective weight was only 11 g at that water depth. In several experiments this has caused a marked reduction or loss of the AEP to a standard click. Removing the object restores the response; the block and restoration can be repeated at will. In some experiments objects placed approximately on the parietal fossa did not block the AEP but they usually altered its form and/or reduced its amplitude. It appears that placement is fairly critical and the type of object is important.

A special sound source was employed to test for differences in regional sensitivity. A small speaker was fitted through a funnel-like coupling into one end of a heavy plastic tube 2 cm in diameter and 16 cm long. This could be hand held or clamped and was used with the speaker in air, and the open end of the tube under about 1 cm water, within 2 or 3 mm of the skin, normal to the body surface. When this local acoustic stimulator was held over the parietal fossa, clicks evoked a good response in suitable loci of the brain using peak sound pressure levels of about 10 dB re 1 μbar (84 dB SPL), as estimated by a hydrophone placed 3 mm below the speaker tube. The same stimuli applied to the shark's surface nearby – over the head behind the parietal fossa, or to the side, or over the lateral line – evoked nothing (Fig. 10), while small responses were evoked over the front of the head as well as just lateral to the parietal fossa.

2. Role of the Ramus Neglectus

In three experiments we attempted to cut the nerve twigs to the maculae neglectae bilaterally. First, loci responsive to acoustic stimuli were found – in experiment 8-3 two loci, one in the medulla, one in the mesencephalon; in shark 8-5, one locus in the mesencephalon; and in shark 8-7, one locus in mesencephalon. Then the electrodes were fixed in place with dental cement and retested to show that they were still responsive. Testing for response at stages of the operation showed the AEP to click still present. However, when the nerve twigs were judged to be really interrupted, the AEP's were greatly reduced (Fig. 11), although AEP's to flash and electric stimuli were unchanged. This operation is difficult, especially on the species available in La Jolla. The dorsal approach necessitated by our recording arrangement did not allow direct visualization of the ramus neglectus during surgery. Only by postmortem inspection could it be confirmed that the nerves were in fact cut. In shark 8-3 it was found that in addition the dorsal

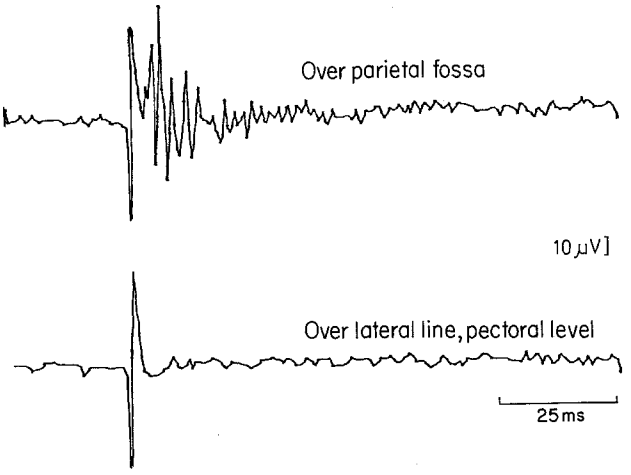

Fig. 10. Localized stimulation with a weak click delivered through a 2 × 16 cm tube with the open end held close to the body surface, under water. The first deflection is an artifact of the stimulus. Note absence of response in lower trace

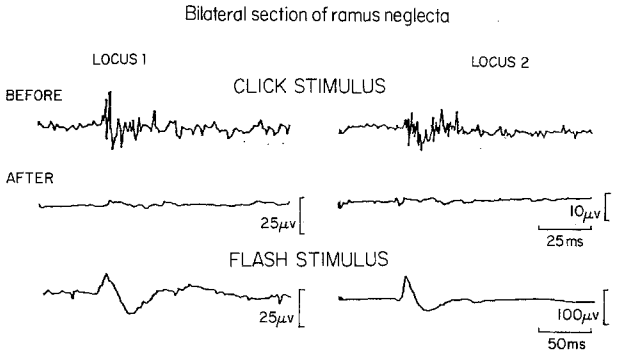

Fig. 11. Experimental section of the ramus neglectus, the nerve supplying the macula neglecta. Recording from two sites, one in the mesencephalon (Locus 1) and one in the medulla (Locus 2), with a click stimulus, averaging 16 sweeps, before and after bilateral section of the nerve. See text for details. Whereas the acoustic AEP is practically abolished, the photic response is still quite normal

