Coding Properties of Two Classes of Afferent Nerve Fibers: High-Frequency Electroreceptors in the Electric Fish, *Eigenmannia*

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THE PROPOSITION THAT neurons can encode information about the intensity of stimuli in other ways than by the familiar gradation of intervals between impulses has been put forward (5, 6, 12). Perkel and Bullock (23) catalog the known and the candidate codes by which neurons can represent information in streams of nerve impulses. For none of these, other than the familiar frequency coders, is a quantitative characterization available of the behavior of the impulse train as a function of intensity.

Electroreceptor units in the lateral line nerve of weakly electric fish have been particularly useful in expanding our understanding of coding types (8, 17, 18, 23, 28, 29). These receptors, first predicted by Lissmann (21) and found by Bullock et al. (9), Hagiwara et al. (17), Fessard and Szabo (16), and Szabo and Fessard (27) are of several kinds. Two main classes are *1*) tuberous or high-frequency-sensitive receptors that fire synchronously with the electric organ, and *2*) ampullary or low-frequency-sensitive receptors that fire independently of the electric organ discharge (1, 2, 26).

Among the synchronous receptors in "wave" species (species with high repetition rate, quasi-sinusoidal electric organ discharge) Bullock and Chichibu (8) distinguished two subclasses of receptors: *a*) one that codes stimulus intensity by latency or phase shift (they could not tell which), and *b*) one that codes by altered probability of missing cycles of the electric organ discharge. The latter subclass had already been

found by Hagiwara and Morita (18). Latency coding was also examined in mormyrids by Szabo (26) and Szabo and Hagiwara (29). Bennett (1) called the synchronous or high-frequency (tuberous) receptors "phasic" from their adapting response to d-c steps, but this name is no longer suitable. To physiological stimuli, like objects in the fish's own a-c field the response may be maintained or may partly adapt.

These two subclasses of nonconventionally coding neurons, together with the high and regular frequency of electric organ discharge in certain genera of Gymnotidae, offer particularly favorable opportunities to examine the coding properties of neurons. Quantitative analysis of the synchronous receptor types, thus far, has only been preliminary because stimulation has relied on dielectric and conducting objects introduced into the water or electric current superimposed on the fish's own field. The resulting fields are so complex that specification of the actual stimulus was impossible.

In the present study the stimulus situation is simplified by first silencing the fish's electric organ with curare, then substituting an artificial sinusoidal field which could be systematically varied in frequency, amplitude, and waveform.

The first question to be faced with the improved method, and one of the main purposes of this paper, is whether there are really two distinct classes of electroreceptors in terms of their properties as coders or only a certain variability in a single population. Since the first experiments showed that all synchronous units encountered, of

both presumptive classes, have both a low stimulus intensity range of graded probability of following and a high intensity range where following is 1:1 and only phase shift is graded, the hypothesis had to be set up that there is but one population with some continuum of properties. This report gives evidence that there are in fact two classes. The purpose of the bulk of the paper is to describe quantitatively these two classes of afferent units which differ in their manner of encoding stimulus intensity in the physiological range.

We shall refer to the two classes as type T or phase coders and type P or probability coders. These names refer to the main dynamic parameter encoding the stimulus voltage in the presumed physiological range of intensities. The main variable of response in this range in the probability coder class of unit is the probability of missing some cycles of the stimulus. The phase coder class fires 1:1 and therefore has only phase or latency as a parameter in the physiological range. This feature is preserved in higher order phase coder neurons (25). The name adopted is partly arbitrary since both phase and latency change, not only with intensity but with frequency, as will be shown.

MATERIALS AND METHODS

Specimens supposed to be of the species *Eigenmannia virescens,* 10–20 cm long, were obtained from tropical fish importers. They were anesthetized with MS 222 Sandoz (tricaine methanesulfonate), 2 ppm initially and during the experiment with a 10 times smaller dose. Flaxedil was injected intramuscularly in a dose of 0.01 mg/10 g. The fish were artificially respirated by a water stream led into the mouth. The fish was gently held on its side by gauze in a flat wax mold on top of a narrow plastic platform in the middle of a 20 x 48 cm rectangular plastic tank. The lateral line nerve was prepared behind the operculum. Opercular movements were mechanically suppressed by a small lever. The water was maintained at 26–27 C.

Single units were recorded with a sharpened tungsten wire coated with aged E 33 insulating varnish (Insl-X Co.) The indifferent electrode was a silver wire placed around the recording electrode like a small ring. This arrangement reduced the stimulus artifact. Spikes were recorded with a conventional amplifier and stored, together with the stimulus, on an Ampex FR-1300 tape recorder in FM mode, at 15 inches/

sec. Stimulation was performed by a pair of gold-plated, round-tipped 4-mm-diameter electrodes placed in the bath. The electrodes, 4 cm on either side of the fish, were placed transversely across the anterior half of the fish. In some low-frequency and waveform experiments local stimulation was used with one electrode near the skin of the fish.

The stimulus voltage was generated by a Data Royal F2030A signal generator coupled to the stimulating electrodes by a General Radio 578A bridge transformer. The transformer introduced a phase lead of 20° at 30 Hz; therefore, the stimulus was monitored by a second pair of electrodes in the bath both as to phase and amplitude changes. The latency of the spikes was measured from a zero crossing of the stimulus sinusoid from positive to negative to a consistent part of the next following spike. The phase of the spike was defined relative to the indicated zero crossing (0°) for every stimulus frequency. The latency was measured using a precision interval plotter (10). The normal sensitivity used was 1 msec full scale giving a resolution of 20 μsec. Only spikes with a 5:1 or better spike-to-noise ratio and less than 10% fluctuation in amplitude were used in order to facilitate latency measurements. A total of 76 units was recorded from 14 fish; 46 T units and 30 P units were identified; 20 units of each type were recorded extensively to allow quantitative conclusions.

RESULTS

To simplify comprehension of the terminology and of the following detailed account, Fig. 1 presents in summary form the main properties of the two classes of units, in diagrammatic form.

In the physiological situation (uncurarized; ref 8) the two receptor types were readily distinguished by their responses to conducting objects in the water. In the curarized animal with artificial a-c stimulation they were found to have very similar though not identical responses. The dynamic range of intensity coding under these conditions was found to exceed considerably what has been stated previously. Apparently the intensity modulation of the electric organ field caused by the objects used, as experienced by the receptors in the uncurarized animal, is relatively small. In other words, a large fraction of the dynamic range is never used under physiological conditions.
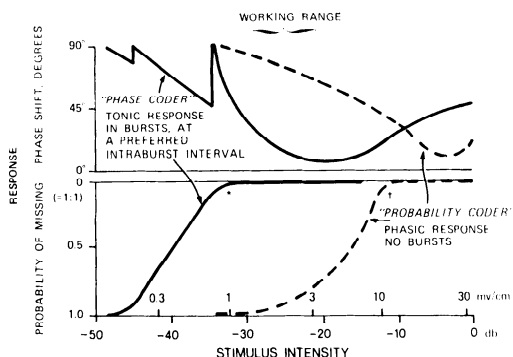
The physiological range of intensities is

FIG. 1. Schematic representation of the type T (phase coder) and type P (probability coder) responses, each measured by both phase shift and probability of missing. Both T and P units have a probability coding range and a phase coding range of intensities but they differ in these ranges. The working range is taken from measured natural signals, i.e., the normal electric organ discharge as modified by objects. *, the 1:1 following threshold of the type T unit; †, the 1:1 threshold of the type P unit.

that over which the receptors are normally driven by the electric organ field, as modified by the presence of objects. It is important, for distinguishing and even designating the two receptor types, to determine where this range lies within the wider dynamic range. One way to estimate this voltage gradient is to measure first the mean resting discharge frequency of the P type (probability coding) receptor in the uncurarized animal (i.e., driven by the electric organ). This value is typically 50 impulses/sec (range 30–75) for fish with about 300 electric organ discharges/sec. The range of transverse voltage gradients of artificial fields of sinusoidal form at the same frequency, needed to yield on an average 50 nerve impulses/sec in the curarized preparation, covered about 10 dB, partly depending on the position of the receptive field along the fish's body. The mean value was about −30 dB on our arbitrary scale; this corresponds to 1.37 mv/cm across the anterior half of the fish directly between electrodes (calibrated with the fish not in place). Using this intensity in the curarized animal we found it always possible readily to distinguish between phase and probability coders (defined in the introduction and DISCUSSION). It will be convenient to refer to this intensity as a single value represent-

ing the usual physiological strength as a reference point for comparison of the electric forces driving the receptors; it will often be referred to as the reference intensity or physiological intensity. Several factors influence the measured value, including water conductivity, loading of the electric organ field by the stimulating circuit, homogeneity, and axis of the stimulating field and condition of the receptors (see note in ref 19); all these factors have been discussed by Bullock, Hamstra, and Scheich (10).

Type I, phase coding class of afferent fibers

WEAK STIMULI—SPUTTERING RANGE. Units of this class in a curarized fish follow a maintained 300-Hz sinusoidal a-c stimulus at the electric organ reference intensity (−30 dB) with a 1:1 nerve impulse discharge. If the intensity is below an absolute threshold, all firing stops. Phase coders typically do not show spontaneous discharge. Some units have been observed to fire spontaneously at about 10 impulses/sec. If intensity is increased in steps of 1 dB, response curves as shown in Fig. 2A (at two stimulus frequencies) are obtained. The probability of firing increases with intensity. At 250 Hz, the probability is proportional to the log of intensity. At 350 Hz, near a probability of 0.5, the curve flattens out over about 3 dB. The interval histogram here shows that there is a high probability of firing to every other cycle of the stimulus (compare Fig. 7). The curve continues with a steeper slope at higher intensity.

The intensity range of graded probability was small—less than 1 log unit (20 dB) of intensity, and notably below the estimated physiological range (1.37 mv/cm), especially at the lower stimulus frequency. It terminates abruptly at the intensity that just causes 1:1 following and hence a plateau of the curve of mean frequency.

