The Jamming Avoidance Response
of High Frequency Electric Fish

II. Quantitative Aspects

Theodore H. Bullock, Robert H. Hamstra, Jr., and Henning Scheich

Department of Neurosciences of the School of Medicine
and Neurobiology Unit of Scripps Institution
of Oceanography, University of California, San Diego

Summary. 1. Using a frequency difference (ΔF) clamp to maintain a stimulus and frustrate the normal escape from a jamming frequency, the response is found to be a characteristic function of the ΔF between stimulus and fish (Figs. 2, 3, 4, 6). It is graded on both sides of a best ΔF of about 3 Hz ($= 0.3\%$ in *Sternarchus*, 1.0% in *Eigenmannia*). There is no systematic response when $F_{\text{stimulus}} = F_{\text{fish}}$, regardless of phase.

2. The J.A.R. is graded with intensity (voltage gradient) of the stimulus over a range of more than 100-fold; higher intensities cause some reduction (Fig. 5). The threshold for longitudinal stimulation under certain conditions is lower than 0.25 μV (peak to peak)/cm; for transverse stimulation as in most of the present experiments 0.5 μV (peak to peak)/cm.

3. The best ΔF is the same when added to the fundamental of any harmonic (response detectable at least to the fifth) (Fig. 6). Stimulation around a subharmonic does not elicit the J.A.R.

4. A response to a small ΔF, e.g. 0.2 Hz, can begin within $< 1/4$ cycle of the beat frequency and, without "hunting", shift in the correct direction.

5. Stimulating with an optimal beat-frequency by amplitude modulation (AM) of a stimulus at F_{fish}, with an AM frequency of 3 Hz, can cause a response though it is confused as to sign. If the stimulus frequency unmodulated is at a $\Delta F = -20$ Hz and therefore almost ineffectual, AM at 17 Hz will cause a response upwards and at 23 Hz downwards (*Eigenmannia*). These results and the following suggest the fish performs the equivalent of a Fourier analysis and responds to sidebands according to their ΔF.

6. In a 300 Hz *Eigenmannia*, if an ineffective stimulus at 280 Hz ($\Delta F = -20$ Hz) is frequency modulated (FM) sinusoidally at 17 Hz between peaks of about 274 and 286 Hz, the fish gives an upwards J.A.R.

7. By curarizing *Eigenmannia* to silence its electric organ we can apply a phase modulated stimulus – a carrier wave whose cycles are systematically phase shifted by a few degrees back and forth at a few Hz. This also causes a response, though only in one direction.

8. If a stimulus is slowly frequency modulated (FM $<<\Delta F$) e.g. between $\Delta F = +$ and -6 Hz sinusoidally or triangularly at 10 to 100 sec per cycle, the fish responds and the $F_{\text{fish}}/\Delta F$ plot traces an hysteresis loop. This is quite well predicted by an analog computer model embodying the best ΔF curve and the time course of response to a ΔF step stimulus (Fig. 7).

Unclamped behavior is similar (Figs. 9, 10) and predictable from the responses to step stimuli in the clamped condition.

10. A block diagram putting the distinguishable properties into sequence is offered (Fig. 11).

A. Introduction

In the preceding paper the general properties of this response were described. It was shown to be a uniquely suitable example of animal behavior for close analysis. A normal social response, it is quite dependable, reproducible, free of habituation, apparently rather simple and potentially completely describable with a modest number of measurements.

The present paper undertakes such a quantitative characterization, leading towards a knowledge of the significant parameters, the dynamics, and the distinguishable steps in processing input and determining output. Following this there will be papers on (a) the quantitative characterization of two chief types of receptors involved, (b) similar analysis of certain midbrain units of the torus semicircularis that are probably relevant in the brain processing, and (c) the properties of a few other types of central responses to J. A. R. stimuli.

B. Materials and Methods

The species and technics used are described in the preceding paper.

C. Results

I. Systematic Study of J.A.R. Parameters Using the Frequency Difference Clamp

1. Effect of ΔF

Use of the device that electronically maintains a constant difference (ΔF) between the stimulus frequency (F_{stim}) and the fish's frequency (F_{fish}) has the consequence that the normal (unclamped) escape or jamming avoidance (increase in ΔF) is frustrated. In a suitable range of values, the clamped, constant ΔF evokes a shift in the fish frequency (Fig. 1; preceding paper, Fig. 2) that rises to a plateau, much like the unclamped response (see Section B, below). This plateau suggests there is another and opposite force acting on the fish, besides the avoidance tendency, perhaps a function of the difference between the fish's present frequency and his resting frequency. The greater this difference the stronger is the equivalent restoring force tending to shift the fish back towards its resting frequency. The J.A.R. under clamp is the result of the balance between these two forces.

When the preselected ΔF is administered to the fish by turning on a suitable voltage, maintaining the ΔF for some 20 or 30 seconds and then
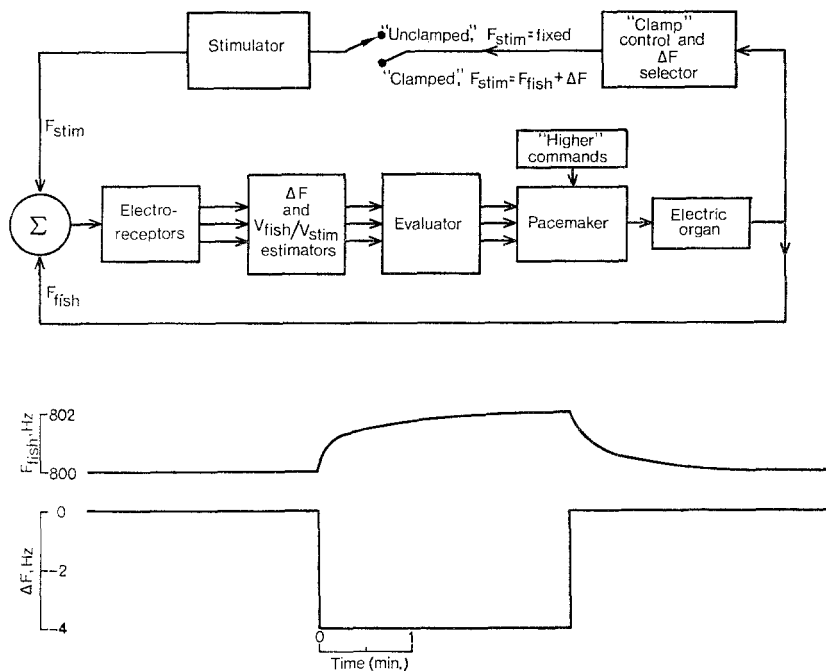
Fig. 1. Schematic diagram of the system, showing the ΔF clamp and a diagrammatic J.A.R. See also paper I, Fig. 5

switching to a new ΔF value for a like period, responses like those in Fig. 2 are recorded. Systematically changing the ΔF, for example by alternating between $+$ and $-\Delta F$ of various values (Fig. 2), the J.A.R. is smoothly graded. A just detectable one is something less than 0.01% change in frequency (equivalent to 0.1/μsec change in interval in *Sternarchus*), in response to a stimulus of about the same percentage, depending on the voltage. The maximum response is usually two or three percent at the best ΔF. At larger ΔF values the response declines; it is a barely detectable frequency shift when ΔF is about 30 Hz.