sacculus in the right ear had a large cut through it and in the left ear a small cut but probably not damaging the saccular nerve. The endolymphatic space had been ruptured in each case, so that it cannot be ruled out that saccular and other receptors were impaired along with the macula neglecta. In shark 8-5 the findings at post mortem were much the same. In shark 8-7, however, a reduced but definite click response remained after the operation. Postmortem examination revealed bilateral cuts through the dorsal saccular walls rupturing both endolymphatic spaces and one cut ramus neglectus. The ramus neglectus on one side remained intact.

Discussion

The main finding of these experiments is physiological evidence of acoustic sensitivity and a *central acoustic processing system in sharks*. The behavioral evidence has been convincing (Kritzler and Wood, 1961; Olla, 1962; Nelson and Gruber, 1963; Davies et al., 1963; Wisby et al., 1964; Banner, 1967), especially with the modern work of Nelson and coworkers (Nelson, 1967; Nelson and Johnson, 1972; Kelly and Nelson, 1975) and Myrberg and coworkers (Myrberg et al., 1969, 1972, 1975). The only physiological work believed to be relevant is that of Fay et al. (1974) who recorded frequency-following potentials from the vicinity of the macula neglecta in response to vibratory stimuli applied through a small rod touching the head. We cannot be sure this has anything to do with normal acoustic reception because of the nature of the stimulus. The lowest estimate of behavioral threshold is that of Nelson (1967), who gave values for lemon sharks (*Negaprion*) in the range of −13 dB re 1 μbar. This may not be the relevant measurement. According to Banner (1967, 1972), Nelson and Johnson (1972), Corwin (1977) and Popper and Fay (1977) sharks are probably using the displacement (velocity wave) component of the acoustic disturbance, not the pressure component. The amplitude of the particle displacement can be calculated for open-water, far-field stimuli, if certain conditions are met; values of 0.02–0.06 Å have been published (Van Bergeijk, 1964; Myrberg et al., 1969). It is however difficult to equate these stimulus conditions with those in a small or even a large tank (Parvulescu, 1967; Tavolga, 1977). In practice displacement can not be calculated from a pressure measurement, especially in a small tank. Also unknown is the axis of the displacement relative to the preferred axis of the ear. We have not had available a velocity (displacement) wave hydrophone with which to measure the sound fields in our tanks directly. Our stimuli, expressed in μbars, cannot be interconverted for comparison with the behavioral sensitivity estimates of previous authors. With the same caveat we believe that sharks are much less sensitive than the most sensitive of the gas bladder teleosts but this comparison to be fair must be qualified by limiting it to the "normal" expected acoustic situation in "well behaved" environments, such as open water, since the elasmobranchs are almost certainly sensing displacements and many of the teleosts with gas bladders are clearly sensing pressure. Someone has usefully likened this to comparing the sensitivity to dead fish of anosmic sea gulls and blind cats – one of which sees and the other smells the fish. Fay (1978) gives threshold values by electrophysiological criteria of −38 dB re 1 μbar for the

goldfish, compared to our value for the AEP of −8 dB re 1 μbar. The last value would of course be lower with more averaging. Sharks may be about as sensitive to sound as teleosts that lack gas bladder specializations for acoustic reception (Chapman and Sand, 1974; Popper and Fay, 1973).