STRONGER STIMULI—1:1 RANGE, WITH PHASE CODING. At an intensity of about −44 dB, on the average, the type T or phase coding units reach the 1:1 firing threshold. The range of variation among different neurons was about 10 dB, which may be attributed to the different distances of the receptors from the electrode. The only response to
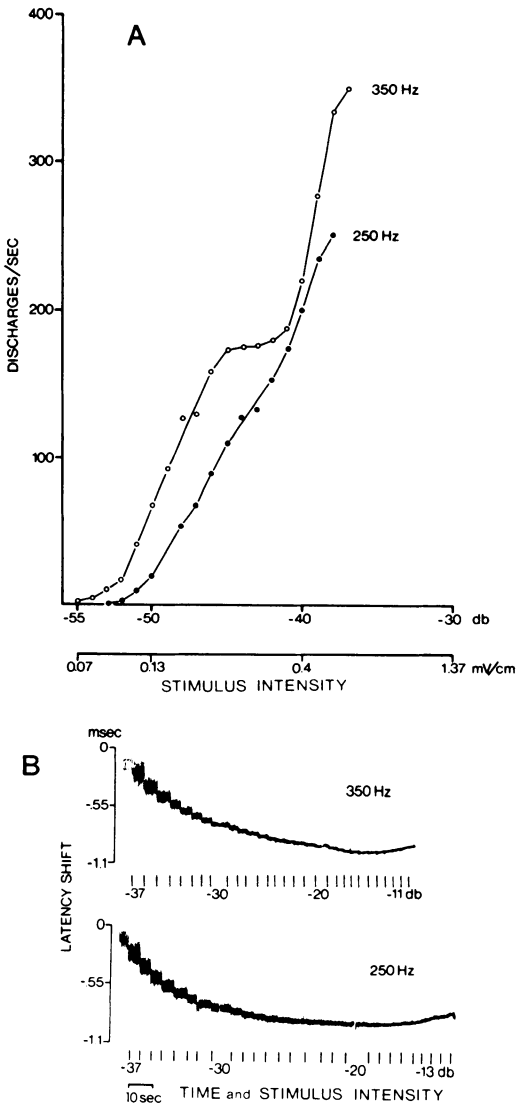
FIG. 2. *A:* phase coder in the probability range, response as function of stimulus intensity for two frequencies of stimulation. The response is tonic so that discharges per second are counted in the steady state. Note the plateau at half the maximum response frequency with stimulation at 350 Hz. *B:* phase shifts of the same unit above the 1:1 firing threshold. Original strip-chart records of a high-resolution interval plotter (10) measuring the delay between a constant phase of the stimulus and the neuronal spike. The amplitude of the stimulus is increased in 1-dB steps; note the stronger stimulus range than in *A*. Latency decreases with increasing intensity of stimulation. In figures which follow and which plot phase, this kind of interval plot was used, averaging the phase over several hundred cycles by eye; jitter well above threshold is about 0.03 msec and the reliability of the estimated value is within about 0.02 msec = 2.4° of phase.

further increase of intensity is then a clear decrease of latency. The changes in response to intensity steps are tonic in nature (Fig. 2*B*). The latency change is greatest just above the 1:1 threshold and decreases smoothly up to about 20 dB above this threshold where it reaches a minimum latency. Further increase of intensity causes a smooth increase of latency, which can almost reach the 1:1 threshold value (not shown in Fig. 2). The response curves are plotted in Fig 3*A* for two frequencies for the same unit as in Fig. 2. The maximum latency shift is higher for 250-Hz stimulation. Expressed as phase, for both frequencies the maximum shift is between 70 and 90°. The shapes of the response curves for the two frequencies are slightly different. The physiological intensity is typically in the middle of the phase shifting response range.

COMPARISON WITH NONSINUSOIDAL STIMULI. In the previous section sinusoidal waveforms were used. The natural stimulus to the receptors, the electric organ discharge, is a distorted sine wave (18). The head goes positive relative to the tail, during discharge, for somewhat less than 50% of the full cycle. In order to test stimuli of different harmonic content, a comparison was made between sine, triangular, and square waves of equal period. The result is shown in Fig. 3*B*. The three curves have similar shapes but the square wave has a 2-dB lower 1:1 threshold. These effects are not due to characteristics of the stimulus isolation transformer used. The similarity in shape is evidence that the T unit in this frequency range acts like a band-pass filter, and that the important component of the stimulus is the fundamental; the harmonics must contribute somewhat since the curves are not identical.

Information about the maximal range of phase shift with the natural electric field intact was obtained by stimulation with a silver plate in the uncurarized animal (Fig. 4). Simultaneously some information is provided about the receptive-field structure of a type T unit right behind the gills. Figure 4 shows diagrammatically the experimental arrangement and the phase shift to the stepwise back and forth movement of a 1.5 x 1.5
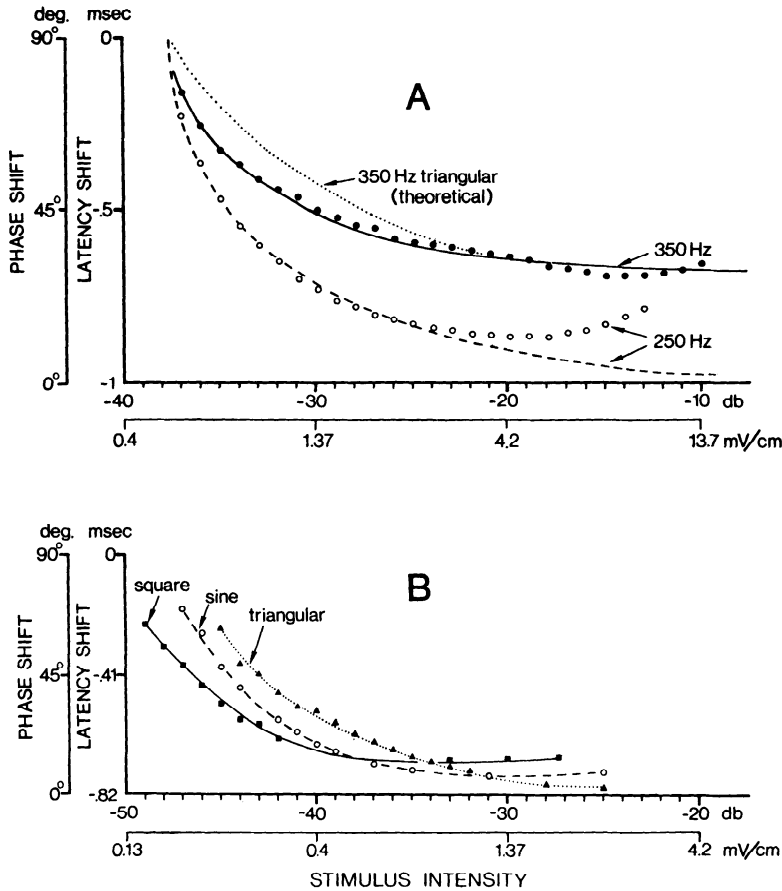
FIG. 3. Phase coder in the phase range. *A:* phase shift, expressed as latency, plotted as function of intensity of stimulation; the same unit as in Fig. 2. Symbols represent the experimental values. The solid and the dashed lines represent the phase shift calculated for the respective sinusoidal frequencies under the assumption that the unit fires at a constant threshold above zero. The dotted line represents the theoretical values for a triangular 350-Hz wave in order to show the effect of the shape of the stimulus. *B:* measured phase shifts of a unit which was stimulated with square (50% duty cycle), sine, and triangular waves of 300 Hz. The three curves have different 1:1 thresholds but very similar shapes, indicating a lowpass filtering of the original stimulus.

cm silver plate along the fish's body. The maximum response span obtained was about 75°, which corresponds roughly to the result obtained in the curarized animal. The hysteresis shown depends on several factors including rate of movement and size of plate.

GAIN AND PHASE AS A FUNCTION OF FREQUENCY. The receptor can be brought just to follow a sinusoidal stimulus, 1:1, over a wide range of frequencies. This is a kind of threshold and therefore a measure of sensitivity or gain. Plots of frequency versus threshold for the 1:1 criterion are given in Fig. 5*A*. The

symbols represent results from two *Eigenmannia* with resting frequencies of the electric organ before curarization at about 200 and 300 Hz. Note that the plateau ranges of highest sensitivity are in the region of natural frequencies, and that there is apparently some tuning to the individual, barring coincidence. The slope of the 1:1 threshold below the natural frequency of the fish is uniformly about 12 dB/octave, like that of a second-order high-pass filter. The high frequency slope falls steeply at 35–40 dB/octave.

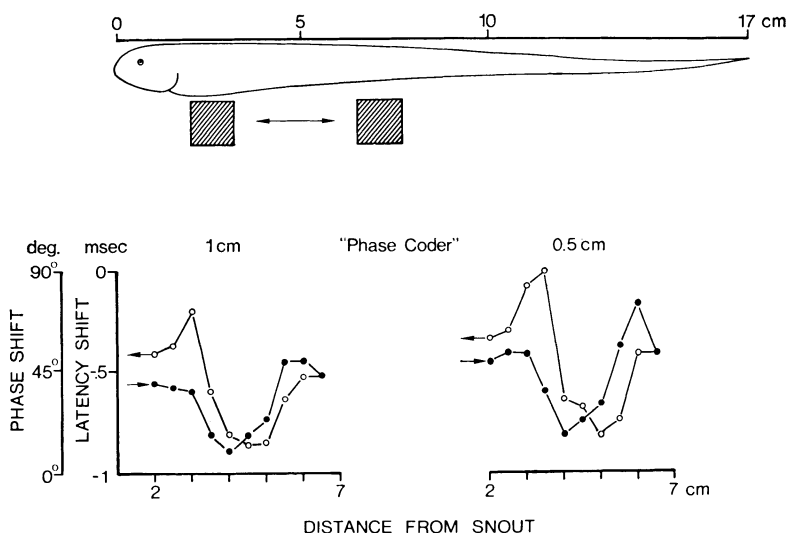At the high-frequency end (usually 700–800 Hz), 1:1 following fails; a stage of sput-

FIG. 4. Stimulation of a phase coder with a silver plate. The phase shift is plotted as a function of the position of the headward edge of the plate (leading in one direction, trailing in the other). The distance was 1 and 0.5 cm from the surface of the fish in order to obtain maximal stimulation.

tering gives way to firing on every other cycle. At about 1 kHz complete silencing of the unit supervenes.

Below 140 Hz the unit tends to fire repetitiously to each stimulus cycle, depending on the intensity. The absolute frequency response limit is near 1 Hz, when some 20 spikes/cycle can be fired. A more complete description of the behavior with low-frequency stimulation is given below in a section so headed.

