

FREQUENCY SELECTIVITY IN THE AMPULLARY SYSTEM OF AN ELASMOBRANCH FISH (*SCYLIORHINUS CANICULA*)

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SUMMARY

1. Frequency characteristics of the electroreceptive system in *Scyliorhinus canicula* were determined both by electrophysiological recording of the primary afferent responses and by optical recording of respiratory reflexes after electrical stimulation.

2. The frequency response of the primary afferents shows a maximum gain at about 5 Hz, with slopes of +2.3 and -3.4 dB octave⁻¹ at the low and high frequency side respectively. The phase changes from +60° at 0.03 Hz to -120° at 15 Hz.

3. The sensitivity curve determined by recording the respiratory reflex has a plateau from 0.1 to 1 Hz, with slopes of +2.8 and -11.4 dB octave⁻¹. The highest sensitivity for sinusoidal electrical stimuli was 40 nV cm⁻¹ peak-to-peak, in the frequency range 0.1 to 1 Hz.

4. We suggest that the difference between the two curves reflects the convergence of primary afferents on to secondary neurones.

5. We conclude that the low pass filter properties are not imposed by the time constant of the ampulla wall.

6. The low frequency slope found in the behavioural curve presumably represents the slope of the receptor-cell-synapse complex.

7. The Lorenzian ampullae apparently act as peripheral filters with different tuning curves; these must play a part in frequency discrimination.

INTRODUCTION

Undisturbed embryos of *Scyliorhinus canicula* make swimming movements inside their egg capsules for minutes on end. They respond to stimuli of all kinds by 'freezing': the swimming movements are interrupted and the respiration is suppressed completely. After a couple of seconds or even a minute the respiration is resumed, movements spread over the whole body and turn into swimming undulations again. Such reactions persist to some extent after birth. Free dogfish

usually lie down on the bottom of the tank if not in search for food, but the 'freezing' can still be recognized after stimulation as a change in gill movement. We used this respiratory reflex in new-born dogfish to determine the frequency response of the electrical sense. We found an unexpected change in the threshold at a frequency of about 1 Hz. This sharp fall in reactivity made us check the literature, but we could not find any support for our findings.

Waltman (1966, p. 27) studied the electrical properties of the ampullary canals of Lorenzini and inferred from physical measurements that a 1-cm long canal (diameter 0.04 cm) has a cut-off frequency of about 300 Hz. In our new-born dogfish neither heads nor ampullae were longer than 1 cm, which made us expect that the frequency range of these small dogfish (10–20 cm in length) should exceed the 1 Hz limit we found. Murray (1974, p. 143) inferred from electrophysiological recordings of the primary afferents that the ampullae are most sensitive to pulses of 0.1–1 s duration, i.e. to frequencies of 1–10 Hz. Dijkgraaf & Kalmijn (1963, p. 441) found a maximum sensitivity for square wave stimulation at 5 Hz. The response criterion they used was the 'eyeblink' reaction. Kalmijn (1974, p. 105) studied, among other things, the feeding reactions of *Scyliorhinus* to artificial electric dipoles of different frequencies. He found the animals were most sensitive to stimulus sources that emanate fields with frequencies below 8 Hz, with a maximum from d.c. to 4 Hz.

The discrepancy between these findings might be caused by the different technical approaches used. On the other hand the difference observed between the frequency characteristics of the primary afferents and the behavioural reactions might reflect properties of the information-processing brain. We might expect variations in sensitivity to occur near the respiratory frequencies. The neural responses caused by the respiratory potentials will dominate and mask all responses elicited by the usually relatively weak environmental stimuli (see e.g. Dijkgraaf & Kalmijn, 1966, Fig. 5, p. 192). A similar phenomenon is seen in the neural recordings of catfish electroreceptor primary afferents (Peters & Buwalda, 1972, p. 34). The brain must somehow take this into account. This might be done, for example, by rejecting the common mode signals (cf. Montgomery, 1984), but common mode rejection alone cannot account for the discrepancies described above.

We here present two frequency characteristics of the ampullary system of *Scyliorhinus canicula*: one of the primary afferents, i.e. before the stimuli are processed by the brain, and another of the respiratory reflex, i.e. after processing by the brain. The difference between these two will tell us about the information-processing properties of the intermediate neural circuitry.

