pattern" in response to strong aversive stimuli. These fishes also become immobile periodically when adjusting for imposed buoyancy displacement (*7*). Another behavioral pattern is marked erection of the dorsal fin (Fig. 1*A*). This display is a common "alarm reaction" in a fish when other animals encroach on its position, or when defensive-aggressive action is imminent. The same fin erection may be found among the reflexes for buoyancy adjustment of swimbladder volume.

A bass which is stationary, or in the immobile state, is approached by topminnows as though it were an inanimate object. One topminnow may swim by within a few centimeters if the bass remains still. Topminnows are always alert to any fish when it makes a fin display or moves, but these topminnows showed no strong aversive reactions to the bass even though it is a predator. However, no topminnow remained beside or touched a normal bass; a marked contrast to their symbiotic behavior with the parasitized fish.

When the parasitized bass had been in the cylinder 48 hours, live topminnows were added as food. Two of these, including a *Fundulus*, were eaten within 3 hours. Feeding behavior did not involve extensive pursuit. The bass usually remained at a "fix position" (*6*) until a topminnow swam to within a few centimeters of it. The bass then advanced slightly; poised, as the topminnow hesitated; then lunged, mouth agape, to eat the topminnow. This was typical feeding behavior for all bass (Fig. 1*A*).

Symbiotic cleaning was first noticed on the day after topminnows were placed in the cylinder. The bass seemed to be paralyzed or insensitive. A *Fundulus* attacked the fins and the velum of the opercular margins. The bass rested on the bottom in an awkward posture, in slightly negative hydrostatic buoyancy. The topminnow sometimes wrested those parts it was biting with enough force to lift the bass bodily from its place. The bass remained completely passive and parasites were suspected. It did retain normal eye reflexes. Since the attacks of the topminnows seemed to focus on parasites the bass was kept in the cylinder so that the behavior of the animals and their interrelationships could be studied and recorded by cinematography. Cleaning activity occurred at intervals of an hour or more and lasted a few minutes. Each of three remaining topminnows in the cylinder participated during the 3-day period of observation. All topminnows and most parasites had been eaten before the 4th day. The bass and parasites were examined post-mortem (*4*).

Before the topminnows showed this cleaning behavior, the bass had usually remained stationary, but not immobile, at its "fix position" for 10 minutes or more. If it was moving, it became stationary as a topminnow approached with a series of darts and pauses. At the beginning of the approach, the bass sometimes exhibited a transient alarm reaction. As the approach continued, the bass settled to rest on the bottom with fins fully relaxed. This state of generalized motor inhibition is comparable to the "immobilization pattern" of buoyancy displacement.

The topminnow then explored about the surface of the bass, tearing off pieces of copepods. Biting around the head disturbed the bass no more than biting at the fins (Fig. 1*B*). If the topminnow worked about an opercular margin, that operculum would gape sufficiently for the topminnow to reach its inner surface. If a wresting action displaced the body of the bass or tilted it, there was usually no compensating fin action. When the topminnow eventually moved away, the bass returned to its normal orientation. The postural reflexes and buoyancy responses of this bass always responded normally to hydrostatic manipulation (*6*, *7*).

Mutuality and cooperation in the cleaning relationship is emphasized by the fact that the bass exposed vulnerable gill regions. This "host" behavior is like exposure of the open mouth for cleaning, which Limbaugh reported (*1*). He saw, in the natural habitat, that gobies entered the mouths of groupers, hogfishes the mouths of barracuda; and while blacksmiths were being cleaned by señoritas they "would remain motionless in the most awkward positions—on their sides, head up, head down or even upsidedown."

There are three possibilities to explain the bass-*Fundulus* symbiosis: (i) the specific relationship was a natural one to which the bass was habituated; (ii) a behavioral background for symbiotic cleaning already existed through independent conditioning in both species; or (iii) a spontaneous, new, facile interaction developed under the experimental conditions. The third possibility seems the least probable. Such a complex behavioral interaction, reversing an established predator-prey relationship and becoming complete within 12 hours after the animals came together, supports the hypothesis of previous conditioning. Whether black sea bass and *Fundulus* are in fact symbiotic in their usual environment is unknown. Their established distribution and natural history, together with the observations here reported, make natural symbiosis probable.