When a phase coder is stimulated 10 dB above the 1:1 criterion the phase of firing as a function of frequency can be measured conveniently because of the very small scatter. At each frequency the 1:1 threshold was determined and then 10 dB of intensity added (Fig. 5B). The total phase shift, including any fixed delay, is near 1,000° over the whole frequency range.

The physiological range (between 200 and 400 Hz) exhibits a considerable proportion of the total phase shift. This means that an individual fish, when changing its frequency for any reason, by 1 Hz will introduce a phase shift close to 1° in the type T fiber. This is equivalent to an intensity change of the field of about 2 μv peak to peak (p − p)/cm—well above the behavioral increment threshold of this species, transversely, which was 0.25 μv(p − p)/cm (11). Therefore phase changes due to frequency altera-

tions are, in principle, able to interfere with the intensity response of the phase coders, if there is no compensatory mechanism.

Some delay time due to conduction of the spike from its site of initiation to the electrode is contained in the measured delay. A high-amplitude short rectangular stimulus causes a spike in somewhat less than 2 msec, depending on the position of the receptor. This delay may not represent only conduction time; the pulse presumably excites an electroreceptor sense cell which, in turn, synaptically excites the nerve fiber. The phase lag due to a constant delay is a linear function of frequency; at 250 Hz a 1-msec delay would yield 90° lag and a 2 msec, 180°. At 500 Hz the values would be doubled. The equivalent phase shifts due to fixed delays will have to be subtracted from the original data when we wish to consider the true phase shift in relation to gain (see Fig. 13 and DISCUSSION).

MODE OF FIRING IN SPUTTERING RANGE. *a*) When stimulated in its low-intensity range, below the level of 1:1 following, the type T unit is still bound to a distinct phase of the stimulus for each intensity, though firing at a lower mean rate than the stimulus frequency. A way to show this is the poststimulus time histogram (PSTH) generated by triggering a cumulative computer at a

constant phase of the stimulus. **Figure** 6*A* shows the development of the spike distribution with increasing probability of firing. The adapted state of the unit is used. The distributions shift their modal time of fir-

FIG. 5. Frequency response of phase and probability coders. Sensitivity of the 1:1 firing criterion as a function of frequency of stimulation. *A:* open circles and open triangles represent two units of a fish with 200-Hz discharge of the electric organ. Filled circles and triangles are from two units of a 300-Hz fish. *B:* phase shift as a function of stimulation frequency. The type T and type P units marked with open circles in *A* and *C* are selected. The total phase shift is more than 900° for both units. The high-frequency end above 110 Hz is very similar in both units. At the low-frequency end the difference between the two units becomes marked and reaches 130° at 30 Hz. *C:* probability coder. Sensitivity of the 1:1 firing criterion as a function of frequency in three type P units. The three units give the range of low-frequency slopes.

ing relative to the stimulus. The shift is in the direction of shorter latencies as the stimulus intensity increases.

The latency shift is smooth and not in appreciable steps over most of the range (−47 to −40 dB). The total shift over the sputtering range can be up to 60° for about 15 dB of intensity change. The standard deviation becomes smaller with higher intensity; that is, the phase is more stable at high mean firing rates.

A little below the 1:1 following threshold (−38 dB), the unit develops a second small distribution of spikes about 0.5 msec later, close to the phase at which the unit fires in the lower part of the phase coding range. This reset is relatively sudden. A proportion of the spikes over some decibels of stimulus voltage (ca. −39 to −40 dB) still persists at the earlier phase. A double distribution is therefore the starting point for the 1:1 range. The interval histograms of Fig. 7*A* show that this "resetting" coincides with the change of mode of firing from a minimum of twice the stimulus interval to some responses at the stimulus interval (see also below, DISCUSSION, and Fig. 6).

b) A remarkable feature in the sputtering range of the type T unit is the likelihood of a "preferred" or modal interval that is stable over a wide range of mean firing rates. The interval histograms (Fig. 7*A*) show a hump of responses already with very low intensity at the position of twice the stimulus interval. Longer intervals occur also but only in a small proportion. The firing occurs in bursts; and the preferred intraburst interval is twice the stimulus interval. The interburst intervals are of much greater length than the histogram time scale. The shrinking interburst interval is the determining factor for the increasing probability with intensity. The only change in the intraburst interval is the switch over to firing more or less often for successive instead of alternate stimulus cycles. This occurs, as one might expect, shortly below the 1:1 following level and coincides with the resetting of the phase. The implications of this coincidence are considered in the DISCUSSION. It will be shown in a later section that the bursting property constitutes one of the crucial differences between the type T and type P units.
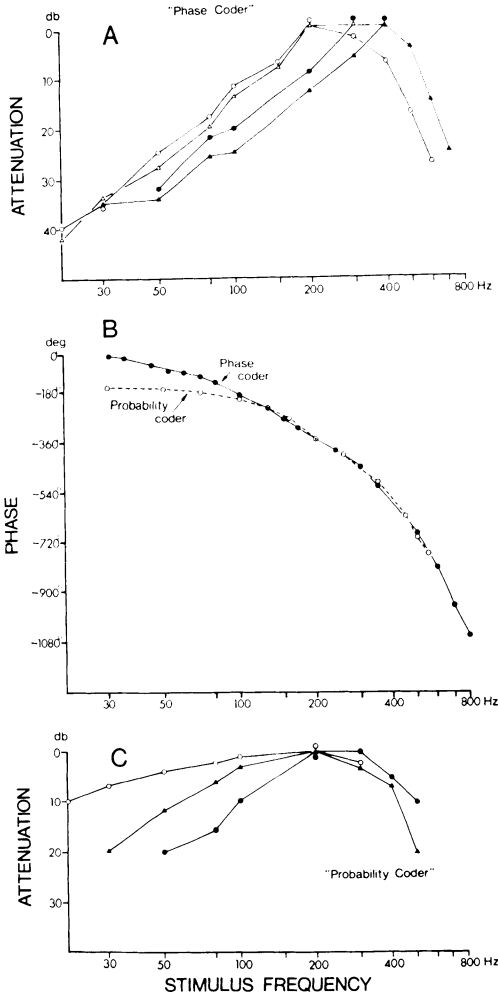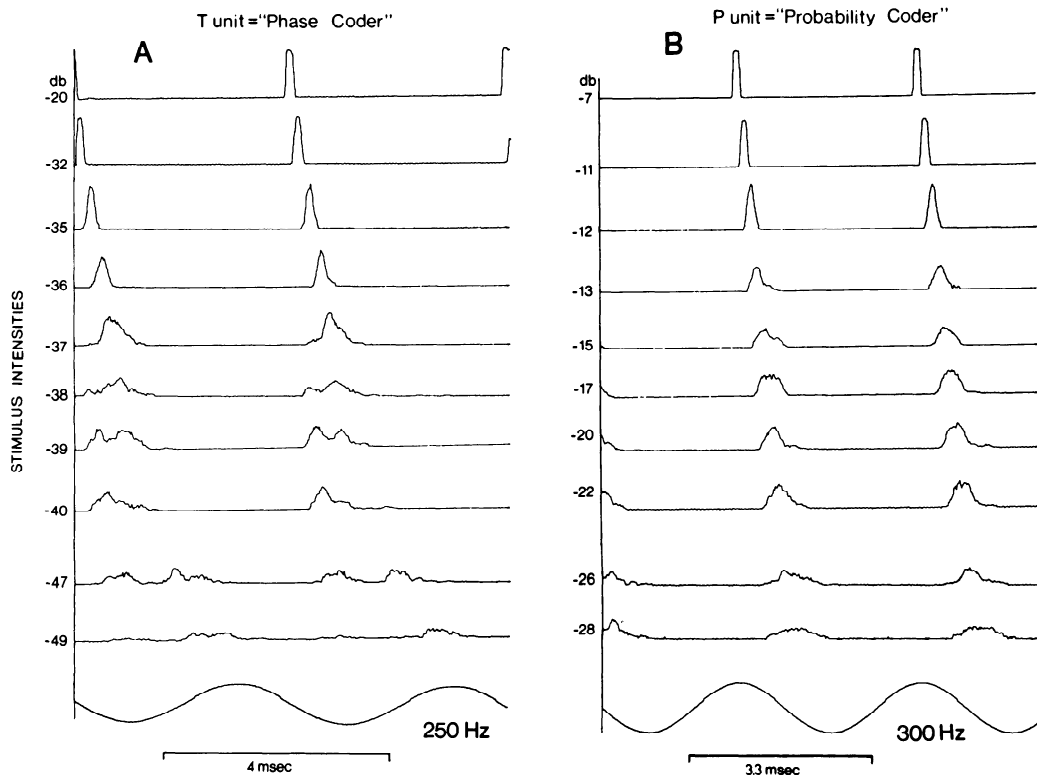
FIG. 6. Phase shift in the probability and phase range for both **T** and **P** units, plotted by a cumulative computer as poststimulus time histograms (512 sweeps). *A:* type **T** (phase coder), as in Fig. 2. The 1:1 threshold is between −38 and −37 dB. During the probability range (−49 to −38 dB) two resets to a later phase occur, from where the phase shift to higher amplitude starts again. The second reset with a bimodal distribution occurs at the transition from 2:1 to 1:1 mode of firing (see interval histograms Fig. 7*A*). *B:* type **P**, the same probability coder as in Fig. 7. The 1:1 threshold is between −12 and −13 dB. The total phase shift over sputtering and 1:1 range is approximately 90° and in the sputtering range (−28 to −13 dB) about two-thirds of this.

Type P, probability coding class of afferent fibers

WEAK STIMULI—SPUTTERING RANGE. At the physiological intensity (−30 dB) it is easy to identify the probability coders. Stimulated at 300 Hz the response is less than one spike for every stimulus cycle. Only with an increase of intensity to about −20 or −10 dB is a probability of 1.0 reached.

When the intensity is decreased in small steps, the firing rate changes typically in a partially adapting or phasic-tonic way. As in Fig. 8*A* the units respond to a decrease of intensity with an undershoot of firing rate, to speed up again with some time constant to a steady state (19). The time constant cannot be fitted usually by an exponential function. An average time constant is 3 sec, but the initial adaptation can be

much faster. The response to an intensity increase starts with an overshoot and adapts back to a tonic plateau, with a similar time constant. It should be noted from Fig. 8 that the over- or undershoots do not normally go to either 1:1 or zero firing over a wide range of stimuli. The type **P** unit could be used equally well to encode intensity increase and decrease.