The form of this stimulus response curve (Fig. 3) is rather characteristic among specimens and quite reproducible within the same individual over and over. For example, there is often a characteristic shoulder on the negative slope or a broader or sharper maximum in different individuals.

There is always a "best frequency" and it falls between $\Delta F = 2.5$ and 6 Hz, usually between 3 and 4.5 Hz for both genera of fish. The peak may be quite sharp and the slopes on either side quite straight. In Fig. 3
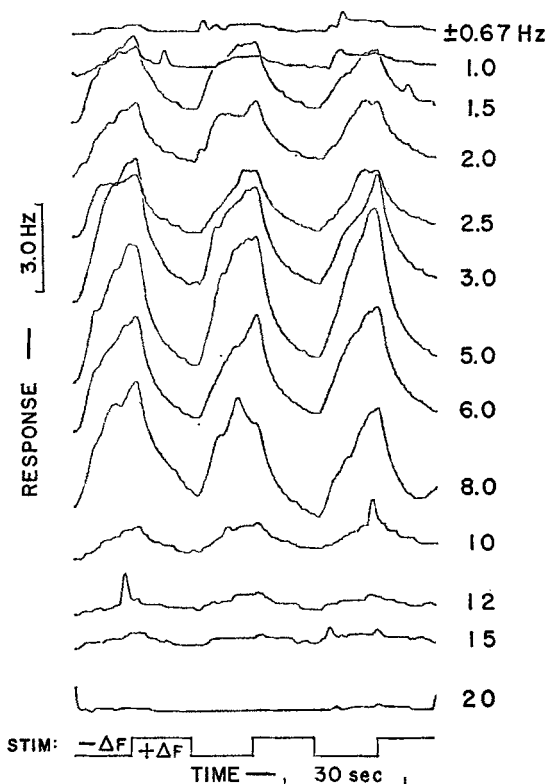
Fig. 2. Stimulation by steps of ΔF, alternated by computer, every 15 sec between equal values (as shown at right) above and below the fish's frequency. Exactly three stimulus cycles on each sweep; one starts each sweep; first sweep started by an arbitrarily chosen cycle in a continuous series. Averages of two successive sweeps; -50 db = submaximal intensity. The sharp, short peaks are due to an unusually high incidence of chirps in this fish. *Sternarchus*; resting frequency = 904 Hz

both slopes are about 6 db per doubling of ΔF. In other cases the peak may be rounded and the slopes curved, as in Fig. 6.

The same kind of relation can be established at a given intensity either by switching the ΔF symmetrically above and below the fish's frequency (F_{fish}), as in Fig. 2 or by switching between two values on one side of F_{fish}. If these values are in ΔF steps of constant proportion, for example 0.5 and 1 Hz, 1 and 2 Hz, 2 and 4 Hz, 4 and 8 Hz, 8 and 16 Hz, a response is obtained as shown in Fig. 4. The response increases while the steps are on the positive slope of the stimulus/response curve (Fig. 3), then decreases when the step is between two values of nearly the same effectiveness equidistant from the best frequency, and finally reverses
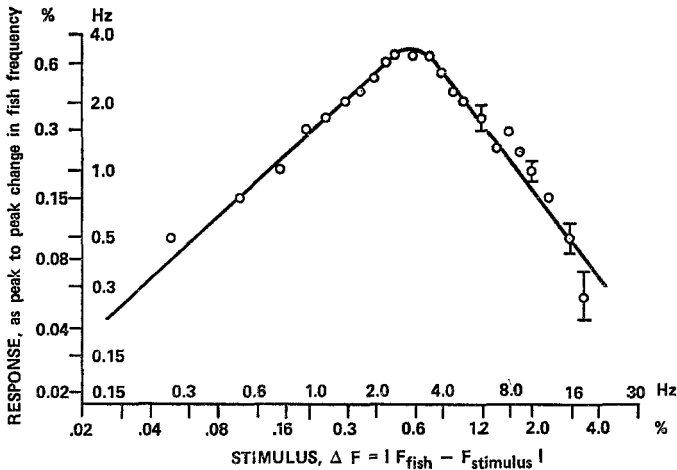
Fig. 3. Response as function of ΔF. The stimulus was applied as alternately plus and minus ΔF of the given (clamped) value, each held for 26 seconds, sufficient for about 90% of an eventual plateau response. Vertical bars are estimated confidence limits. *Eigenmannia* of resting frequency 500 Hz

in sign as the step moves to the negative slope of the curve. If the ΔF steps are graduated for example 0 to 1, 0 to 2, 0 to 4, 0 to 8, 0 to 16 Hz, the result is again predictable from the preceding relation; the response grows to a maximum at about 0 to 4 Hz and then declines to no detectable response when step is too large, usually about 16 to 20 Hz.

These curves show no response at zero ΔF and this is true regardless of the phase relation between the fish frequency and the stimulus frequency and over a wide range of intensity. However, sometimes especially at high intensity there may be a succession of small irregular fluctuations of the fish frequency when a stimulus is applied at zero ΔF especially on the first application. It gives the impression that the fish is "hunting". This irregular response is not due to lag or errors in the clamp circuit.