In everyday terms, the threshold clicks delivered by the 2 × 16 cm plastic tube with a small speaker in one end, sound to us when the other end is held near the ear not loaded by the usual 10 mm under water (71 dB SPL) "weak" to "moderate", not "faint" or "loud". Submaximal tone bursts from the large speaker 66 cm above the water sound to us "moderate" to "conversational", not "very loud" (88 dB SPL at the water surface). The shark in our experiments has an additional, disadvantageous air-water coupling, and may normally depend upon a larger area of its surface receiving a coherent wave than is the case with the local stimulation via the plastic tube.

Our conclusion – certainly little more than an opinion – is that the stimuli used in these experiments are not unreasonably intense, as acoustic events, and are much weaker than many normal, biologically significant acoustic signals in the sea. In this regard we also suspect that the sharks' thresholds might be lower in a quiet region of the open ocean (30 Hz to 3 kHz noise −50 dB re 1 μbar; Wenz, 1962) than they were in our laboratory tanks (30 Hz to 3 kHz noise 8 dB re 1 μbar) due to the decreased masking by the ambient noise level.

The evidence presented makes the possiblity that we are dealing with *lateral line receptors unlikely*. The intensity of the stimuli is low compared with the effective stimuli used in many, though not all studies of those receptors (Dijkgraaf, 1963; Harris and van Bergeijk, 1962; Tavolga, 1977). Moreover, the greater sensitivity over the parietal fossa than over the lateral line (Fig. 10), the susceptibility to local damage within the labyrinth (Fig. 11), and the high best frequency (ca. 300 Hz) all argue against a major participation by the lateral line.

The physiological evidence, although preliminary and based on few animals, is supportive of the proposals of Lowenstein and Roberts (1951), Lowenstein (1971), Tester et al. (1972), Fay et al. (1974) and Corwin (1977) that the *macula neglecta is especially important* for acoustic reception. It does not exclude the possible, perhaps concurrent, participation of some other maculae as well. It does suggest that refinements in surgical technique and in methods of reversibly blocking the ramus neglectus instead of cutting it, when used with favorable species, may well settle this question quite cleanly.

The physiological evidence, again preliminary and not definitive, is supportive of the proposal of Lowenstein and Roberts (1951), Tester et al. (1972), Fay et al. (1974), Popper and Fay (1977) and Corwin (1977) that the *parietal fossa is an important portal* for entry of sound to the sense organ. Under our conditions the further proposal could not be tested that the fossa is important in determining the direction of a sound source (see Corwin, 1977).

The methods used here, especially the forms of stimuli, have not brought out the *integrative capacities* of the brain centers for acoustic processing. Presumably there is at least spatial summation for enhanced sensitivity, for enhanced directionality and possibly for movement detection as well as for discriminating types of sounds by the temporal structure and frequency content. Frequency-following suggests the physiological substrate for the behavioral finding (Nelson, 1967) that these lowest of vertebrates known to hear have frequency analyzing capacities.

The *values for best frequency* for AEP amplitude – 200 to 300 Hz, are much higher than reported values obtained by the criterion of peak power of the most attractive sound source in the field (Myberg et al., 1976). Many factors could play a role in influencing each of these two methods of measurement. The animal may well hear sounds without showing either an AEP or behavioral attraction. Body size, species differences, differences in synchrony of cell firing in favor of the higher frequency tones are among the factors that might influence the AEP-measured threshold. Even if AEP is related to audibility, there is not a priori expectation that the most audible pure tone frequency will be the most attractive in the field, mixed with other frequencies. There is, however, good correspondence between our AEP-measured frequency threshold curves and those determined behaviorally in the lemon shark, *Negaprion brevirostris* (Nelson, 1967). Both measures place the best frequency in terms of absolute sound pressure at about 300 Hz and the high frequency cut-off between 300 and 600 Hz. At the low frequency end the AEP-measured threshold is elevated below 100 Hz with significant elevation at 40 Hz, while Nelson (1967) reported a relatively flat threshold down to 10 Hz. It is possible that this difference reflects the contribution of the lateral line detectors or differences in the frequency composition of the background noise. In both cases background noise levels approached the measured thresholds and may well have influenced the shape of the audiograms.