The tonic firing rate of the type **P** or probability coder plotted against stimulus voltage is a complex function; we may recognize two sections of different slope (Fig. 8*B* "adapted"). The average slope of the lower section is 1 impulse/sec per 50 μv/cm of intensity change. When stimulated with 300 Hz, from an intensity sufficient to cause 100–140 impulses/sec, to the 1:1 threshold the slope is steeper, on an average 1 im-
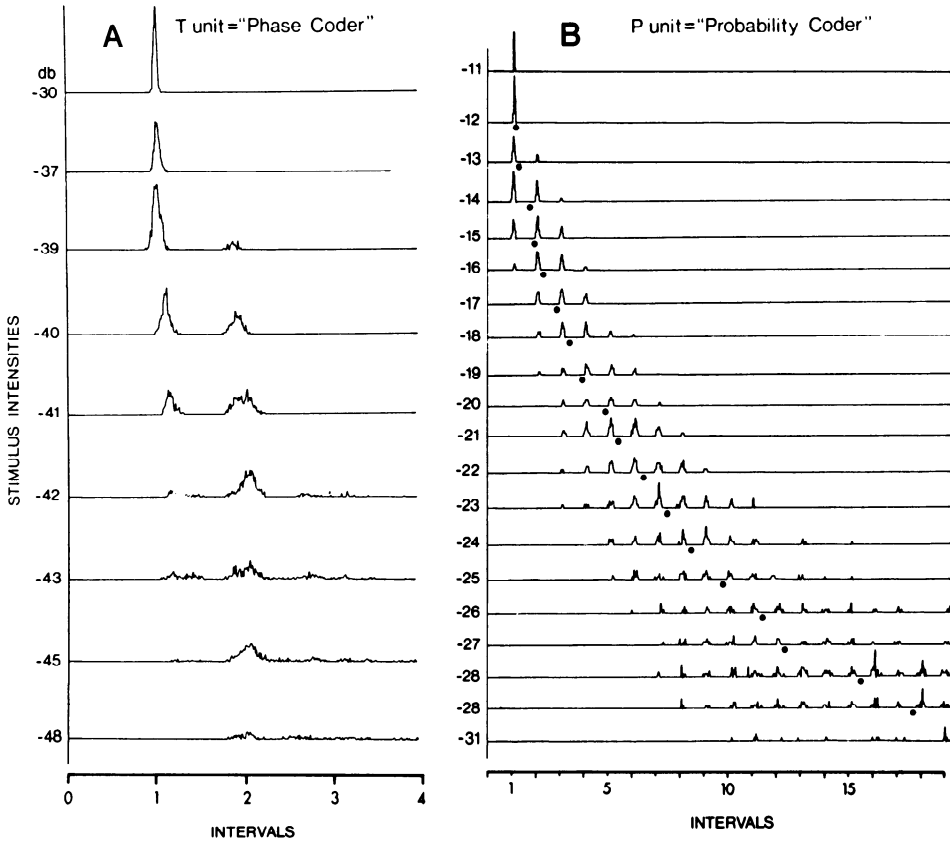
FIG. 7. Distribution of intervals for the probability ranges of a type T and a type P unit. *A:* type T unit exhibits a preference for firing in pairs at low stimulus intensity (the long intervals between pairs are off scale and differ for each intensity); firing begins at −38 dB. One interval is 4 msec. *B:* type P unit exhibits a systematic change with increasing amplitude from a widely scattered distribution of many multiples of the stimulus interval to a narrow distribution of unit intervals. It is remarkable that the single intervals are so well separated indicating a narrow phase range of firing. One interval is 3.3 msec. Dots are explained in the text (DISCUSSION, PROBABILITY CODER).

pulse/sec per 25 µv/cm change. Thus we have the case where a neuron has two different linear response ranges for lower and higher intensities. The inflection is sometimes near the reference intensity; that is, the physiological range of stimuli may include the change in slope so that the response to equal steps of intensity upward and downward may be asymmetrical, the response to intensity increase being larger.

In comparison to the phase coder the absolute threshold of the probability coder is about 10–15 dB higher. This is one of the major differences between the two types of afferent fiber. Typically, the dynamic range in which probability encodes intensity is greater for the P or high-threshold units so that 1:1 threshold is >20 dB higher than for the T units (Fig. 1).

STRONGER STIMULI—1:1 RANGE, WITH PHASE CODING. Above −15 dB on the average the P unit fires 1:1 and grades intensity by phase shift, like the T unit (Fig. 8C). The response to a stepwise intensity increase again shows a transient reduction of latency, which declines exponentially to an intermediate plateau latency with a time constant of 3 sec. Figure 8C shows a series of responses to steps of intensity, first upward and then downward. The phasic and tonic components are both at their largest in the first few steps above the 1:1 threshold and then increment more slowly. The broad maximum or plateau is usually reached within 5 dB, less than half the dynamic range of the type T or phase coder units. The total span of phase shift can come close to 90°, but in most cases does not.
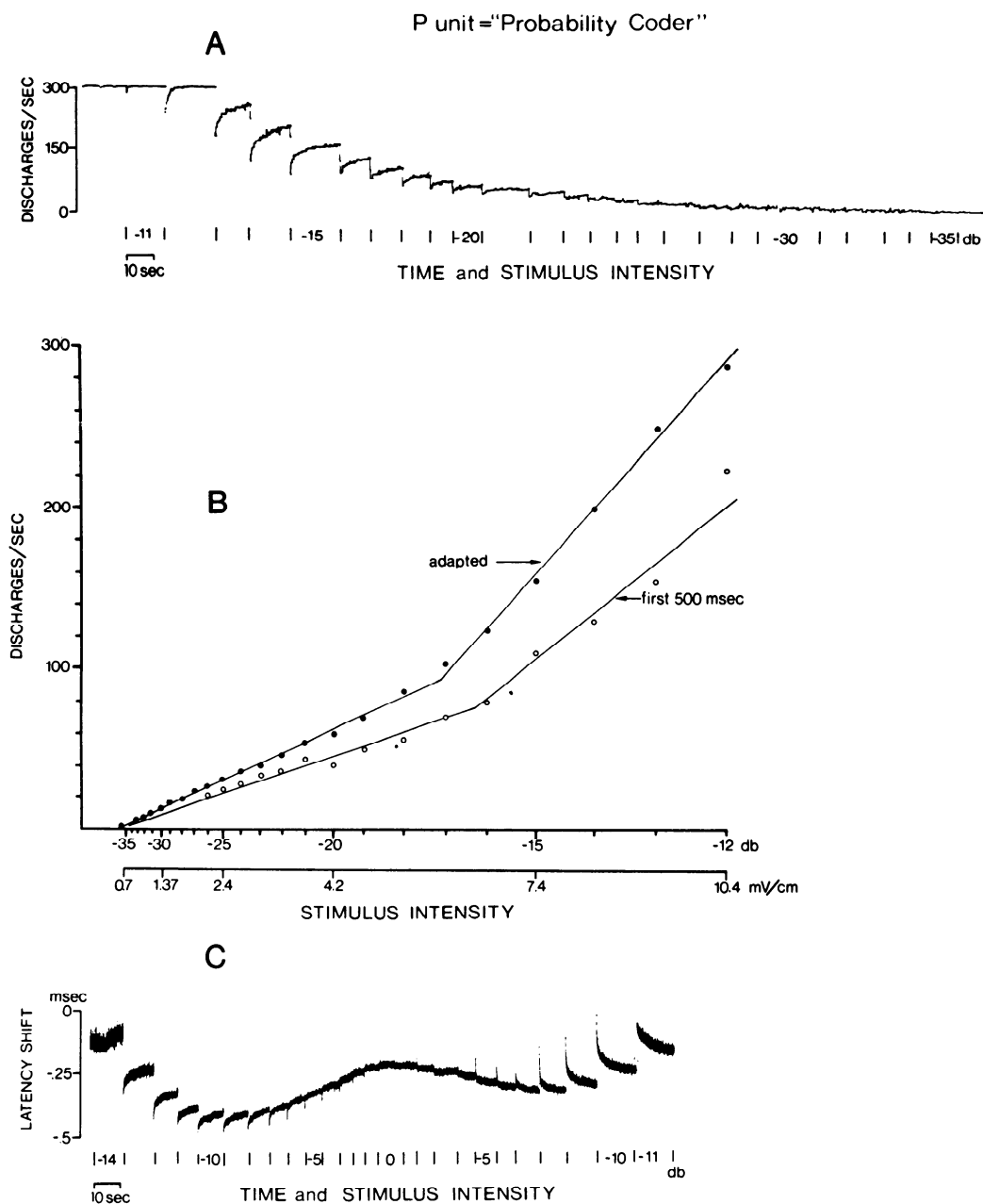
FIG. 8. Responses of the type P unit in the probability and the phase range as a function of stimulus intensity. *A:* probability range. Original record of an interval plotter measuring the mean spike interval with a bin width of 200 msec. The record is calibrated in discharges per second. The stimulus frequency was 300 Hz and the intensity was decreased in steps. The record represents the right-hand continuation of the record in *C.* The time constant of the phasic response is about 3 sec. *B:* discharge rates of the above responses are plotted against stimulus intensity on arithmetic coordinates. Data can be fit by to linear slopes. One-to-one firing in the adapted state starts at −12 dB. (Compare interval histograms in Fig. 7*B.*) *C:* interval plotter record showing the phase shift, measured as latency of the unit relative to the stimulus, above the 1:1 firing threshold in response to intensity switching. The intensity is first increased then decreased. Note the hysteresis of the phase shift. The time constants of the phasic responses are similar to those in the probability range (see *A*).

After the maximum, further intensity increase leads to a reversal in both the phasic and tonic responses, i.e., latency increases.

The stepwise decrease of intensity in Fig. 8C shows a high-intensity reversal of the phasic component. It is noteworthy that the response for any given stimulus intensity is mostly not the same in the increasing and in the decreasing series (hysteresis), the responses in the decreasing intensity series are equivalent to lower intensities in the increasing series. This effect is perhaps due to the unphysiologically high intensities the receptor has recently experienced. We did not test whether the hysteresis would disappear if a much longer time was allowed at each step, but it is possible that there is at high intensity a specially slow adaptation phenomenon. Hysteresis is also shown in Fig. 4 in the phase coder during the back-and-forth shift of the silver plate.