MATERIALS AND METHODS

Animals

All experiments were done with *Scyliorhinus canicula*. For the preliminary behavioural work we used specimens and their young that had been kept in our aquaria for more than 2 years. These fish came from the Atlantic coast (Arcachon,

France). Most of the experiments were performed at the Station Biologique at Roscoff (Atlantic coast, France) where we had a choice of healthy, naive animals. A final set of experiments was done at Utrecht with dogfish brought back from Roscoff.

For the electrophysiological recordings of the primary afferents we used larger dogfish, 40–65 cm in length. For the behavioural work we used both large (40–65 cm) and small (10–20 cm) specimens. During the experiments all the animals were kept in glass or plastic containers at temperatures of 14–16°C. The fish were fed on pieces of squid or beef. They all ate regularly and breathed slowly and regularly in spite of their confinement. The small animals usually remained motionless for hours on end at the bottom of the experimental tank. The larger ones had to be confined in a nylon wide-mesh net during stimulation and recording. The dimensions of the experimental tanks were 40 × 60 cm or 55 × 90 cm. The water depth was 20 cm. The behavioural experiments were performed both in daylight and with indoor illumination. Sometimes the tank was covered with a black plastic sheet to prevent the fish from being alarmed.

Stimulation

Electrical stimuli were generated by a Wavetek 112B function generator followed by a voltage-to-current converter, which guarantees a controlled stimulus current. The electrodes consisted of cylindrical silver surfaces (diameter, 5 mm; length, 15 cm) coupled to the water in the tank by an array of salt bridges in order to eliminate eddy currents due to inhomogeneities on the electrode surface.

During the behavioural observations, stimuli were presented as single cycle sinusoids. Now and then, mostly at frequencies above 1 Hz, a train of sinusoids was presented. At other times the stimulus was linearly increased from zero to its maximum value. Care was taken that neither erroneous d.c.-stimuli (e.g. from drift), nor switching artefacts were presented to the animals. We ensured that the experimental tank was earthed by the stimulus reference electrode only. The tank water was aerated by means of an air pump and air stone. For the electrophysiological experiments we used continuous sinusoidal stimuli.

The head of the fish was always exposed to a uniform field, perpendicular to its body axis. To avoid habituation during the behavioural studies we varied the strength and the frequency of the stimuli randomly. An inter-trial interval of 5 min proved satisfactory.

Recording

Data were recorded on a stereo cassette tape recorder, ITT 730. Action potentials were recorded directly; low frequency signals, such as stimuli and respiratory movements, were recorded *via* a home-built FM-modulator. The reactions were also recorded on a pen recorder (Hewlett Packard 7702 B) during the experiments.

The respiratory movements of the dogfish were detected by a phototransistor which measured the changes of light reflection from the moving gill covers. The phototransistor was placed in the centre of a Perspex pipe which acted as a light guide. As a rule this device was placed a few cm from the animal in order to avoid unwanted field disturbances at the skin surface. A response to a stimulus was defined as a decrease in respiratory amplitude (during the stimulus) of more than twice the standard deviation of the ten beats preceding the stimulus.

For the electrophysiological recording of the primary afferents we followed the procedure that is described in Dijkgraaf & Kalmijn (1966). Instead of insulating the electrode and recorded nerve (n. oph. sup. V + VII) with silicon rubber and paraffin we used cocoa butter according to Peters, Bretschneider & Schreuder, (1975). Cocoa butter insulates electrically, fixes mechanically and can be applied in liquid form.

The gross activity of the nerve fibres was recorded by means of standard electrophysiological equipment. Full wave rectification of the signal and subsequent smoothing by a leaky integrator ($\tau = 5.6$ ms) (Thompson & Patterson, 1973, p. 212) gave a well-defined measure of both the average resting discharge of the bundle of primary afferents and the modulation of the resting discharge after stimulation, i.e. the response. The amplitude changes of the smoothed signal were taken as the measure of the response. We did not present stimuli above 20 Hz to be certain that the remainder of the stimulus artefact would not distort the outcome.