It is of interest in respect to the *evolution of the acousticolateralis system* and its central representation that we can now affirm the special modalities believed to be derived from the lateral line, in particular the acoustic and the electric, have separate central foci

of maximal responsiveness. They are apparently contiguous and overlapping and the same seems most likely to be true for the other subdivisions, the position and acceleration modality of the vestibule and the mechanoreception of the lateral line.

In view of the growing evidence that *several afferent modalities* send projections into the telencephalon, even in the elasmobranchs (Ebbesson, 1972; Schroeder and Ebbesson, 1974; Ebbesson and Northcutt, 1976; Northcutt, 1977, 1978) it is noteworthy that the acoustic system also projects to the forebrain. Moreover its locus of best response is near but distinct from that of the electroreceptor system, a closely related modality. We believe the acoustic, like the electric and photic AEP's recorded in the forebrain are due to intrinsic activity of that region, and are not the signs of field potentials from distant structures in the brain stem. We have not used the techniques of current density analysis. However, we note that (1) the acoustic and electric evoked potentials in good loci in the telencephalon are almost as large as they are in good loci in the mesencephalon, more than 10 mm away, and that the intervening loci are nonresponsive. (2) A movement of the electrode by as little as 1 mm can lose 90% of the amplitude. (3) The latencies and recovery times are in general longer in the telencephalon.

The *form of the acoustic AEP* is worthy of note in comparison with familiar evoked potentials in the mammalian cortex. At all levels from medullary to telencephalic it is a complex series of waves, mostly only 2 to 4 ms in duration, the series lasting for upwards of 100 ms. It is suggestive of well synchronized unit spike discharges that follow the oscillations of the acoustic stimulus. The AEP to electric field stimuli which are pulses or steps of DC, without oscillations, are quite similar. As already noted in tectal evoked potentials to a light flash (Platt et al., 1974), early stages in an afferent pathway are capable of exhibiting "late" waves – up to more than 500 ms after a brief stimulus. We do not see large amplitude very slow waves, that is, deflections more than 100 ms wide and many tens of microvolts, at any level from medulla to forebrain. The very high amplitude optic evoked potentials in the tectum are rarely longer than 20–30 ms. Slow wave components of any of the modalities studied are seldom larger than 10 μV.

Finally, it should be pointed out that in relation to the *comparative neurology of ongoing potentials* ("EEG", "brain waves"; Bullock, 1945, 1974) the elasmobranch telencephalon and mesencephalon are characteristic of vertebrates in general. That is, the power spectrum of ongoing activity is broad, with substantial energy above the background noise at all frequencies in the band 1–30 Hz, with a maximum below 15 Hz. In view of the structure of the telencephalon and the mesencephalon in these animals (Northcutt, 1977) it must be said that this characteristic vertebrate EEG does not depend on distinct lamination.

The facilities of the Mid-Pacific Marine Laboratory on Enewetak Atoll, Marshall Islands, were made available by Dr. S. Smith of the University of Hawaii. Grant support came from the National Institute of Neurological and Communicative Disorders and Stroke and the National Science Foundation.

References

Andrianov, G.N., Brown, H.R., Ilyinsky, O.B.: Responses of central neurons to electrical and magnetic stimuli of the ampullae of Lorenzini in the Black Sea skate. J. comp. Physiol. **95**, 287–299 (1974)

Banner, A.: Evidence of sensitivity to acoustic displacements in the lemon shark, *Negaprion brevirostris* (Poey). In: Lateral line detectors. Cahn, P.H. (ed.), pp. 265–273. Bloomington, Indiana: Indiana University Press 1967

Banner, A.: Use of sound in predation by young lemon sharks, *Negaprion brevirostris* (Poey). Bull. Marine Sci. **22**, 251–283 (1972)

Bennett, M.V.L.: Electroreception. In: Fish physiology, V. Hoar, W.S., Randall, D.J. (eds.). New York: Academic Press 1971

Bullock, T.H.: Problems in the comparative study of brain waves. Yale J. Biol. Med. **17**, 657–679 (1945)