As compared to the type T unit the dynamic range of the P units in this phase-shifting range is narrow. Unphysiologically high intensity is required to reach and exceed the 1:1 firing in the type P receptors.

GAIN AND PHASE AS A FUNCTION OF FREQUENCY. As in the case of the phase coder, the 1:1 threshold for the adapted state can be measured over the whole frequency-response range (Fig. 5C). The range of 1:1 following does not usually exceed 500 Hz. Further increase in frequency leads to partial missing, followed by silencing. The plateau range of lowest threshold is around 200 Hz. The attenuation for both the high and low frequencies is quite variable in different units. The sensitivity at low frequency falls at 3–10 dB/octave.

The phase shift of one unit in Fig. 5C (open circles) is shown in Fig. 5B. The phase was measured in the adapted state at 10 dB above 1:1 threshold for each frequency. The phase shift above 110 Hz is quite similar to that of the phase coders. As expected from the low-frequency end of the gain data, the phase change in this frequency range is very small. At 30 Hz the phase difference between the two types of units is about 130° (compare the section on low-frequency stimulation and Fig. 9). In contrast to the gain data the phase curves for probability coders are more uniform.

Since the P unit is phasic the filter functions measured for the adapted state reflect only part of the filter properties. These are more fully explored in a dynamic situation (unpublished observation).

MODE OF FIRING IN SPUTTERING RANGE. a) For both neuronal types the high-intensity domain has been called explicitly the "phase coding range." This subdivision is, however, merely due to the fact that after the 1:1 threshold is reached, nothing besides phase information can be provided by the neuron. In the so-called probability range (sputtering range) both neuronal types also change phase of firing with stimulus intensity. (Compare section on type T units.) Figure 6B shows a set of PSTHs for a type P or probability coder in the probability range. The frequency of stimulation was constant and is shown below. The intensity was increased stepwise and the tonic response is displayed. Again, as in the phase coder a smooth phase change is obtained. There is, however, no reset before the 1:1 threshold. The phase shift is therefore continued above this threshold. The total span of phase shift yielded in this way in the probability coder over both ranges is roughly 90°, of which up to 60° is in the probability range.

b) The distribution of modal or preferred intervals in the probability range, for the adapted state, is given in Fig. 7B. Below the 1:1 threshold the preferred intervals of firing smoothly increase as intensity decreases. The distribution of intervals as a whole also becomes broader. Even at low intensity the unit still fires at distinct multiples of the stimulus interval; i.e., the responses do tend to smear (see also PSTHs, Fig. 6A). In contrast to the phase coder, we do not see bursting in the probability coder; the modal intervals smoothly shift with the mean frequency as intensity changes; there are no long intervals off the scale.

Special behavior of both types with low-frequency stimulation

The low-frequency domain for both types of afferent units can be defined as the range where multiple spiking is obtained in each sinusoidal stimulus cycle. The upper limit of this range varies slightly among units and the two types of coders, but is generally
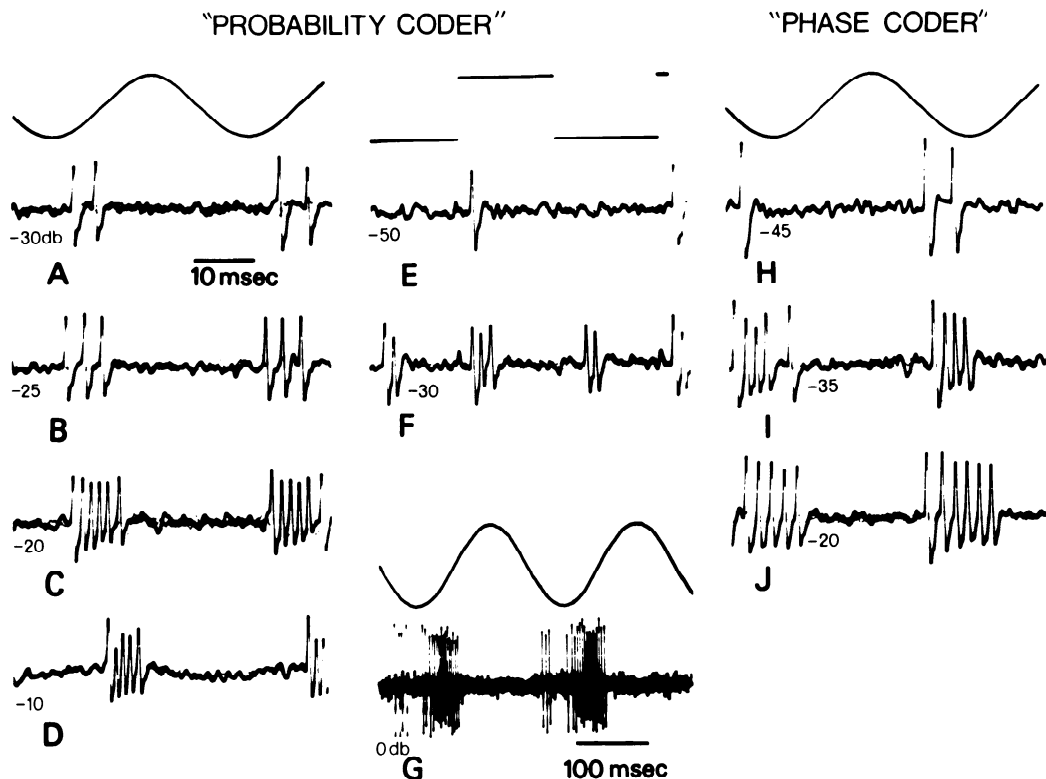
"PROBABILITY CODER"   "PHASE CODER"

FIG. 9. *A–D:* 30-Hz stimulation of a type P unit with different stimulus intensities. Traces represent superimposed single sweeps. The time variation of occurrence of the first spikes, relative to the stimulus, from *A* to *C* is typical for stimulation below 50 Hz and can be reduced by averaging. Only the high-intensity phase to the right in *D* is significant; there is also a reduction of the spike number. Note the decreasing interspike interval with higher intensity of stimulation. *E–F:* same unit with 30-Hz square-wave stimulation; there is a spike response also to the negative step of the stimulus but with longer latency (positivity upward as in all traces). *G:* stimulation at 5 Hz; up to 25 spikes/cycle. *H–J:* 30-Hz stimulation of type T unit. Compare the phase of the first spike in *A–D* with *H–J.*

between 100 and 140 Hz; it is a little lower with weaker stimuli. With sinusoidal stimulation an increasing number of spikes per cycle is elicited by lowering the frequency. A higher number of spikes will also appear when the intensity is increased at low stimulus frequency. Figure 9*A–G* shows the main phenomena for a probability coder with sinusoidal and square-wave stimulation and for a phase coder (*H–J*) with sinusoidal stimulation (both at 30 Hz). In the probability coder the 1:1 threshold was reached at −38 dB and 300 Hz. At 30 Hz 1:1 firing was obtained at −33 dB and 2 stable spikes/stimulus cycle appeared at −30 dB, 3 discharges at −25 dB, 6 discharges at −20 dB, dropping to 5 spikes at the high level of −10 dB. Accompanying the increase of spike number is a decrease of interspike

interval which seems to be fairly regular from the first to the fifth interspace. The minimum interval obtained at −10 dB is 1.2 msec, which is even smaller than the minimum interval for 1:1 firing at the upper frequency limit for following sinusoidal stimuli; i.e. 700 Hz. With 5-Hz stimulation a maximum of 25 spikes/stimulus cycle could be obtained (Fig. 9*G*). Figure 9*D* illustrates the response with high-amplitude stimulation; the number of spikes in a train decreases and a considerable phase lag is introduced in both types of unit.

Object detection by capacitance

The two receptor types have been discussed in this paper as largely equivalent, providing similar information with a different code except in the social situation with

two superimposed electric fields when the two coding types have quite different roles (10, 11, 24). But another conceivable situation where the two receptors would report different information might obtain if they can react differently to objects with capacitative reactance.

Considerations of object detection up until now have been limited to detection of changes of ohmic resistances in the water. Certain objects in the fish's environment, however, probably have in addition capacitative qualities sufficient to alter the voltage gradient and the phase of the electric organ field. If so and if electric fish can assess these qualities, the suggestion has arisen (joint discussions with A. J. Kalmijn) that it would be a useful additional way to discriminate objects, perhaps especially living organisms. Cell membrane capacitance has been found in most tissues to be of the order of 1 $\mu f/cm^2$ (14). Muscle tissue (Table VB, ref 22) has a dielectric constant in the 100–1,000 Hz range of 100×10^3 to $1,000 \times 10^3$; this means that 1 cm^2 electrodes 1 cm apart would see about 0.01–0.1 μf. However, we do not know the values for whole organisms or objects that *Eigenmannia* is likely to encounter, and hence what capacities are available for it to detect. No behavioral evidence for this capability is known to us either. We can now report, however, that the specific receptor properties adequate to detect and estimate capacity are clearly there.

The influence of a capacitor on alternating currents is recognized by a change in intensity and a change in phase of the current relative to the voltage. Both are frequency dependent. Therefore, the capacitative nature of an object can be identified in two ways: by measuring the intensity change caused by an introduced object while varying the frequency, or by measuring simultaneously the intensity and phase change when the frequency is stable.

The following experiments show that *Eigenmannia* has a stable enough electric organ frequency and a suitable dual set of receptors to perform in principle the second operation; that is, the two coding types of receptors are different in such a way as to permit assessment of capacity, if the brain reads this information.

Noncurarized, anesthetized *Eigenmannia*

were prepared for electroreceptor unit recording. Two metal electrodes 2 mm in diameter and 2 mm long were placed 7 cm apart in the water on one side of the fish; one 2 cm from the skin opposite the site of the receptor, the other 6 cm from the skin opposite the side of the tail. The electrodes were connected together, one by one, by any of a set of resistors or capacitors, thereby shunting the conductance of the water locally. The impedance of each capacitor was calculated at the fundamental of the fish's electric organ frequency, treated as a sine wave, and the influence on receptor units compared to that of the pure ohmic impedances.