According to Smolders, Folgering & Bernards (1975) the square root of the average number of spikes contributing to the gross activity is proportional to the amplitude of the rectified and smoothed gross activity. In this paper we shall consider the average spike frequency to be proportional to the gross activity (linear relationship). We checked (R. C. Peters & F. Bretschneider, unpublished) that errors introduced by this simplification were negligible. The phase shift between stimulus and response was either measured directly from the traces of the pen recorder or by means of cross correlation of the stimuli and smoothed gross activity (Hewlett Packard, 3721 A Correlator).

RESULTS

Frequency response of the primary afferents

We measured the amplitude characteristics of four fish, 40–65 cm in length (Fig. 1). All these fish came from the Roscoff coast. The slopes of the curve were estimated by calculating the regression lines through the points below 0.6 Hz and above 10 Hz. The slopes of these lines are +2.3 and -3.4 dB octave⁻¹ at the low and high frequency side respectively. The -3 dB points are at 0.9 and 18 Hz. Maximum sensitivity is at 5–8 Hz.

Since we used single sinusoids during the behavioural experiments we also looked at the response of the primary afferents to a single cycle stimulus. An example of the gross activity to stimulation with a single sinusoid of 33 s (0.03 Hz) is given in Fig. 2A. The response shows three clear peaks, which illustrates the high-pass properties of the ampullary system.