Bullock, T.H.: Comparisons between vertebrates and invertebrates in nervous organization. In: The neurosciences, Third study program. Schmitt, F.O., Worden, F.W. et al. (eds.), pp. 343–346. Cambridge, Mass.: M.I.T. Press 1974

Bullock, T.H.: Processing of ampullary input in the brain: Comparison of sensitivity and evoked responses among elasmobranch and siluriform fishes. J. Physiol. (Paris) (in press) (1978)

Chapman, C.J., Sand, O.: Field studies of hearing in two species of flatfish *Pleuronectes platessa* (L.) (family Pleuronectidae). Comp. Biochem. Physiol. **47**A, 371–385 (1974)

Corwin, J.T.: Morphology of the macula neglecta in sharks of the genus *Carcharhinus*. J. Morph. **152**, 341–362 (1977)

Corwin, J.T.: The relation of inner ear structure of feeding behavior in sharks and rays. Scanning Electron Microscopy/1978 v. II, 1105–1112 (1978)

Davies, D.H., Lochner, J.P.A., Smith, E.D.: Preliminary investigations on the hearing of sharks. S. African Assoc. Marine Biol. Res., Oceanog. Res. Inst., Invest. Rep. (7), 10 pp. (1963)

Dijkgraaf, S.: Sound reception in the dogfish. Nature **197**, 93–94 (1963)

Ebbesson, S.O.E.: New insights into the organization of the shark brain. Comp. Biochem. Physiol. **42**A, 121–129 (1972)

Ebbesson, S.O.E., Northcutt, R.G.: Neurology of anamniotic vertebrates. In: Evolution of brain and behavior in vertebrates. Masterton, R.B., Bitterman, M.E., Campbell, C.B.G., Hotton, N. (eds.), pp. 115–146. Hillsdale, N.J.: Lawrence Erlbaum Associates 1976

Fay, R.R.: Coding of information in single auditory-nerve fibers of the goldfish. J. Acoust. Soc. Am. **63**, 136–146 (1978)

Fay, R.R., Kendall, J.I., Popper, A.N., Tester, A.L.: Vibration detection by the macula neglecta of sharks. Comp. Biochem. Physiol. **47**A, 1235–1240 (1974)

Harris, G.G., van Bergeijk, W.A.: Evidence that the lateral line organ responds to near-field displacements of sound sources in water. J. Acoust. Soc. Am. **34**, 1831–1841 (1962)

Kalmijn, A.J.: The detection of electric fields from inanimate and animate sources other than electric fields. In: Handbook of sensory physiology, III/3: Electroreceptors and other specialized receptors in lower vertebrates. Fessard, A. (ed.), pp. 147–200. Berlin, Heidelberg, New York: Springer 1974

Kelly, J.C., Nelson, D.R.: Hearing thresholds of the horn shark, *Heterodontus francisci*. J. Acoust. Soc. Am. **58**, 905–909 (1975)

Kritzler, H., Wood, L.: Provisional audiogram for the shark, *Carcharhinus leucas*. Science **133**, 1480–1482 (1961)

Lowenstein, O.: The labyrinth. In: Fish physiology, V. Hoar, W.S., Randall, D.J. (eds), pp. 207–240. New York: Academic Press 1971

Lowenstein, O., Roberts, T.D.M.: The localization and analysis of the responses to vibration from the isolated elasmobranch labyrinth. A contribution to the problem of the evolution of hearing in vertebrates. J. Physiol. (Lond.) **114**, 471–489 (1951)

Murray, R.W.: The ampullae of Lorenzini. In: Handbook of sensory physiology, III/3. Fessard, A. (ed.), pp. 125–146. Berlin, Heidelberg, New York: Springer 1974

Myrberg, A.A., Jr., Banner, A., Richard, J.D.: Shark attraction using a video-acoustic system. Marine Biol. **2**, 264–276 (1969)