Similar results are obtained by a form of self stimulation (Bullock, 1969), using each electric organ discharge to trigger a stimulator. As in other species (Larimer and MacDonald, 1968; Bullock, 1969) a 1 msec stimulus pulse is quite ineffective if delayed by a millisecond or more (*Eigenmannia*). At moderately high intensity (60 db above threshold for J. A. R.) one can elicit increased wandering in pacemaker frequency (up to ca. 1%) if the stimulus pulse is delayed less than 0.2 msec relative to an arbitrary point on the early, rising phase of the fish discharge.
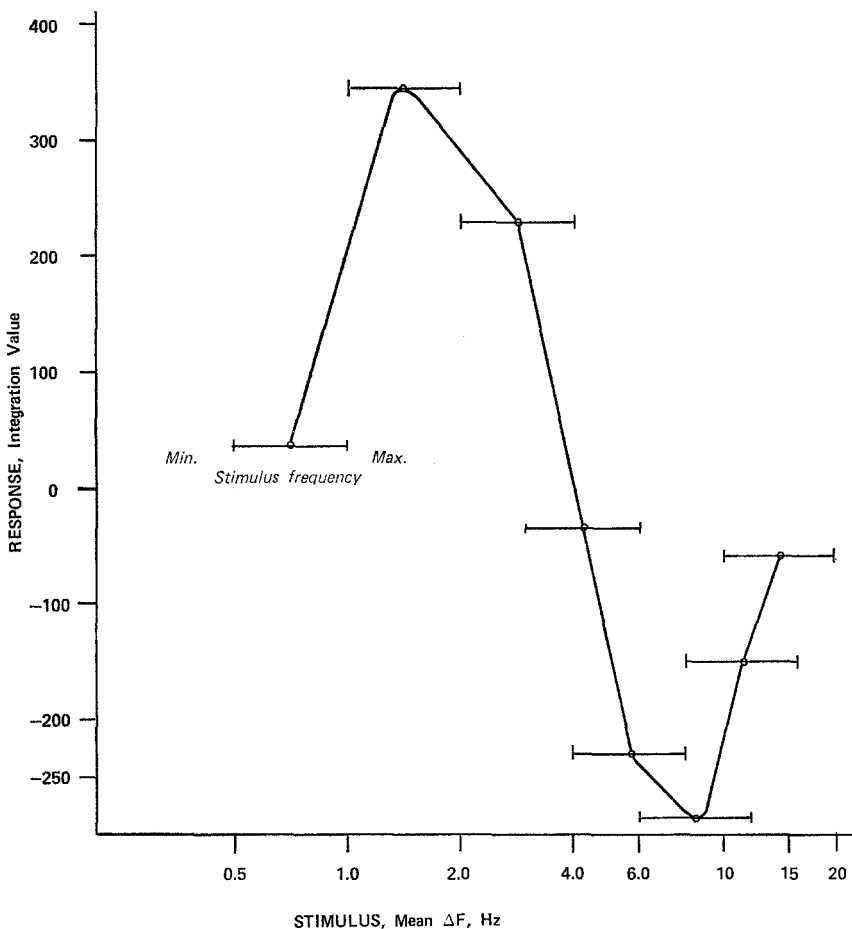
Fig. 4. Stimulation by alternating between two ΔF values, both below the fish's frequency, delivered under computer control in geometric progression of size of the ΔF step, at a fixed, moderate intensity and low repetition rate. Computer averages of six successive responses to each stimulus; *Sternarchus*. The stimulus/response relation is plotted by measuring the response as the computer-integrated area under the curves of the fish frequency; positive values represent upwards, negative values represent downward frequency shift, always taking the lower ΔF as the starting point. Records resemble those of Fig. 6 but with a reversal of sign as the more effective ΔF changes from second to starting value

It is noteworthy that the responses of *Sternarchus* are at least usually and perhaps invariably upwards from the fish's resting frequency whereas those of *Eigenmannia* are commonly symmetrical around the resting frequency. If the initial step of the stimulus cycle is a frequency just below the fish's, both species respond by shifting upwards but if the

initial step is a frequency just above the fish's *Sternarchus* will usually show no response whereas *Eigenmannia* shows the same response in the downward direction. Larimer and McDonald (1968) reported downward shifts in *Sternarchus*. Some individuals of *Eigenmannia* are more or less asymmetrical in this respect and some may even respond solely by shifts in the downward direction. When we see a small downward response in *Sternarchus* we have reason to consider the fish has not had time since the last upwards response to return to resting frequency; this can some-times take 10 min. It is significant that this return is the same in time course in the presence of a positive ΔF stimulus (which should produce a downwards J.A.R.) as it is upon the simple removal of a negative ΔF stimulus.

2. Effect of Wave Form of Stimulus

Comparison of the effectiveness of square, sinusoidal, triangular and sawtooth wave forms as the stimulus shows very little difference. We have routinely used sine waves. The natural wave forms of these genera are distorted sine waves, i.e. the discharge is a rounded wave about one half of the duration of the discharge period.

3. Effect of Intensity

Increasing the voltage of the stimulus increases both the excursion of the response to any given ΔF and also the span of ΔF's which are effec-tive. Plotting the response as peak-to-peak F_{fish} shift against the inten-sity as stimulus voltage on a loglog plot (Fig. 5), a wide range of slopes is observed. The dynamic range is usually 60 db and often 70 db above threshold. A straight line is sometimes obtained over most of the range. Stronger stimuli usually depress the response and this depression increases smoothly over an additional 20 db at least. We have not delivered stimuli stronger than 90 db above threshold for technical reasons.

The absolute threshold, using naked eye detection of fish frequency shifts that follow regular alternation between plus and minus values of the best ΔF steps is about –115 db on the arbitrary scale of the figure. This is 0.5 microvolts (peak to peak)/cm across the fish measured in the water, not close to the electrodes, and in the region of maximal stimulus gradient in the transverse axis. Since this is quite sensitive compared to other measurements in gymnotids (see p. 47–48) we have repeated it a number of times in different fish of both genera. Some individuals are 10–20 db less sensitive. The threshold voltage gradient was found to be still smaller if oriented in the antero-posterior axis. Placing the electrodes about five centimeters beyond head and tail and comparing the steepest part of the gradient occupied by part of the fish (here the head region) with the transverse gradient, in its steepest central region, and using
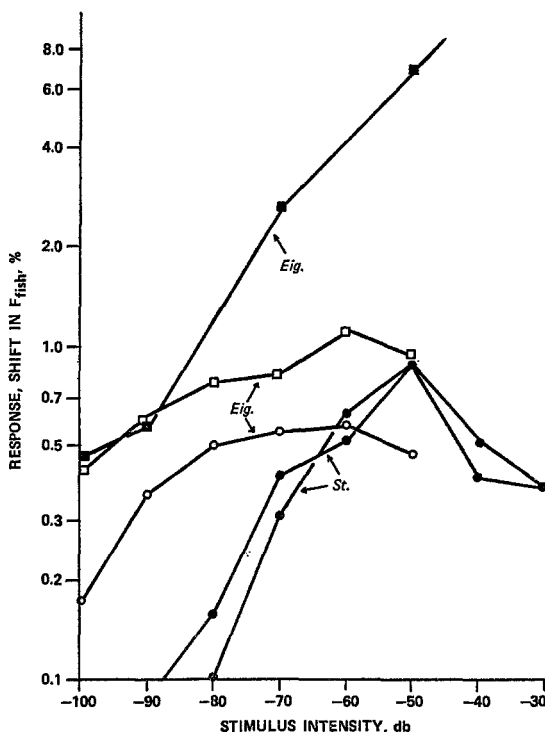
Fig. 5. Response as function of intensity. The stimulus frequency was slowly modulated in a triangular form with a period of 50 sec to a maximum ΔF of 3Hz for the upper curve, from *Eigenmannia* (Eig.); of 20 sec to $\Delta F - 3$ Hz for the next two curves, from another *Eigenmannia*, and of 33 sec to a maximum ΔF of 5 Hz for the lower pair, from *Sternarchus* (St.). Each point is the average of three successive responses measured as the maximum shift in fish frequency (peak to peak). Extrapolated lines based on off-scale data