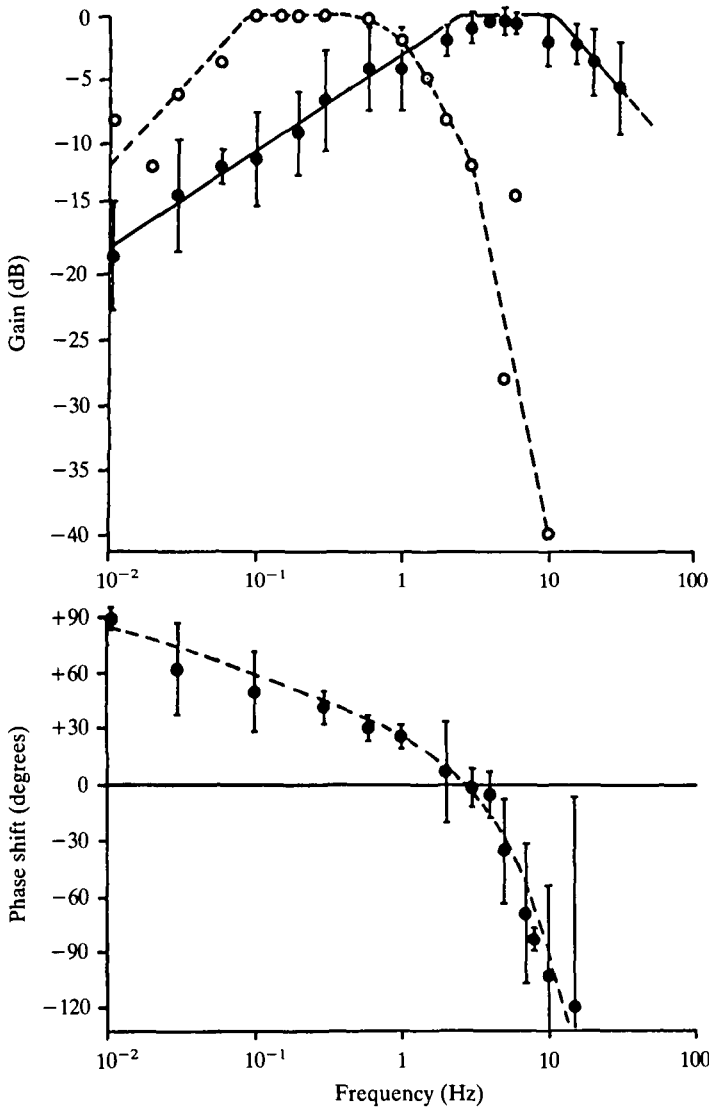


Fig. 1. Frequency characteristics of the electroreceptive system in *Sycliorhinus canicula*. (A) Amplitude characteristics of primary afferents (filled circles) and 'respiratory reflex' (open circles) in uniform horizontal electric fields perpendicular to the body axis. The curve of the primary afferents was measured at stimulus strengths between 1 and $3 \mu\text{V cm}^{-1}$. Bars are standard deviations. The 'respiratory reflex' curve is the inverse threshold curve from Fig. 3. 0 dB corresponds here to 40 nV cm^{-1} peak-to-peak. According to Kalmijn (1974) the curve of 'turning-towards-electric-dipole' would run at the 0 dB level from d.c. up to 4 Hz; Kalmijn found the -6 dB point at 8 Hz. (B) Phase characteristic of primary afferents. The point at 0.01 Hz consists of only two samples. The other points are the average values from four animals. The values are corrected for phase shift induced by the integrator. Bars are standard deviations.

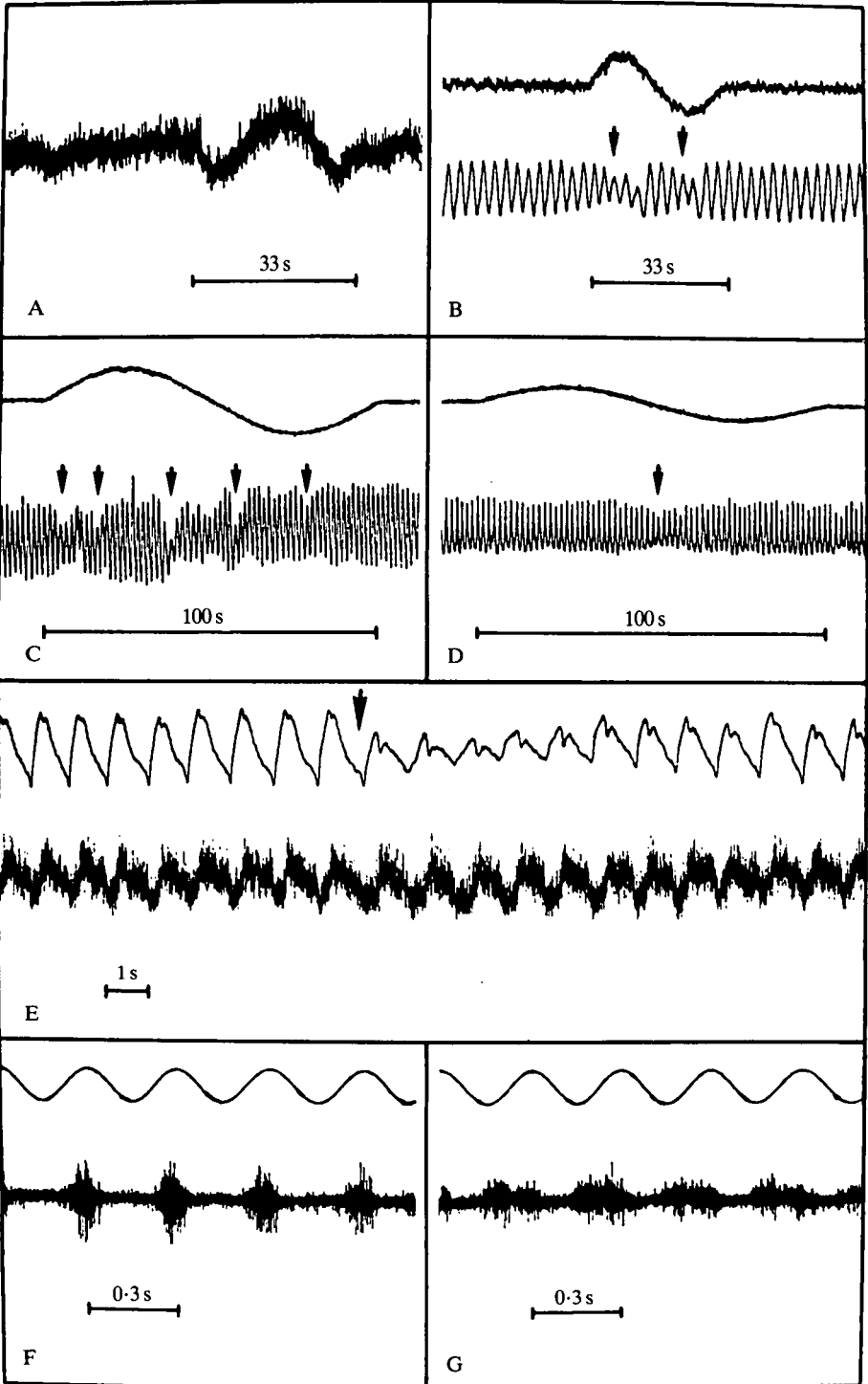


Fig. 2

A simultaneous recording of gill movements and smooth gross activity (Fig. 2E) shows that even a strong reduction in gill movement apparently does not notably influence the gross activity.