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## Electroencephalograms of Sharks

Abstract. *Patterns of electrical potentials recorded from the brains of sharks exhibit definite relationships to chemical and visual stimuli. Forebrain potentials reflect olfactory processes. Both restrained and free-swimming sharks exhibit mesencephalic responses to light and neural triggering of respiratory reflexes from the medulla. Early evolution of typical vertebrate brain functions, with emphasis upon chemoreception, is indicated.*

Patterns of electrical potentials from the brains of sharks are of interest from two main standpoints. First, the elasmobranchs illustrate more primitive features in the evolution of the vertebrate brain than any other examples which have been subjected, thus far, to electroencephalographic (EEG) study. Consequently, EEG analysis might reveal features of brain function established some 350 million years ago, during the Devonian Period (*1*). Second,

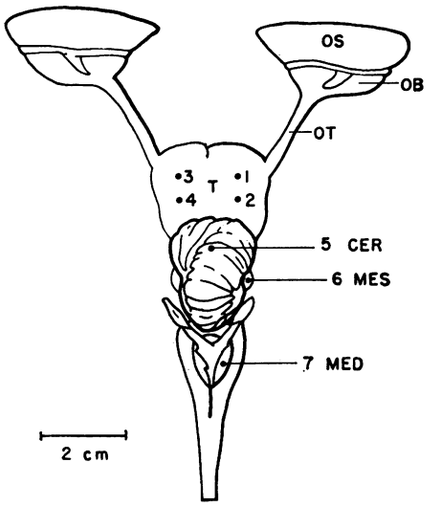


Fig. 1. The brain of the lemon shark, *Negaprion brevirostris*. Numbers indicate recording points. Abbreviations: *OS*, olfactory sac; *OB*, olfactory bulb; *OT*, olfactory tract; *T*, telencephalon; *CER*, cerebellum; *MES*, mesencephalon; *MED*, medulla.

the shark brain exhibits prominent development of regions concerned with chemoreception—the complete olfactory apparatus, for example, attaining approximately two-thirds of the weight of the brain in many species (*2*). Abundant observations have established the major roles of the chemical senses in the orientation, feeding, and attack behavior of sharks, but lack of electrophysiological studies on these senses impedes more detailed analyses of the underlying neurophysiological mechanisms (*3*). The studies described here were undertaken to determine the EEG patterns characteristic of various lobes of the brains of unanesthetized sharks, with particular reference to patterns from the olfactory lobe associated with olfactory stimuli.

Thirty-two lemon sharks (*Negaprion brevirostris*), six bonnet sharks (*Sphyrna tiburo*), and eight nurse sharks (*Ginglymostoma cirratum*) were studied. Both sexes were included in the experiments, and each shark weighed between 2.5 and 14 kg. The narcotic MS222 (1:1000 by weight) was administered to the gill openings as an initial temporary anesthetic (*4*); this was followed by an intravenous injection of D-tubocurarine (2.6 mg per kilogram of body weight) to prevent muscle activity. After electrode insertion, the MS222 was washed out by flowing sea water through the mouth and gills.

Bipolar silver–silver chloride electrodes were used, each wire being 0.1 mm in diameter. The two insulated wires were held rigidly apart within a single supporting sleeve inserted through holes drilled in the cartilaginous skull of the anesthetized shark. Recording positions are shown in Fig. 1. These electrodes recorded the same potential changes as two pointed wick electrodes touching the same region, thus ruling out pressure effects from the electrodes. Connections were made to 2 to 4 recording positions at a time, making it also possible to record between single leads in different lobes (*5*). Depth recordings were made by means of single electrodes inserted measured distances below the brain surface, as in the method of Schade and Weiler (*6*). Electrode placement was similar for recordings from unrestrained free-swimming sharks, with wire leads in these sharks sutured to the animal's skin and covered with Silastic-RTV (Dow Corning) adhesive and sealant.

Potentials were recorded on a Garceau electroencephalograph, with paper speed of 3 cm/sec, and were photographed from an oscilloscope screen at a speed of 10 cm/sec. A Grass PS-2 photostimulator and an underwater speaker delivering pure tones from a Krohn-Hite model 430-AB audio oscillator were used for light and auditory stimulation. Tygon tubing, fitted into a nasal opening, delivered sea water from a constant pressure source at a rate of 0.3 ml/sec to one nasal sac. A two-way petcock permitted change-over to a flow of sea water plus chemical test solution, without appreciably interrupting the flow or changing its pressure (*7*). The results varied according to the site from which the recording was obtained.