constant current stimulation, the longitudinal gradient was found to have a lower threshold by about 6 or 7 db.

In the most sensitive fish the threshold longitudinally was less than 0.25 µV (peak to peak)/cm (*Eigenmannia*, $\Delta F = 3$ Hz, alternately $+$ and $-$, for 15 sec each, water resistivity $= 6$ k $\Omega \cdot$cm; without averaging; response $=$ shift of $F_{fish} \pm 0.01 \%$ for 8 out of 10 consecutive stimuli). Note that the water was more resistive than the optimal; according to the findings on the influence of salinity (paper I) the voltage threshold will be lower by 8 ot 10 db in common aquarium or tap water of 1—2 k $\Omega \cdot$cm.

These measurements were done in the 3.9 l chamber described (26 × 12.5 × 12 cm), which means the electric fields were constrained

by the walls. We do not know whether a free field (unconfined) or a more restricted one like the narrow spaces they prefer for rest periods would lead to higher sensitivity.

Most measurements were done with a constant voltage stimulator. Therefore a very low resistance was permanently connected across the stimulating electrodes, usually carbon rods just protruding through the walls at a single point on each side or end. We find that substituting a constant current stimulator (high series resistance) has no effect or lowers the threshold by no more than 3 db, which is of doubtful significance. This change inevitably altered another factor also that might conceivably affect the sensitivity, namely, any stray d.c. potentials due to the electrodes. Because we could not readily compare current vs voltage source without altering d.c., we capacity-coupled the current source, thus eliminating any d.c. We have not attempted to study the effects of d.c. on the J. A. R.

Converting the usual, virtual point source, transverse field into a virtually homogeneous transverse field (constant current source), we found the threshold not appreciably affected. This conversion was accomplished by leading the current from each stainless steel electrode in a large reservoir of aquarium water through 81 polyethylene tubes 305×1.587 mm i.d. filled with the same water, to the fish's chamber, the openings of the tubes arranged on two grids 57×250 mm and at least 48 mm from the fish on each side.

4. Effect of Harmonics and Subharmonics

Sinusoidal stimuli at frequencies close to subharmonics of the fish are ineffective even at moderate intensity i.e. 40–50 db above threshold for best frequencies. Thus stimuli which are centered around one-half of the fish's frequency but alternate between a few Hz above and a few Hz below this value give no response at any ΔF. If the stimulating voltage is not sinusoidal but sawtooth in form there is a response at a ΔF of ± 3 Hz from the submultiple of the fish but this might be expected from the fact that this wave includes its own second harmonic which will be close to the fish's frequency and therefore effective–if we assume the fish analyzes the mixed waves it receives by detecting each component frequency in the power spectrum. Square wave and triangular stimuli close to one half of F_{fish} are weak or ineffective, as might be expected from their lack of the second harmonic.

The same experiment centered around the second or third harmonic of the fish's frequency however gives a good, though weak, response and a just detectable one can be seen up to the fifth harmonic (Fig. 6).

It might be expected that the best ΔF is some proportion of the stimulus frequency and therefore would increase, for example from 3 Hz
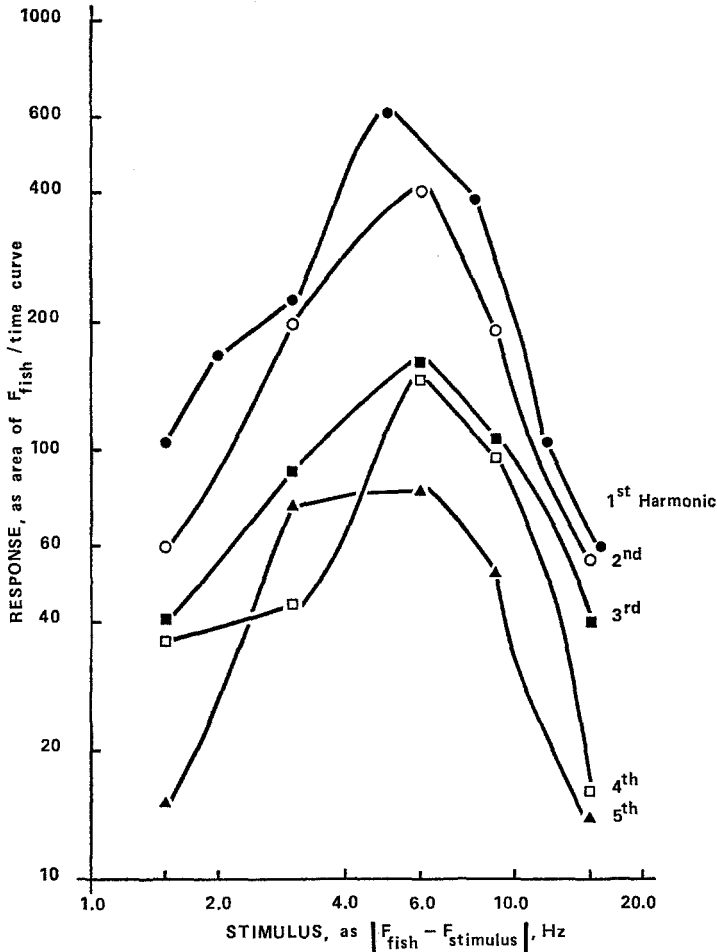
Fig. 6. Stimulus/response relation like that of Fig. 7 for the same ΔF's centered around the fundamental and first five harmonics of the fish's frequency. *Sternarchus*. Note that the best stimulus ΔF is about the same for all

at the fish's frequency to 6, 9 and 12 Hz at the second, third and fourth harmonics. Fig. 6 shows that this is not the case and the best ΔF remains at about the same value through the fifth harmonic where the response is very feeble.