In some recordings, however, the respiratory response interfered strongly with the response to sinusoidal stimuli. At other times, we found that slight changes in the orientation of the fish with respect to the electric stimulus field introduced a phase shift at identical stimulus frequencies. Fig. 2F–G shows how after the position of a dogfish was changed (by rotation) with respect to the electric field another group of ampullae with different frequency properties became stimulated. After our return to Utrecht we used another three specimens to assess the relevance of this finding. It turned out that the frequency-dependent phase change of the afferent response was associated with the filter properties of the electrical receptor and was independent of the fish's orientation in the electric field (Fig. 1B).

Threshold curve of the respiratory reflex

For the determination of the threshold curves of the respiratory reflex we used six large dogfish, 40–65 cm in length, and five small ones, 10–20 cm in length. The small animals reacted promptly by holding their breath on electrical stimulation, especially after being fed. The larger animals gave us more problems. Sometimes they reacted to the stimulus; at other times they were very unresponsive, even to stimuli that had elicited clear responses earlier. Something similar might have bothered Dijkgraaf (Dijkgraaf & Kalmijn, 1963) when he studied the eyeblink reaction of *Scyliorhinus*. We tried to condition the respiratory reflexes by means of light flashes and even electric shocks, but *Scyliorhinus* either became habituated to the punishments or became so stressed that it would not respond at all. The most conspicuous feature of the respiratory reflex is not the rather small change in frequency, but the strongly diminishing amplitude (Fig. 2E).

The most complete curve is shown in Fig. 3. The line representing the threshold is drawn through the lowest stimulus values at which a reaction could be evoked. The stimulus values are given as peak-to-peak values. To be able to compare this curve with the frequency characteristic of the primary afferents, we made a Bode plot of the

Fig. 2. Some examples of 'respiratory reflexes' and gross activity of the primary afferents. (A) Response of primary afferents, after rectification and smoothing, to stimulation with a single strong sinusoid of 0.03 Hz. Time constant of integrator, 58 ms; (B), (C), (D) upper trace, stimulus; lower trace, respiratory movements. Arrows denote respiratory reflexes. (B) Double reaction to a stimulus of 0.06 Hz, 60 nV cm^{-1} peak-to-peak. (C) Multiple reaction to a stimulus of 0.01 Hz, 200 nV cm^{-1} peak-to-peak. (D) Single reaction to a stimulus of 0.01 Hz, 100 nV cm^{-1} peak-to-peak. (E) Response of primary afferents (lower trace) and simultaneous recordings of gill movements (upper trace) to a stimulus of 0.03 Hz (not shown here). The recordings show clearly that strong reductions in gill movements do not change the responses to the respiratory potentials. Arrow shows onset of stimulus; (F), (G) Neural responses of primary afferents to alternating currents of 3 Hz. A slight change in the position of the fish (15° rotation) influences the discharge pattern. If all the ampullae were to have identical frequency responses one would expect a polarity change but not a phase shift of less than 180° . Recordings F and G demonstrate frequency selectivity at the level of the receptors.