A number of phase coders and of probability coders in the lateral line nerve have been tested with this arrangement. Figure 10 shows a typical set of results for a unit of each type. The P unit was firing at a low probability as usual in the physiological
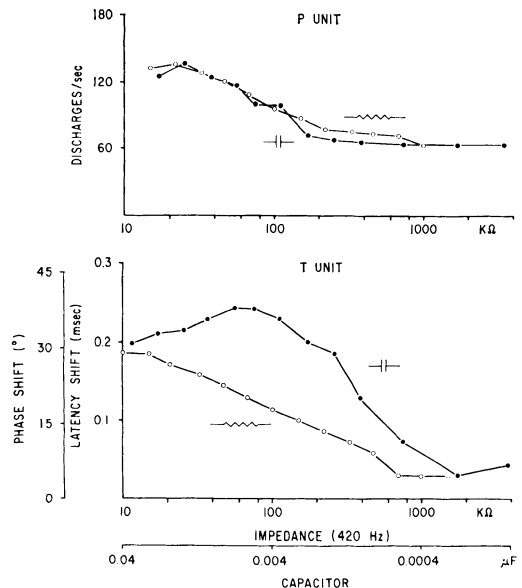

FIG. 10. Responses of P and T units in the lateral line nerve to shunting a portion of the electric organ field by resistors or capacitors. Electrodes 7 cm apart in the water oriented in parallel to the field lines and one electrode 2 cm from the receptor. Resistors (open circles) or capacitors (filled circles) connected across electrodes. The impedance of the capacitors is given at 420 Hz, the fundamental of the electric field. *A:* P unit response measured as mean firing rate in the adapted state; points are average rates in 20-sec samples. *B:* T unit response measured as phase lead (upward) by visual averaging of the interval plot.

range; i.e., with its own electric organ as stimulus. The mean rate of firing increased with shunting and did so equally for pure ohmic and pure capacitative shunts. The slope depends on the position of the electrodes in the field.

The T unit was firing 1:1 as usual and only changed phase. The phase shift was quite different for the ohmic and the capacitative shunt. The ohmic shunt must be working by the same effect as that of Figs. 1, 2, 3, 4, 6A, and 7A, due to its alteration of the local voltage gradient. The capacitative impedance must be causing phase shift in two ways, partly by the same amplitude effect and partly because the electric organ field is phase shifted; the effects will be additive.

The P unit shows a phase shift also which is much less sensitive to amplitude and has much more phase jitter so that, as before, we may suppose its useful encoding is by mean firing rate.

Although the smallest capacitor detectable depends on electrode position, the example of Fig. 10 shows that a capacitor of 1.0 megohms at 420 Hz = 0.0004 µf in the geometry of electrode employed causes a reliable phase shift in the T unit. Moreover, the results show that the phase shift of the field is detectable in the T unit with smaller capacitors than the amplitude shunt since the latter component of the capacitative effect is given by the ohmic curve (threshold ca. 500 kilohms). The combined effect of a capacitor well above threshold (e.g., 380 kilohm = 0.001 µf) is a phase lead equal to that caused by a pure resistive shunt of 70 kilohms.

To gain some impression of the relative magnitude of the threshold value of 1 megohm, the water resistance between the same electrodes was 4 kilohms. The T unit therefore responds to a local inhomogeneity caused by adding a relatively small capacitative impedance. The other and preferable way to evaluate is by comparison with the capacitative component of the complex impedance of interesting natural objects—roots, alive and dead, prey, conspecifics, etc. Figures are not available as yet to make this comparison.

While either receptor type is influenced by capacitative shunts, neither one alone can usefully signal that there is a capacitative as distinct from an ohmic impedance in the water. The T unit is completely ambiguous, since it only shifts phase. The P unit theoretically could signal uniquely because its phase shift due to the phase shift of the field in the presence of a capacitor might, in principle, be recognized as different from a similar phase shift due to an ohmic shunt if the analyzer in the brain labeled a predetermined relation between phase shift and mean firing rate as one type of stimulus and another relation as another type of stimulus. We will not here discuss second-order complications such as changes in electric organ frequency. But this theoretical ability of the P unit is severely limited by its relatively flat phase shift to intensity change, its phase shift merely due to change in firing rate and, most of all, its serious phase jitter.

However, the two receptor types complementing each other can signal capacity unambiguously. If they converge in the brain, a suitably decoding integrator need simply compare whether the phase shift in the constant-high-frequency T channel bears a certain relation to the firing rate change in the phase-jittery P channel to distinguish capacitative from ohmic shunts. Mixed impedances will give values between the curves of Fig. 10 but, without frequency change, the relative contribution of capacitative and resistive components cannot be analyzed.

DISCUSSION

Two types of afferent units

The findings reported in this quantitative characterization of a random sample of afferent fibers in the lateral line nerve of *Eigenmannia* appear to justify the distinction between two classes among the units firing in synchrony with the electric organ. This confirms the qualitative description of Bullock and Chichibu (8). The main differences that define them are summarized in Fig. 1. Clearly, they are quite similar and overlapping, though distinguishable on many criteria. We suspected at the outset that they were two ends of a continuum either preexisting or merely stages in the survival, under our conditions of prepara-

tion, of a single class. But the lack of intermediates, the stability of properties, the occurrence of both types in the same preparation in random sequence, and the considerable number of units seen for a sufficient time to verify identification convince us that the classes exist in the normal animal. This assumes that light MS 222 anesthesia and a small dissection far from the receptor are not deleterious.

The following sections discuss selected aspects of general neurologic interest and which are important for the development, in a later paper, of central and system consequences of this input.

Constant threshold

The normal intervals of firing, around 3 msec, lie in the relatively refractory period. Since the threshold is falling progressively one might expect that stimuli of different amplitudes would intersect this recovery curve and trigger the impulse at different voltage levels. The evidence is, on the contrary, that the threshold voltage remains the same for different amplitudes of periodic stimulation at a given frequency. The following analysis shows that this is to be expected in the general case of a progressive recovery interrupted by a periodic stimulus that causes a single spike for each cycle.
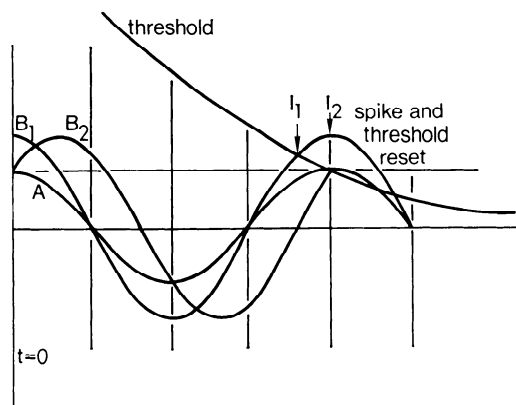
Consider the initial condition of a stimulus just strong enough to reach threshold on each cycle (Fig. 11, curve A). The time interval between spikes equals the stimulus

FIG. 11. Graphical deduction of the constant threshold principle of firing in the relatively refractory period of the T unit. For explanation see text (DISCUSSION, *Constant threshold*).

period. The stable point of intersection between signal and threshold corresponds to the maximum of the signal (I_2, 90°) since the phase there is equal to that at $t = 0$. Any signal of smaller amplitude would need more than one complete cycle to fire a spike; i.e., the relationship would not be 1:1.

Let us now consider a sudden amplitude increase leading to curve B_1. The point of intersection is then I_1. It is obvious that the phase of the stimulus at I_1 does not correspond to the initial phase at $t = 0$; therefore, on the next cycle the spike starts out at a different phase. The phase of the stimulus at which the spike is fired will undergo a variation over a number of cycles, predicted by the initial phase, the frequency and the amplitude of the stimulus, and the slope of the recovery curve. It is in fact a series approaching a stable phase.

We are only interested here in the steady-state conditions; i.e., the stable phase relationship between stimulus and spike which is usually reached. This relationship is graphically deduced in curve B_2. The phase of the signal B_2 in $t = 0$ and I_2 is identical. The important result is that the firing threshold for both signal amplitudes (A and B) is a constant. This result allows a simple mathematical description of the phase at which the spike is fired as a function of amplitude of the input signal, if the frequency is known. For any signal with higher amplitude than the one with initial conditions the spike will be fired at an earlier phase. The upper limiting case is a signal of infinite amplitude which will fire the spike 90° earlier. The agreement of our findings (Fig. 3*A*) with this expectation means that there is no accommodation in the first few milliseconds in these receptors.

PHASE CODER. In Fig. 3*A* the phase shift of an afferent unit was plotted as a function of stimulus intensity for 250 and 350 Hz sinusoidal input. It was shown that the span of phase shift in either case was somewhat below 90°. For these response properties the hypothesis has been tested that the unit fires at a constant threshold voltage. The phase shift has been calculated for a sinusoidal input signal. The heavy and dashed lines represent the calculated phase func-

tion and apparently fit the data in the 350-Hz case. The theoretical function reaches 0° asymptomatically for infinite amplitude and therefore cannot account for the reversal of the phase shift which occurs at high amplitude (not shown) for 350 Hz. This phase reversal starts at a lower field intensity when the frequency is lower (250 Hz). Therefore the span of phase shift reached with lower frequencies is generally smaller than 90°, but the shape of the response curve in the lower part is not distorted. It was not tested expressly whether receptors in the rare fish with extreme low frequency, for instance 200-Hz natural electric organ discharge, would reach 90° at that low frequency. The curves presented here are from an animal of 330 Hz before curarization. Most of the receptors tested for the constant threshold principle stem from fish between 300 and 400 Hz and show a full 90° span of phase shift with a shape similar to the theoretical (Fig. 3A).

PROBABILITY CODER. We will here extend the threshold principle into the range of less than 1:1 following to the adapted state of the probability coder. This unit is remarkably linear in the low-intensity range of partial missing, which is not true for the phase coder in the equivalent (probability) range.

In the probability range the stimulus intensity is obviously small enough not to allow every membrane potential excursion to reach threshold. Under the constant-threshold principle, when the signal amplitude is small there must exist a stable threshold amplitude after multiples of one cycle of a given input frequency have elapsed. Let us assume that after two cycles a certain small signal reaches threshold just at the maximum. The spike intervals would be then twice the normal interval and the unit would fire at half the input frequency. We can now increase the amplitude of the input signal and would observe a phase shift of the spike relative to the stimulus. This time however we would not reach 90° phase shift because at a certain amplitude the earlier cycle will already reach threshold, thus doubling the firing frequency in one jump. Compare the behavior of the phase coder at

this point, as shown in MODE OF FIRING IN SPUTTERING RANGE.