It may be relevant that the wave form of the fish's own discharge is rich in harmonics. The normal electric organ discharge was examined in a single *Sternarchus* of typical waveform; the second harmonic was found to be only 5 db down from the fundamental while the third was down 15 db, the fourth down 20 db, the fifth 33 db and the sixth 38 db.

5. Time Course

The latency of the response from the moment of a frequency switch at optimal ΔF and moderately high intensity e.g. 50 db above threshold is typically 150–250 msec. The latency is sensibly the same over a wide range of intensity, even within 6–10 db of threshold. The error of estimation of take-off point in feeble responses is serious but it appears that within about 5 db of threshold the latency may be increased to as much as 1.5 sec, though it is usually not over 0.8 sec. There is no apparent difference between different ΔF's (except those barely evoking responses), upwards and downwards shifts, or earlier and later J.A.R.'s in a series.

A significant corollary of this is that a very small ΔF, such as 0.2 or 0.1 Hz, can evoke a response with a latency around one second or even half a second, therefore within a fifth or smaller part of a cycle of the beat frequency, without any sign of hunting for the correct direction of the J.A.R. It does not require even one full cycle of the beat to give information adequate to respond with the correct direction and magnitude.

Rise and fall times as well as symmetry of response are mentioned in the preceding paper, Section A.1. There is a clear tendency, especially on the falling-frequency phase of the response in *Sternarchus*, to approach the final frequency asymptotically. There is commonly evidence of more than one process or time constant especially on the rising phase in *Sternarchus* where a rapid frequency shift is followed by a slow one. There are often wobbles, up and down, during this slow approach to plateau, whereas the fall toward resting frequency is smoother.

A kind of flicker fusion frequency can be demonstrated. If stimuli of the form discussed so far are used, i.e. square FM, alternating between two ΔF values, the frequency of switching can be increased. With 50% of the cycle at each ΔF, the flicker fusion frequency or modulation frequency where J.A.R. just disappears is about two complete cycles per second; one half second hardly gives time enough for a response and relaxation. A brief stimulus or a brief gap in a maintained stimulus can cause a detectable response shift down to durations, at optimum ΔF and intensity, of the order of 0.2 sec.

If the stimulus has a slow onset either in intensity or in ΔF and creeps up gradually over many seconds or minutes, there is nevertheless a good response; the system does not exhibit accommodation. When a long maintained stimulus is removed, the tonic response may take two minutes or more to decline back to resting frequency. The rate of change of frequency can be small and yet systematic and progressive, i.e. larger than the ongoing background of irregular fluctuations; in a typical *Sternarchus* recovery from a J.A.R. of 0.8% the frequency was still falling back toward resting level at 400 sec following OFF, by $3 \times 10^{-4}\%$ per second.

6. Sinusoidal Amplitude Modulation (AM) and Frequency Modulation (FM)

If a stimulus at the fish's frequency (the frequency difference clamp maintaining a zero ΔF) is amplitude modulated sinusoidally, to a depth between 10 % and 95 %, at a frequency of 3 Hz, the fish will sometimes give a clear sign of disturbance by a transition from a constant frequency to a fluctuating one. Irregular increases and decreases of a few seconds duration give the impression that the fish is "hunting", much as it does with zero ΔF at high intensity. This may be understood from (a) the fact that such stimulation provides energy in sidebands at both 3 Hz above and 3 Hz below F_{fish} (Panter, 1965, Chapt. 5; Schwartz, 1970, pp. 203ff.) and (b) the plausible possibility that some fish are slightly asymmetrical in the degree of effectiveness of the $+3$ and the $-3\,\Delta F$. *Sternarchus* especially can be expected to give a response to this stimulus, because of its asymmetry of response in escaping only in the upwards direction; and this we have seen.

If the sinusoidal AM is imposed on a stimulus frequency that is ineffective, for example 20 Hz below F_{fish} and the AM frequency is for example 17 Hz, the fish gives a good response upwards. This stimulus has a power spectrum with power at the fundamental (20 Hz below the fish), and sidebands 3 Hz and 37 Hz below the fish. The response can probably be attributed to the -3 Hz ΔF stimulus. The sideband at -37 Hz ΔF is presumably without effect. If the AM frequency is 23 Hz the response of *Eigenmannia* is equally good but in the downwards direction. This stimulus has the same fundamental and sidebands 3 Hz *above* the fish and 43 Hz below it; the latter is presumably ineffective. If the stimulus fundamental is closer to the fish and is itself an effective stimulus, for example 10 Hz below the fish, an AM frequency can be found which just neutralizes this response, for example 15 Hz at a certain depth of modulation, equivalent to a weak $+5$ Hz ΔF stimulus neutralizing a stronger -10 Hz ΔF stimulus. The sideband at 25 Hz below the fish is presumably ineffective.

FM gives similar results when the peak frequency deviation is small, for example 6 Hz. In experiments on *Eigenmannia* of about 300 Hz this means the stimulus, whose fundamental is, for example, 20 Hz below F_{fish}, is modulated sinusoidally between 274 and 286 Hz, at a modulation frequency of, for example, 17 Hz. Such a stimulus has a power spectrum with a fundamental at 280 and principal sidebands at 263 and 297 Hz, out of phase with each other (Panter, 1965, Chapt. 7; Schwartz, 1970, pp. 324ff.). The similarity of the fish's response to the AM case with -3 Hz and -37 Hz sidebands indicates that either the reversal of phase of one sideband relative to the other is without significance for this behavior or that a sideband 37 Hz from F_{fish} is ineffective and agrees

with the previous experiment in showing the sidebands evidently act for the fish like independent sine wave sources.

Phase modulation is a closely related form of stimulation (with the same power spectrum as FM) which can be used if we silence the fish's own electric organ and substitute a completely controlled electric field. As explained in the preceding paper, section B 1, we can curarize and abolish the electric organ discharge in *Eigenmannia* but record the frequency of the pacemaker with a microelectrode in the medullary command center. We then deliver into the water the output of an oscillator of a type (Data Royal F230A) which can be triggered cycle by cycle. By sinusoidally shifting the delay of the trigger back and forth at a few Hz by a peak value of 1.6 msec, or 180 degrees at 400 Hz, around a mean delay, a phase modulated stimulus is produced. This contains the same principal bands in the power spectrum as would three oscillators, one at the fundamental (mean) frequency, which is uncritical, one above and one below by $+$ and $-\Delta F$, plus some smaller sidebands due to the triggering method of modulation. But the time course of the instantaneous frequency is different, going up and down several times a second, much like the case of slow sinusoidal frequency modulation. When this form of stimulus is given to a curarized *Eigenmannia* the result is a definite response; the pacemaker in the medulla shifts frequency usually in the direction of increase but in some specimens, of decrease. The amount of shift depends on the phase modulation frequency and is greatest at 6 to 8 Hz. The meaning of this for the mechanism of the J.A.R. is discussed in section IV below.