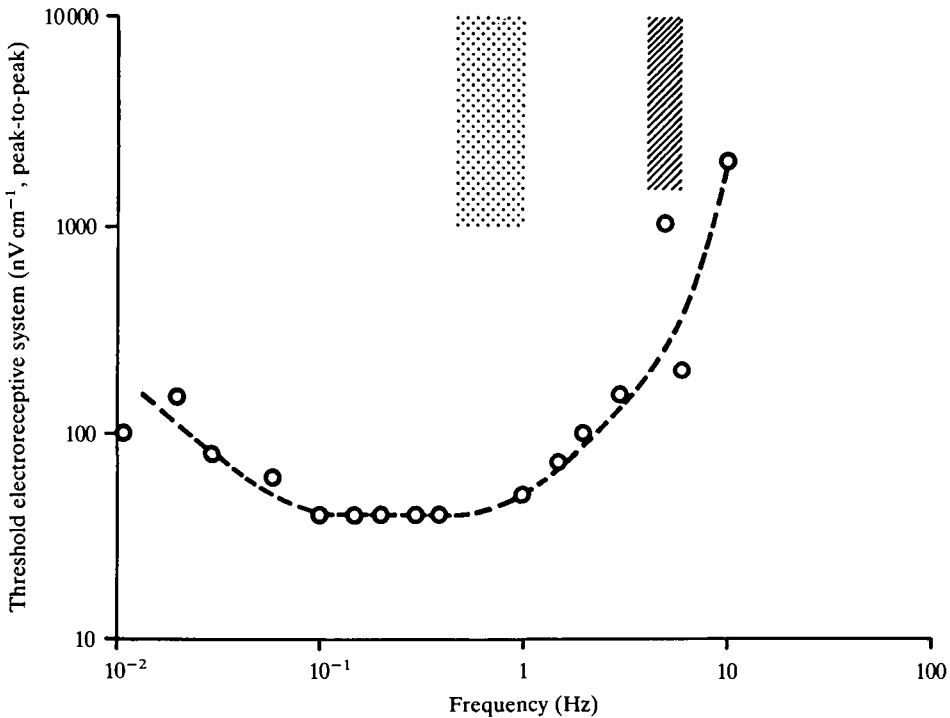


Fig. 3. Threshold curve of the 'respiratory reflex' of *Scyliorhinus canicula* after stimulation in horizontal uniform electric fields perpendicular to the body axis. The values on the y-axis are given as peak-to-peak values. The threshold represents the lowest value at which a response would be evoked in animals in a 'reactive' state (see text). The hatched area indicates the stimulus strengths used by Dijkgraaf & Kalmijn (1963) for evoking the 'eyeblink' reflex. The dotted area represents the range of the respiratory potentials.

inverse threshold (Fig. 1A). The low frequency and high frequency slopes are $+2.8$ and -11.4 dB octave $^{-1}$ respectively. The -3 dB points are 0.07 and 1.5 Hz.

During low frequency stimulation we found a two-fold or three-fold reaction near the threshold (Fig. 2B,C). We assumed that this might be the result of the differentiation of the single, low frequency, sinusoidal stimuli by the receptor system. This hypothesis is supported by the frequency characteristic of the primary afferents (Fig. 1).

The difference between the low frequency slopes of the primary afferent curve and of the behavioural curve is not significant at the 5% level; neither is the difference between the high frequency slopes (Sokal & Rohlf, 1981, p. 507: unplanned comparisons among a set of regression coefficients).

DISCUSSION

Responses of the primary afferents

Our electrophysiological results are in good agreement with the data of Murray (1974, p. 143), and of Dijkgraaf & Kalmijn (1963, p. 441). Waltman's findings of

turnover frequencies of 9 Hz for a 10-cm long canal wall and 300 Hz for a 1-cm long canal wall (diameter 0.04 cm) seem to be too high to account for the low-pass frequency limit based on electrophysiological studies. The conclusion is that the low-pass characteristic of the electroreceptors is dictated by the nerve fibre-receptor cell complex and not by the time constant of the canal walls.

The high-pass characteristics found so far ($+2.3$ dB octave⁻¹) must also be a basic property of the receptor cells and their synapses. According to Bretschneider, de Weille & Klis (1985) such slopes are properties of fractional order systems.

The strength of the stimuli used in these electrophysiological experiments is about 1000 times the threshold we found from the recordings of the respiratory reflexes. Dijkgraaf & Kalmijn (1963) also evoked the eyeblink reaction with stimuli of this magnitude (see Fig. 3). Because these stimuli are rather strong one might wonder whether the system still responds linearly at these stimulus amplitudes. From control experiments in which we compared gradually increasing stimuli with the gross activity responses we inferred that the receptors were not saturated.