If the amplitude decreases more and more, the intervals between spikes will increase as multiples of one cycle. Firing will still occur however at a "constant" amplitude which is specific for the number of cycles that have elapsed and the value the threshold has reached during this time.

It is physiologically reasonable to believe that the recovery curve can be influenced by the recent mean frequency of firing, i.e., can be appreciably depressed by two or more short intervals between spikes and, contrariwise, enhanced by one or more missed cycles. Such longer term effects, even if we consider only the number of spikes in the last few stimulus cycles, permit us to expect intermediate ratios. Thus, a certain stimulus amplitude might tend to cause every third interval to be missed (making a series 110110110) or a weaker stimulus might cause a succession like 1010010100101001, assuming no jitter.

The theoretical case predicts a single most likely interval or two adjacent intervals (or higher order fixed successions) between spikes for each given amplitude. From Fig. 7B we find that the interval histograms of the probability coder are always a distribution of several preferred intervals. We can justify the use of the mode of the distribution of preferred intervals for a test. The first requirement is that the interval histograms, as in Fig. 7B, and the PSTHs of Fig. 6B show narrow distributions corresponding to only small phase variation.

As explained above, we expect from the constant-threshold concept that the stimulus amplitude which will, for instance, yield half as many spikes as there are stimulus cycles, will also show a preferred spike interval of the length of two cycles. We have checked the applicability of the constant-threshold concept in this way: for all fractions of 300 discharges/sec with 1 in the numerator, i.e., $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{4}$, etc., the corresponding stimulus amplitude is looked up in Fig. 8B. It is then compared in the interval histogram table of Fig. 7B to see whether at this particular decibel point the modal interval corresponds to the expected value, which is the reciprocal of the firing fre-

quency. For instance 150 spikes/sec are reached between −15 and −16 dB, which corresponds to a mode of every other interval in Fig. 7B; 100 spikes/sec are reached at −17 dB, which corresponds to every third interval as a mode in the interval table, etc. As a guideline to every decibel point, the theoretical modal interval is calculated as the reciprocal of the number of discharges. These values are then marked as filled circles under the histograms of Fig. 7B. It is shown that the receptor follows the constant-threshold prediction to a good approximation.

It should be pointed out that the constant-threshold behavior of *Eigenmannia* is equivalent to finding that the specialized receptor cells have a receptor potential that is faithfully microphonic. This is in striking contrast to the "receptor potentials" so far described in electric receptors. Szabo and Fessard (27), Bennett (1), and Harder (20) have reported complex and varied spontaneous potentials, recorded externally over the receptor opening, in mormyrids and low- and high-frequency gymnotids, including *Eigenmannia*.

NOISE. Phase jitter is evident in Figs. 2B and 6A and is highest just above the 1:1 firing threshold. It can be considered as noise inherent in the receptor to spike-initiating system, if it is not attributable to amplitude noise on the input signal. Since it has less influence on strong responses, it acts like noise of a certain amplitude, independent of the stimulus.

Interval fluctuation in the sputtering or probability range is another possible manifestation of amplitude noise. Let us inquire whether the interval distributions of the probability coder in Fig. 7B could be explained by equivalent noise of a fixed amplitude, and what that amplitude would be. As discussed above, with weak stimuli, multiplication of the basic interval of the electric organ discharge or stimulus can be expected, so that the afferent unit would fire on every second, third, fourth, and fifth cycle, assuming no noise. Specified intermediate successions of adjacent multiples of the basic interval were also held to be theoretically possible, e.g., 110110110 or 10100101001.

Bullock and Chichibu (8) analyzed firing and misfiring sequences in *Sternopygus* for serial dependence and found a significant though weak tendency to uniform run lengths; a perturbation in the direction of randomness is strong but does not wipe out the tendency to regularity. The span of preferred intervals in the histograms of Fig. 7B grows with decreasing intensity of the field in a systematic manner. This feature can be translated into millivolts amplitude of "equivalent noise." The translation is carried out for the above neuron in Fig. 12 (for method see legend). A constant noise level of about 4 mv(p − p)/cm is found which is not present in the water and seems likely to be a property of the receptor cell or the nerve ending. At low stimulus intensity this noise would be bigger than the signal. Since at low intensities firing occurs still at discrete intervals we can specify further that the equivalent noise can only be active during one polarity of the stimulus wave.

The introduction of jitter would have a real value in improving averaged resolution if only the relatively coarse steps were available of every second, third, fourth, or nth cycle; i.e., if the course of recovery is not influenced by the preceding interval or recent mean frequency. But the meager evidence available, which is that cited above, from *Sternopygus,* is in favor of some tendency to runs, such as 011011011 or 111011101110. The jitter nearly obscures this tendency, but we cannot claim that without the jitter small differences in mean rate could not be produced.

Frequency response

Bennett (1) proposed an equivalent circuit for the high-frequency receptor containing a blocking capacitor to account for the phasic response to d-c steps. Our results showed that the proposed filter not only has a high-pass section but that it is a band pass which will largely be specified for the two units in the following section.

PROBABILITY CODER. For the probability coder a reasonable fit of the gain data is obtained with a single section high-pass filter with a 3-dB point at 100 Hz and a low-pass
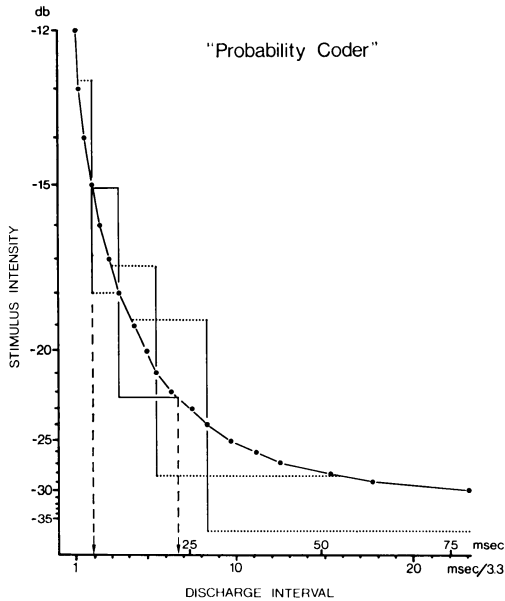
FIG. 12. A method of estimating the equivalent noise of P unit discharge intervals expressed as though due to fluctuation of the electric field in the water. The curve of mean interval for different stimulus intensities (from the unit of Fig. 8) is regarded as describing the recovery of threshold after a spike. A high-frequency stimulus, e.g., 300 Hz, of a given intensity will reexcite, following a spike, when the stimulus crosses the recovery curve; this interval will represent some number of stimulus cycles as shown on the outer abscissa scale. If there is noise this interval will fluctuate. If the natural fluctuation of the unit in Fig. 8 is between 6.5 and 22 msec (vertical arrows), with a mean of 11 msec, the stimulus intensity must have averaged − 18 dB with noise equivalent to the black bar whose solid horizontal projections from its maximum and minimum meet the recovery curve at about − 15.2 and − 22.3 dB. This span can be given as equivalent microvolts per centimeter in the water (see text). Other vertical bars and dotted projections fit the same noise level at other mean intervals.

filter of nine sections with a 3-dB point at 200 Hz (Fig. 13, filled circles). The phase curve obtained for this arrangement starts to deviate from the data points (open circles) above 200 Hz. Transmission delays in the peripheral nerve must account for some phase lag in the upper frequency range. The delay that would account rather well for the deviation is about 1.1 msec, which is in reasonable agreement with conduction times obtained experimentally. The probability coder is probably to be characterized

as having little deviation in phase from that expected of the filter chosen to fit the gain data.

The probability coder adapts with a time constant of about 3 sec. This adaptation could not account for the gain and phase data, since for a time constant of 3 sec a 3-dB point for a high-pass section is obtained at 0.05 Hz. It is therefore not reasonable to assume that a filter section so far beyond our frequency scale has measurable effects on the gain and phase data. For the
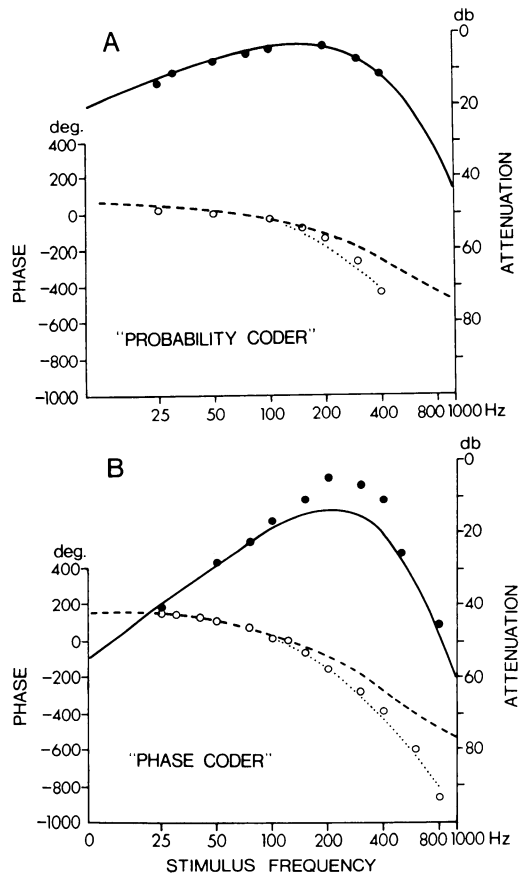

FIG. 13. Selected phase and probability coders fitted by band-pass filters for their gain (filled circles) and phase (open circles) as a function of frequency. The high-frequency end of the phase data in both units deviates from the prediction by an amount reasonably accounted for by the utilization and conduction time. Pointed lines correct filters for 1.1 msec (probability coder) and 1-msec delay (phase coder). In the phase coder the physiological frequency range (200 Hz) has 10 dB higher sensitivity than expected from the slopes of the band-pass filter.

reverse question it must be considered whether the high-pass section described with a 3-dB point at 100 Hz contributes to the adaptation we have measured. A 100-Hz cutoff frequency corresponds to a time constant of 1.7 msec. An adaptation that fast is beyond the resolution of the neuron which is driven at 300 Hz; i.e., the spike interval is minimally 3.3 msec.

Some of the low-pass properties may be explained by the constant-threshold property. The threshold recovery constitutes a limiting factor for higher frequencies since the amplitude of the higher frequency wave has to be increased in order to hit threshold after every cycle (4).