7. Slow Frequency Modulation (FM $< < \Delta F$)

For two reasons this form of stimulation should be of interest in comparison with the square FM or abrupt switching between two ΔF values which we have reported up to this point. One reason is that it may be a common occurrence for the fish since other fish that approach the range of effective ΔF's may themselves slowly shift frequency both as a result of their own J.A.R. and as a result of the spontaneous changes with activity or unknown factors mentioned in the preceding paper, Section A.2 (f). The other reason may be expressed in this form: a slowly changing stimulus offers a way to test the adequacy of our understanding. We may compare the results with expectation from combining the relations established by step stimuli.

A convenient form of stimulation is to increase and then decrease ΔF in a sinusoidal or triangular manner, at various rates slow compared to the mean ΔF. Fig. 7 illustrates such an experiment, plotting the stimulus as ΔF and the response as F_{fish}. Starting from $\Delta F = 0$, as the stimulus rises in ΔF, the J.A.R. is seen, i.e. the fish shifts its frequency downwards. This progresses as the ΔF rises toward the "best frequency"
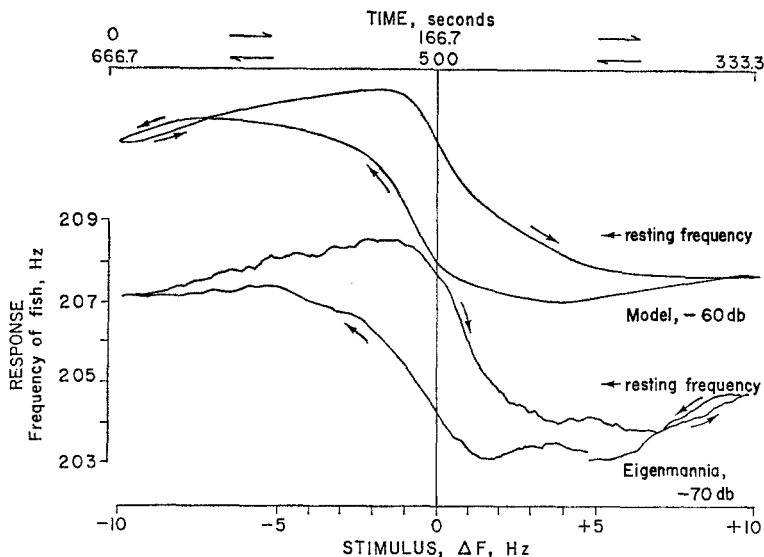
Fig. 7. *Lower*. Stimulus/response hysteresis loop with clamped, slow, triangular modulation of ΔF between -10 Hz and $+10$ Hz at 667 sec per cycle, -70 db. *Eigenmannia*. *Upper*. An analog computer model output based on the rise time and best frequency curves of this fish

of ca. 3 Hz. The lag in attainment of full response carries F_{fish} farther down, even when F_{stim} passes beyond the best frequency to less effective ΔF values and, at the particular FM value used, to a nadir as the stimulus returns through ca. $+2$ Hz. Then, presumably, the combination of decreasingly effective, very small $+\Delta F$ and the time-since-best-stimulus results in a slight recovering of the F_{fish} toward its resting value, which is between the maximum and minimum ordinate values, as though the stimulus were turned off. As the ΔF becomes minus, a similar sequence takes place in the opposite polarity.

Hysteresis loops are traced by the response to this type of stimulus unless the modulation frequency is too slow or too fast. In the case of too rapid FM the loop closes to a horizontal line (no response). In the case of extremely slow FM the loop closes to a sloping line (response independent of direction, no hysteresis). The "flicker fusion frequency" or modulation frequency just high enough to cause no ripple, at moderate intensity, with triangular FM (which is slightly more effective than sinusoidal FM), is about 1 Hz. In the range 0.5–0.005 Hz, the peak to peak response increases about 80–100% (ca. 6 db) for a halving of the modulation frequency. Response magnitude continues to grow at least down to 0.003 Hz. We do not give the change in phase of response with stimulus

modulation frequency because the form of the response changes continuously and is only sinusoidal close to the fusion frequency. Further results related to this kind of experiment are given in the next section.

Whereas qualitatively the hysteresis loop can therefore be expected in a certain FM range, it is a matter of some interest whether the shape and details, like the reverse loop at the end in some fish, can be quantitatively expected. We have therefore programmed an analog computer to simulate a particular fish and respond to the same stimulus regime. The simulation was designed to test the response of a simplified model to slowly rising and falling stimulus frequencies, based only on actual data from a living fish responding to step frequency changes like those used in the preceding sections. The data consisted of (a) the time course of the fish's response and (b) the magnitude of the response as a function of (clamped) ΔF.

For (a) the stimulus was $\Delta F = -3$ Hz and starting from at least 3 mins of rest it was turned on and maintained (ΔF clamped) for 500 sec. The resulting curve of the time course was matched by the analog model as explained below. The model assumes and the fish's response shows symmetrical time courses for on and off responses; the inset in Fig. 8 shows an off response of this fish for 180 sec.

The data for (b) was taken by letting the fish rest for 3 min and then turning on the stimulus at a given ΔF, maintaining it for 30 sec. The change in F_{fish} at 30 sec was taken as the response magnitude for purpose (b), and this value was plotted as a function of ΔF from $+2-$ to -20 Hz. This curve was used in the simulation as described below. In the same session the fish was stimulated with very slow triangular modulation of the stimulus frequency, generating hysteresis loops (Figs. 7) between clamped ΔF values of ± 10 Hz. All these fish responses were taken at a single intensity in the middle of the dynamic range (-70 db).

The Electronic Associates model 680 analog computer was set up, as in Fig. 8. The generator voltage represented the stimulus ΔF. The diode function generator represented the hypothetical ΔF detector and the integrators and their associated potentiometers represented two low pass filters which are supposed to operate on the same signal and are summed in amplifier 005 to form the output. The diode function generator was fitted to the curve of response as function of ΔF (with the use of short segments and ten break points). The integration gains and time constants were adjusted to approximate the curve of response time course, therefore using only two time constants. Either a triangular or a sinusoidal wave was obtained from the generator to plot the hysteresis loops.