Respiratory reflex

The suppression of the respiratory movements is usually dependent on the stimulus strength and the inter-stimulus interval. If the stimuli follow upon each other too closely, habituation of the response occurs. Dishabituation can be provoked if stimuli of another modality are given. In short, the respiratory reflex behaves as a real orienting response (Lynn, 1966; Kandel, 1970; Evans & Mulholland, 1969).

At stimulus frequencies above 1 Hz, we presented both single sinusoids and continuous sinusoids. The spectra of single sinusoids are different from those of continuous sinusoids. From control experiments (unpublished) we concluded that single sinusoids and continuous sinusoids are equally effective in stimulating the sense organs. Nevertheless, we found that single sinusoids resulted in higher behavioural thresholds than continuous signals above 1 Hz. This confirms Dijkgraaf's observations that a kind of temporal summation must occur somewhere in the brain. Differences in perception must be located in the higher centres of the nervous system. We did not pay much attention to these differences as the variations in the reactivity of the animal caused more variability of the results.

Comparison of primary afferent gross activity and respiratory reflex

The two frequency curves described above show that the responses of the whole organism and of the primary afferents are different. The behavioural curve demonstrates clearly that some kind of neural processing takes place in the brain. The most simple model that can account for these differences is the following. The primary afferents converge to secondary neurones, where integration, i.e. summation, takes place. The time constant of the integrating part of this system

cuts the upper bandwidth limit down to about 1 Hz. The loss of gain which such a filter invariably introduces will be compensated by the presumably enormous summation of primary afferent activity. In our simple model, the low frequency slope of the behavioural curve (Fig. 3) must be identical to the low frequency slope of the primary afferent system (Fig. 1A). To account for the existence of a threshold we need another feature in our model. The spontaneously active primary afferents are thought to be threshold-free, so a threshold must be sought in higher order neurones. A threshold could be realized by a secondary (integrating) neurone with no, or only very low, spontaneous activity. Evidence has been found for the existence of such neurones (Andrianov & Ilyinsky, 1973), for example in catfish brain. The high frequency slope of the behavioural curve supports the hypothesis of at least one additional integrating station after the primary afferents.

Comparison of three behavioural frequency characteristics: frequency discrimination

Three different behavioural frequency characteristics of 'outputs' of the electroreceptor system in *Scyliorhinus* have been found, each representing some neural information-processing circuit: the 'eyeblink' system with a maximum gain at 5 Hz (Dijkgraaf & Kalmijn, 1963), the 'freeze' system with a maximum gain from 0.1 to 1 Hz (this paper) and the 'feeding reaction' with maximum gain between d.c. and 4 Hz (Kalmijn, 1974).

The eyeblink reaction has its sensitivity peak at the same frequency as the primary afferents, and is evoked by strong stimuli (cf. Fig. 3). We suggest that this may be due to the existence of secondary neurones with a high threshold which are activated only after strong stimulation.

The 'respiratory reflex' occurs when an animal is confronted with an unusual stimulus. This must be a suprathreshold stimulus, of course. So the behavioural frequency curve of Fig. 3 will probably represent the real frequency dependence of the electroreceptive system. Furthermore, the threshold of 40 nV cm^{-1} is in rather good agreement with the thresholds found independently by Kalmijn (1982) for *Mustelus*. We must take into account that the peak-to-peak values should be halved when they are compared to the d.c.-stimuli given by Kalmijn.

There is a difference between the 'respiratory curve' from our experiments and the 'feeding response curve' from Kalmijn's (1974) experiment in the frequency range below 1 Hz. This is presumably the result of the different experimental approaches. In Kalmijn's experiment the perceived stimulus frequency will depend on the speed at which the animal approaches the stimulus source. So the 'feeding response' should not be used to determine the properties of the ampullary system in this low frequency range, in spite of its biological relevance.

The maximum sensitivity range of our 'respiratory curve' corresponds to the frequency of the respiratory and swimming movements (R. C. Peters, unpublished). This correspondence tells us that the central information processing must have features which enable the organism to distinguish very weak stimuli of 40 nV cm^{-1} from the very strong respiratory potentials of 6000 nV cm^{-1} (cf.

Fig. 3). We think that the different time constants of the ampullary groups (cf. Fig. 2F,G) provide a powerful means for frequency discrimination.

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