Myrberg, A.A., Jr., Ha, S.J., Walewsky, S., Banbury, J.C.: Effectiveness of acoustic signals in attracting epipelagic sharks to an underwater sound source. Bull. Marine Sci. **22**, 926–949 (1972)

Myrberg, A.A., Gordon, C.R., Klimley, A.P.: Attraction of free ranging sharks by low frequency sound, with comments on its biological significance. In: Sound reception in fish. Schuijf, A., Hawkins, A.D. (eds.), pp. 205–228. Amsterdam: Elsevier 1976

Nelson, D.R.: Hearing threshods, frequency discrimination and acoustic orientation in the lemon shark, *Negaprion brevirostris* (Poey). Bull. Marine Sci. **17**, 741–768 (1967)

Nelson, D.R., Gruber, S.H.: Sharks: attraction by low-frequency sounds. Science **142**, 975–977 (1963)

Nelson, D.R., Johnson, R.H.: Acoustic attraction of Pacific reef sharks: effect of pulse intermittency and variability. Comp. Biochem. Physiol. **42**A, 85–95 (1972)

Nelson, D.R., Johnson, R.H.: Some recent observations on acoustic attraction of Pacific reef sharks. In: Sound reception in fish. Schuijf, A., Hawkins, A.D. (eds.), pp. 229–239. Amsterdam: Elsevier 1976

Northcutt, R.G.: Elasmobranch central nervous system organization and its possible evolutionary significance. Am. Zool. **17**, 411–429 (1977)

Northcutt, R.G.: Brain organization in the cartilaginous fishes. In: Sensory biology of sharks, skates and rays. Hodgson, E., Mathewson, R.F. (eds.). Washington, DC: U.S. Govt. Printing Office 1978

Obara, S., Bennett, M.V.L.: Mode of operation of ampullae of Lorenzini of the skate, *Raja*. J. Gen. Physiol. **60**, 534–557 (1972)

Olla, B.: The perception of sound in small hammerhead sharks, *Sphyrna lewini*. M.S. Thesis, University of Hawaii 1962

Parker, G.H.: The sense of hearing in the dogfish. Science **29**, 428 (1909)

Parvulescu, A.: Acoustics of small tanks. In: Marine bio-acoustics, Vol. 2. Tavolga, W.N. (ed.), pp. 1–13. Oxford: Pergamon Press 1967

Platt, C.J., Bullock, T.H., Czeh, G., Kovaćević, N., Konjević, Dj., Gojković, M.: Comparison of electroreceptor, mechanoreceptor and optic evoked potentials in the brain of some rays and sharks. J. comp. Physiol. **95**, 323–355 (1974)

Popper, A.N., Fay, R.R.: Sound detection and processing by teleost fishes: a critical review. J. Acoust. Soc. Am. **53**, 1515–1529 (1973)

Popper, A.N., Fay, R.R.: Structure and function of the elasmobranch auditory system. Am. Zool. **17**, 443–452 (1977)

Schroeder, D.M., Ebbesson, S.O.E.: Nonolfactory telencephalic afferents in the nurse shark (*Ginglymostoma cirratum*). Brain Behav. Evol. **9**, 121–155 (1974)

Tavolga, W.N.: Mechanisms for directional hearing in the sea catfish (*Arius felis*). J. Exp. Biol. **67**, 97–115 (1977)

Tester, A.L., Kendall, J.I., Milisen, W.B.: Morphology of the ear of the shark genus *Carcharhinus* with particular reference to the macula neglecta. Pacif. Sci. **26**, 264–274 (1972)

van Bergeijk, W.A.: Directional and nondirectional hearing in fish. In: Marine bio-acoustics, Vol. 1. Tavolga, W.N. (ed.), pp. 281–299. New York: Pergamon Press 1964

Wisby, W.J., Richard, J. D., Nelson, D.R., Gruber, S.H.: Sound perception in elasmobranchs. In: Marine bio-acoustics, Vol. 1. Tavolga, W.N. (ed.), pp. 255–268. New York: Pergamon Press 1964