The result is shown in the upper half of Fig. 7. A remarkably close agreement exists between the fish and the simulation. This supports the conclusion that no new factor enters with slowly modulated ΔF.
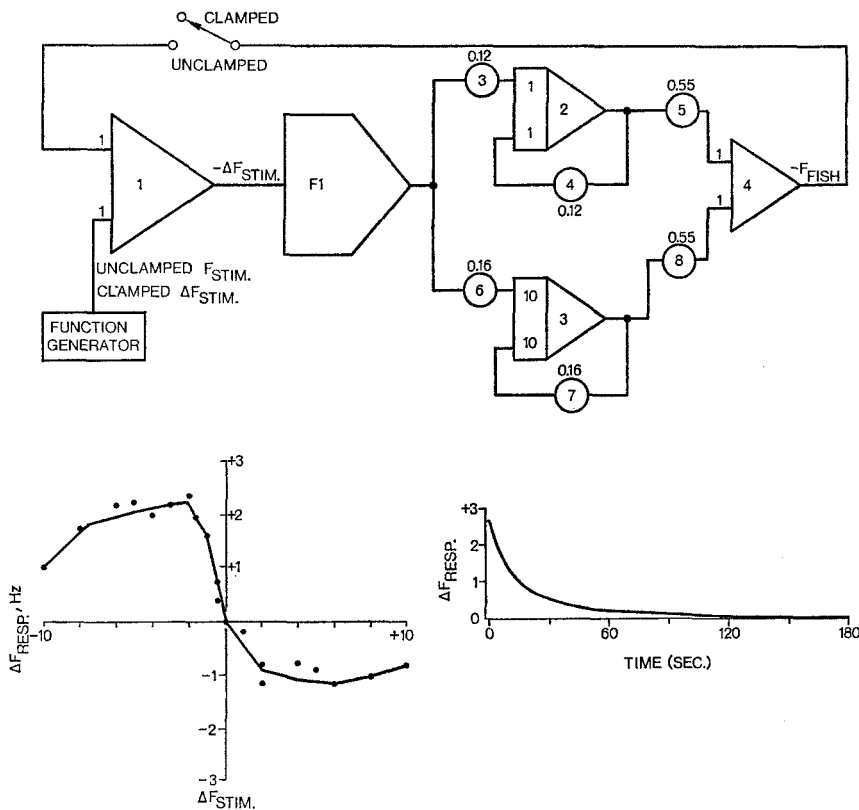
Fig. 8. Block diagram of analog computer simulation of a certain specimen of *Eigenmannia* under stimulation by slowly rising and falling ΔF (clamped) or F_{stim} (unclamped). Insets, below, show the actual responses, to square ΔF-clamped stimuli, of the fish from which parameters were taken and, for the stimulus/response relation, lower left, the method of hand-smoothing for generating the simulation

II. J.A.R. without the Frequency Difference Clamp

Since a ΔF clamp is not natural and frustrates the normal consequence of the J.A.R., namely escaping from the stimulating frequency, it is desirable to describe the response with the normal consequence intact.

As Watanabe and Takeda (1963) first reported, and Larimer and MacDonald (1968) confirmed and extended, if a stimulus is applied consisting of a sine wave at a frequency a few Hz below that of the electric organ discharge of an *Eigenmannia*, at a suitable voltage, the fish responds by shifting over some tens of seconds to a new plateau at a higher frequency. If now the stimulus is switched to a slightly higher frequency still, the fish shifts down to a lower plateau. To find the maxi-

mum response in terms of shift from high plateau to low, the stimulus frequencies must be chosen by trial so that they just bracket the fish, i.e., so that the high stimulus frequency is just above the maximum F_{fish} by about 1 Hz or less, and the low stimulus frequency is just below the lowest F_{fish}. In shorthand terms, the stimulus span must be centered between the fish's floor and ceiling for that voltage, and only about 1 Hz greater than the fish's response. These high and low stimuli are just the starting ΔF; the fish increases the ΔF by its response.

Comparing the maximum response thus obtained with the response under ΔF clamp at the best ΔF, we find no appreciable difference in magnitude. Even the forms or time courses of the responses to step stimuli are not markedly different. At the most effective voltage the best unclamped J.A.R. rises from a very small ΔF to one considerably greater than the ΔF in the clamped case with the most effective ΔF. It is perhaps surprising that the clamped fish, with the best ΔF, maintained, does not shift to a greater degree. It is as though the fish, though driven by the optimal ΔF, nevertheless soon gives up trying to widen the gap, or encounters an equal and opposite restoring force that grows with the change from "resting" frequency. The adequacy of the last postulate is doubtful when we consider that the same peak change in F_{fish} is achieved in the unclamped case, i.e., the same departure from resting frequency, with a different steady state ΔF, one that is numerically greater and therefore less effective. Note that experiments of the type of Fig. 4 do not suggest any considerable change in the best ΔF at different starting values of F_{fish}.

This behavior – the approximate equality of clamped and unclamped responses always measuring peak to peak excursion – is also found in the analog computer simulation. That model incorporates only two parameters, the time course of the clamped response and the relation of magnitude of response to ΔF of the stimulus in the clamped state. The "restoring force" requires no additional input, but is a consequence of the filters in the model.

Fig. 9 shows the effect of different intensities of stimulus on *Eigenmannia* in the unclamped condition. We choose the illustration from the type of stimulus regime that modulates the stimulus frequency in a triangular instead of a square step manner, between high and low extremes that bracket the extreme ceiling and floor frequencies with a constant span. The response is plotted as F_{fish} against F_{stim} (not ΔF). The resulting hysteresis loop therefore differs from Fig. 7: the 45° slope represents $\Delta F = 0$. The larger the J.A.R., the more open the hysteresis loop. With increasing intensity, starting from subthreshold levels, the first appearance of a loop is a sensitive indication of the threshold. With this form of stimulation, as intensity is increased the ineffective ΔF beyond the ends