Two patterns of activity were found in the telencephalon (forebrain). Surface potentials had a dominant frequency of 4 to 9 per second, and amplitudes of 30 to 60 $\mu$v (Fig. 2, *A* to *D*). During chemical stimulation, both the amplitude and frequency of the potentials increased. Figure 2*B* shows such a response recorded between surface positions 1 and 2 (see Fig. 1) during chemical stimulation of the homolateral olfactory sac of a bonnet shark with body fluids of crabs, which are among the normal foods of this species. The effects of stimulation of a nurse shark with 0.1*M* glycine are shown in Fig. 2*D*. These electrical responses are clearly different from the effects of sea water alone and persist several seconds longer than the duration of the stimulus flow (see Fig. 2*C*, which is a continuation of 2*B*).

Depth recordings yielded positive results only in the anterior lateral halves

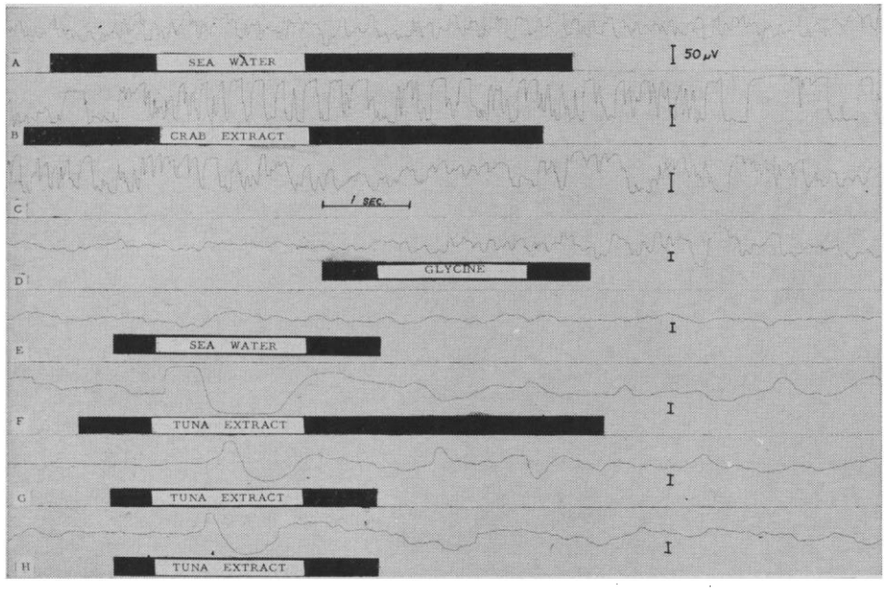


Fig. 2. Electroencephalographic records from forebrains of sharks. Broad horizontal bars indicate periods of chemical stimulation. The time scale, as indicated, is the same in all records. Vertical calibrations 50 $\mu$v in all records. (*A*) Forebrain surface, bonnet shark, during perfusion of olfactory sac with seawater; (*B* and *C*) same, during and following chemical stimulation; (*D*) forebrain surface, nurse shark, with pure chemical stimulus; (*E* to *H*) depth recordings from the forebrain of lemon sharks, stimuli as indicated.

of the forebrain, about 5 mm below the surface at positions 1 or 3, during stimulation on the homolateral side (Fig. 2, *E* to *H*). Filtered extracts of tuna meat evoked large negative potentials, followed by slower potentials of opposite polarity and a gradual return to the unstimulated normal level. Similarity of pattern, with some decrease of amplitude and duration, is usual even when the same stimuli are presented with intervening rinses (for 60 seconds) of sea water, as between Fig. 2, *F*, *G*, and *H*. At these stimulus intensities (probably high for sharks) the duration of the electrical response seems to depend more upon the adaptation of the olfactory system than upon duration of stimulation (see the longer period of stimulation in Fig. 2*F*). Tuna extracts evoked the largest electrical responses. Similar patterns, but smaller amplitudes of potentials, were evoked by most of the chemical stimuli tested, including the DL forms of glutamic acid, glycine, cysteine, and serine, as well as fresh tuna blood, body fluids of lobsters and crabs, and the "amine F" which has been identified as an olfactory attractant for lampreys, fishes, and sharks (*8*).