PHASE CODER. In much the same way a fit of the gain data has been tried for the phase coder. From the slopes of the data two single-section high-pass filters and eight single-section low-pass filters have been combined. By adjusting the 3-dB points for the high-pass sections to 400 Hz and those of the low-pass sections to 150 Hz, the best fit to the slopes of the data has been obtained (Fig. 13B, filled circles). Even so, we do not obtain the steep band-pass property with high gain for the normal frequency range of the electric organ discharge (dots reaching 5 dB). In contrast to this the above combination allows a good fit of the phase data of the receptor (open circles) when a constant conduction time of 1 msec is allowed for.

We conclude that the gain and phase data of some probability coders can successfully be fitted by a band-pass filter through variation among units in the gain data at the low-frequency end occurs and may make the fit less satisfactory (Fig. 5C). The phase coders, on the other hand, are very uniform in gain and phase and a suitable band-pass fit is easy except for the curiously high gain in the physiological frequency range.

From the data on the frequency response, especially on square-wave and triangular-wave stimulation (Fig. 3B), we can conclude that the sequence of processing the incoming signal in the neurons is, first, transformation through the equivalent of a band-pass filter and then spike triggering at a constant threshold of the filter output. This

threshold seems to be independent of frequency only between 300–400 Hz.

Bursting

Since there is a discrepancy between the gain and phase data of the phase coder on the hypothesis of a simple filter, we tend to assume that the considerable threshold decrease in the middle frequency range (10 dB) has a heterogeneous origin. There are a number of synergistic arguments which can be made from independent data. The phase coder in the less than 1:1 following range (probability range) exhibits a bursting mode of discharge, i.e., at low field intensity and low average response rate there is a tendency to fire in bursts at twice the driving interval; this is not compatible with the constant-threshold concept. If we assume an arbitrary number of discharges per second caused by a given stimulus amplitude, a bursting tendency can manifest itself in two ways. One is to increase the number of spikes per burst and compensate by lengthening the interburst intervals. The second way is not to compensate but merely add spikes. We do not know the number of discharges due. In comparing phase coders and probability coders, the probability range covers only 15 dB in the former and about 25 dB in the latter neuron. Since the probability coder was characterized as a linear unit at constant frequency, we conclude that the steeper response curve of the phase coder is more likely to be the result of the second alternative, adding spikes without perfect compensation. Since the response curve is steeper than that of the probability coder, the 1:1 following threshold must be reached in a smaller intensity span, and this may be the consequence of noncompensated addition of spikes.

The time constants of any feedback eliciting bursts should correspond to the frequency range where we find the very low threshold in the gain data; e.g., the neuron of Figs. 2A and 7A driven at 350 and 250 Hz exhibits a preference for every second interval, which corresponds to 175 and 125 Hz. The gain data of Fig. 5A have a maximum at 200 Hz. The tendency to every second interval is stronger in the 350 Hz case as also shown by the plateauing of the response

function in Fig. 2*A*. Since we have not measured systematically whether the bursting tendency increases with higher frequency of the input signal, we cannot precisely decide whether the best frequency threshold decrease and the preferred interval correspond to each other.

Sensitivity of dual receptor system

Both classes of tuberous electroreceptors dealt with in this paper primarily give information about the amplitude of the a-c field at the skin. Since both work on a constant-threshold principle they can both give information about phase changes of the a-c field such as is brought about by the interference of the electric fields of two nearby fish (7, 10, 11). This aspect is dealt with in a subsequent paper.

The aspect of amplitude discrimination can be considered in terms of resolution for various electric organ discharge frequencies. Here an interspecific comparison seems useful. It is obvious in comparison to *Eigenmannia* that, for instance, *Sternarchus albifrons* at about 1 kHz has higher resolution provided by the probability coder, whereas a phase coder with a maximum possible phase shift of only 0.25 msec (90°) would provide little dynamic range. In fact a phase coder has not been found in this species (19). *Sternopygus* (60–100 Hz), on the other hand, might take advantage of the large span of phase shift available as shown by Bullock and Chichibu (8) when the probability coder is already limited in its resolution. In low frequency, irregular species afferent types are found which give a variable number of spikes per electric organ discharge (17, 18). These are similar to the receptor types described here since we could obtain in *Eigenmannia* multiple discharge not only to low-frequency sine- and square-wave stimulation but also to short current pulses. It seems reasonable that coders giving several spikes per organ discharge should only be found in low-frequency species where their information content (dynamic range) can be higher. The frequency range of *Eigenmannia* electric organ discharge probably constitutes an optimum compromise for the usefulness of the two systems together which are described in this paper.

In comparing sensitivity we must distinguish between absolute and increment sensitivity. The absolute sensitivity of the two receptor types is quite different, as Fig. 1 shows. The phase coder has, on an average, a 15-dB lower absolute threshold than the probability coder. In the same physiological intensity range where the phase coder is already above the 1:1 firing criterion, the probability coder just starts its smoothly graded probability of firing. The distinctive and useful coding ranges of the two neurons almost completely overlap (Fig. 1). This is an especially significant result for the biology of the fish because this is the range of normal voltages due to the electric organ discharge.

The relative increment sensitivities of the two receptor types cannot be compared directly since their best response mode is different. The probability coder has a linear increase of number of impulses per second with intensity increase. The sensitivity can be given as 1 extra spike/sec per 25–50 μv $(p - p)$/cm stimulus increase, depending on the upper and lower part of the response curve and the stimulus frequency. Values for *Hypopomus sp.* ranging from 50–1,000 μv/cm for one added spike per burst are given by Hagiwara et al. (17). For *Steatogenys sp.*, 1 extra spike/sec per 100 μv $(p - p)$/cm is said to be added. This figure can be compared with behaviorally established sensitivities of gymnotids of 0.05–5 mv$(p - p)$/cm for *Gymnotus* (3); 0.04 μv$(p - p)$/cm for a trained feeding response in *Gymnotus*—a factor of 1,000 (13) and 0.25 μv$(p - p$/cm for the jamming avoidance response of *Sternarchus* and *Eigenmannia* (11).

A phase coder at -40 dB (400 μv$(p - p)$/cm) just at the 1:1 threshold will show the strongest phase shift to a few decibels of amplitude increase. An increment to -39 dB (50 μv$(p - p)$/cm increase) leads to 27° phase shift. There is no difficulty to resolve by eye a phase shift due to only one-fifth this amplitude change, i.e., 10 μv$(p - p)$/cm and 5.4° phase shift on the chart recorder (see Fig. 2).

For a signal of 300 Hz this 5.4° gives 60-μsec latency shift on the interval plotter record, which is way above the limit of resolution of a higher order phase coder in the

midbrain (torus semicircularis) of the fish (25). Therefore, if we consider any noticeable change in the firing pattern of the next higher neuron as a criterion, we can claim that the first-order neuron is sensitive to an even smaller fraction of a decibel. Any phase change causes a noticeable alteration in neuron activity within a few cycles even in the higher order neurons of the torus. In a comparison we have to keep in mind that the measures of sensitivity are quite different in the two classes of afferents. Sensitivity of the probability coder was arbitrarily expressed as one extra spike per second. This means that much more time will be required to signal a stimulus by these afferents—at least a second if there is no convergence centrally. It appears that one of the features of the phase coder system is high discrimination combined with very fast transmission. We have reason to assume that these properties are involved in social communication, especially the "jamming avoidance response" (unpublished observations) and perhaps also in the detection of moving objects (15).

SUMMARY

After blocking the electric organ discharge with curare, sinusoidal artificial stimulation confirms that there are two distinct types of primary electrosensory nerve fibers in the lateral line nerve in *Eigenmannia,* that fire synchronously with high-frequency stimuli: T units or phase coders and P units or probability coders. In both there is a low stimulus intensity range where a probability of missing of stimulus cycles is graded with intensity (sputtering or "probability range") and a high intensity range where 1:1 firing occurs so that only the latency of the spike encodes stimulus intensity ("phase range").

The phase coder has a 15 dB lower absolute threshold. The estimated physiological working range is in the phase range of the T unit and the probability range of the P units.

The two units respond to stimulus frequencies from about 1 to 800 Hz (phase coder) and 600 Hz (probability coder). Below 100 Hz, multiple discharges are obtained for every stimulus cycle, up to 25 spikes/cycle near 1 Hz.

The frequency response to both coders has band-pass characteristics with a plateau between 200 and 400 Hz. In order just to obtain a 1:1 firing response, the stimulus intensity has to be increased, as frequency is decreased below 200 Hz, at 12 dB/octave for the phase coders and less than 6 dB/octave for the probability coders. Above 400 Hz sensitivity falls for both units between 40 and 50 dB/octave.

Increasing the frequency from 30–800 Hz causes the spike to lag in phase relative to the stimulus by 900° or more; subtracting estimated conduction time this is about 600°. Phase coders can advance by 130° more than probability coders at 30 Hz.

The P units are phasic-tonic units which adapt after the sudden onset of a maintained sinusoidal voltage with an average time constant of about 3 sec, and in the first few hundred milliseconds considerably faster in both their probability range and their phase range. The T units are tonic in both ranges.

In the sputtering range the probability coders show a modal interval equal to the mean and a wider distribution of intervals, at multiples of the stimulus interval as intensity is weakened. The T units, on the other hand, code by longer or shorter bursts in this range; the preferred intraburst firing interval changes mainly from once every two cycles to 1:1.

The increment sensitivity of the two types of units is: 1 extra spike per 25–50 $\mu v(p - p)$/cm of stimulus change for probability coders, 5.4° phase shift (or 60-μsec latency shift) per 10 $\mu v(p - p)$/cm of amplitude change for phase coders, higher order phase coders in the midbrain resolve fractions of this value. The absolute threshold in the phase coder is 70 $\mu v(p - p)$/cm, in the probability coder 10 times higher.

Shunting capacitors across a portion of the fish's field causes P and T units to respond in different ways, the P mainly to the change in voltage, the T mainly to the change in phase of the field. At a distance of several centimeters, capacitors at least down to 0.0004 μf are detected. It is proposed that electric fish detect capacitative reactances which may occur in objects, perhaps especially in living plants and animals. The main features of a model are first

that stimuli must be transformed by the equivalent of a band-pass filter which is specified and second that a spike then arises, in the frequency range of the fish, at a constant-threshold voltage of the filter output, even though this is within the relatively refractory period.

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