Mesencephalic potentials were related to light. During darkness, potentials of relatively large amplitude (80 to 170 $\mu$v) in dominant frequencies of 5 to 11 cycles per second were recorded at the surface of the optic tectum in 19 out of 32 studies of this region. When room lights were turned on during such a recording, the typical "dark" pattern was replaced by potentials of much lower amplitude (less than 40 $\mu$v). The latter potentials were usually difficult to discern in tracings where the amplification had been reduced to a level permitting complete display of the larger potentials (Fig. 3*A*).

In four cases, synchronous potentials were detected from previously "quiet" optic tectum preparations at the frequencies of the light flashes. Two lemon sharks (one free-swimming) showed such potentials at the same frequencies as stimulating flashes up to frequencies of 23 and 24 flashes per second. Figure 3*B* illustrates the response of a free-swimming shark to a frequency of 10 flashes per second, with fluctuations in response amplitude correlated with movements and possibly variations in electrode contact. For comparison, the EEG in Fig. 3*C* was taken from a curarized lemon shark stimulated with

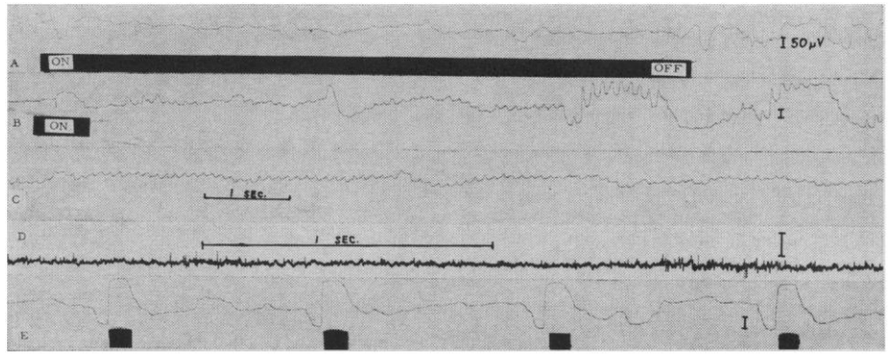


Fig. 3. Electroencephalographic records from the mesencephalon, cerebellum, and medulla. Light stimulation on and off as indicated in records (*A*) and (*B*). (*A*) Mesencephalic response to steady light in lemon shark; (*B*) synchronized mesencephalic responses to light flashes at a frequency of ten per second in free-swimming lemon shark; (*C*) mesencephalic responses during light flashes, ten per second, in curarized lemon shark; (*D*) cerebellar potentials in the bonnet shark; (*E*) medullar potentials in the lemon shark, with gill movements indicated by black markings.

flashes at the same frequency. Two bonnet sharks yielded similar potentials at frequencies up to 15 and 17 flashes per second.

Potentials in the cerebellum were small (20 to 45 $\mu$v) and tended to occur in irregular groupings which could not be correlated with any of the test stimuli used. A typical portion of a rapid sweep oscilloscope record is shown in Fig. 3*D*. These results, unfortunately, do not provide any clues which might illuminate the controversial views on the functions of the cerebellum in elasmobranchs (*2*).

Potentials obtained from the medulla were of relatively large amplitude (150 to 200 $\mu$v) and appear to represent the neural triggering of respiratory reflexes. They are correlated with gill movements (see Fig. 3*E*, in which black markings at the bottom of the record represent gill movements marked by an observer during the medullar recording). When the electrode tips were withdrawn to just outside the medulla, these potentials were no longer recorded, even though the gill movements continued, thus showing that the potentials were not being recorded directly from gill musculature. Nor were any such potentials ever recorded from the medulla of a free-swimming shark in the absence of gill movements. Medullar responses to sound, such as reported to occur sporadically in fish (*5*), were not observed, even at sound frequencies of 400 to 600 cy/sec (up to 40 db, ref. 1 $\mu$bar), which have been reported as the range of maximum sensitivity for sharks (*9*).

Although the Paleozoic elasmobranchs appeared on the evolutionary horizon somewhat later than the first bony fishes, they evolved much earlier than the teleost fishes. Nevertheless, the EEG patterns in modern elasmobranchs and teleosts show remarkable similarities, indicating an early evolution of basic patterns of brain functions in vertebrates. In the larger size of their olfactory apparatus, however, the sharks possess a decided advantage over the teleosts for electrophysiological studies. This may explain the failure of some investigators to detect forebrain responses in teleosts during chemical stimulation of the olfactory epithelium (*7*).

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