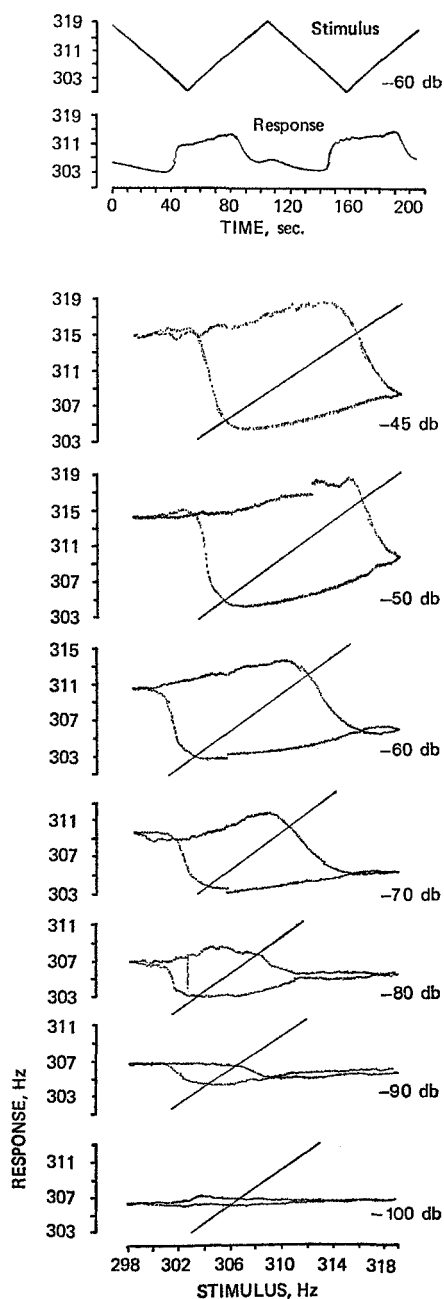T. H. Bullock, R. H. Hamstra, Jr., and H. Scheich:

Fig. 9. Stimulus/response relation as function of intensity in arbitrary db values, no ΔF clamp. Stimulus frequency swept above and below the fish's at a fixed rate, as shown on the records of stimulus frequency (horizontal) against fish frequency (vertical). *Eigenmannia*
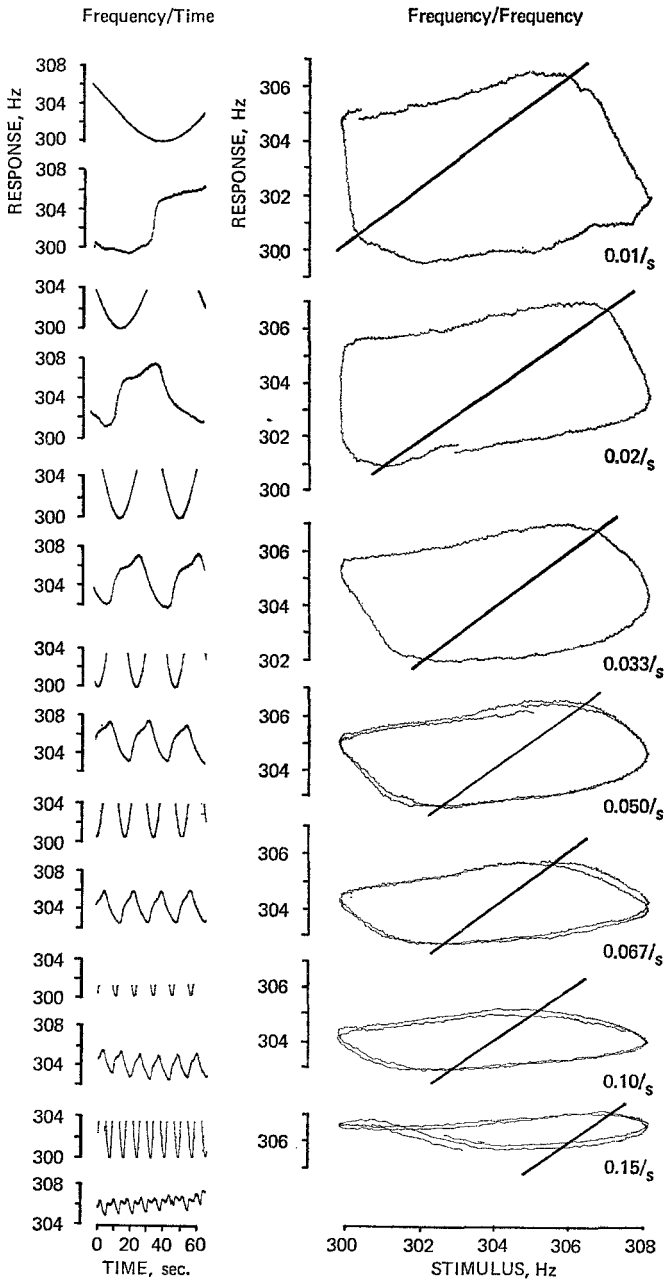
Fig. 10. As in the preceding figure but with intensity fixed and rate of sweeping the stimulus varied. The plots at the left are the two frequencies separated, against time

of the open hysteresis loop becomes smaller and those ends become steeper.

Fig. 10 shows the effect of modulating the stimulus frequency at different rates, at a fixed moderate voltage and minimal span. The response is still increasing at the slowest modulation frequencies, here 1 cycle in 100 sec, but the same is seen at least to 1 cycle in 700 sec. Especially notable is the change in form of the wider loops; they become sharper cornered and steeper at the ends at the lower modulation frequencies. This means that when the fish reaches a floor or ceiling it then reverses its direction of frequency shift, moves *towards* the stimulus frequency then through it to escape on the other side, by a rapidly achieved large amount just as in the analog computer simulation in the previous section (B.7). When the stimulus frequency reverses and begins to chase the fish farther in the same direction, it is already close to its ceiling frequency and the top and bottom limbs of the loop are nearly constant in slope at all modulation frequencies. Thus, unlike the cases of stepped or square FM maintained at fixed levels, or of infinitely slowly modulated frequency, when we use slowly changing FM the condition of $\Delta F = 0$ can occur during an accelerating change in fish frequency.

Comparison of different wave forms of the slow modulation frequency, square, sinusoidal or triangular shows the latter to be typically the most effective for any given voltage, modulation frequency, and span.

When an FM stimulus begins the response takes some time to come to that expected from the hysteresis loop, but this time is less than half a cycle.

Certain modulation frequencies, intensities, and spans may elicit more complex response wave form including one or more reversals of frequency shift near the extreme ends of the stimulus span (Figs. 7, 9); these appear on the hysteresis loop as reverse loops at the upper and lower stimulus frequencies.

Discussion

Fig. 11 is a possible block diagram of the J.A.R. system; it is obviously *not* a model but merely an heuristic scheme to recognize certain relations and components that can be inferred from diverse properties of the behavior observed.

The input to the fish from the electric field in its aquatic milieu may include its own electric organ output mixed with that of other fish or our stimuli simulating another fish plus other lower and higher frequency voltages from animate or inanimate sources in its environment. Only certain frequencies and intensities have been found to influence the J.A.R. These help define the properties of the input analyzing blocks. The present paper does not deal with the mechanisms by which the fish
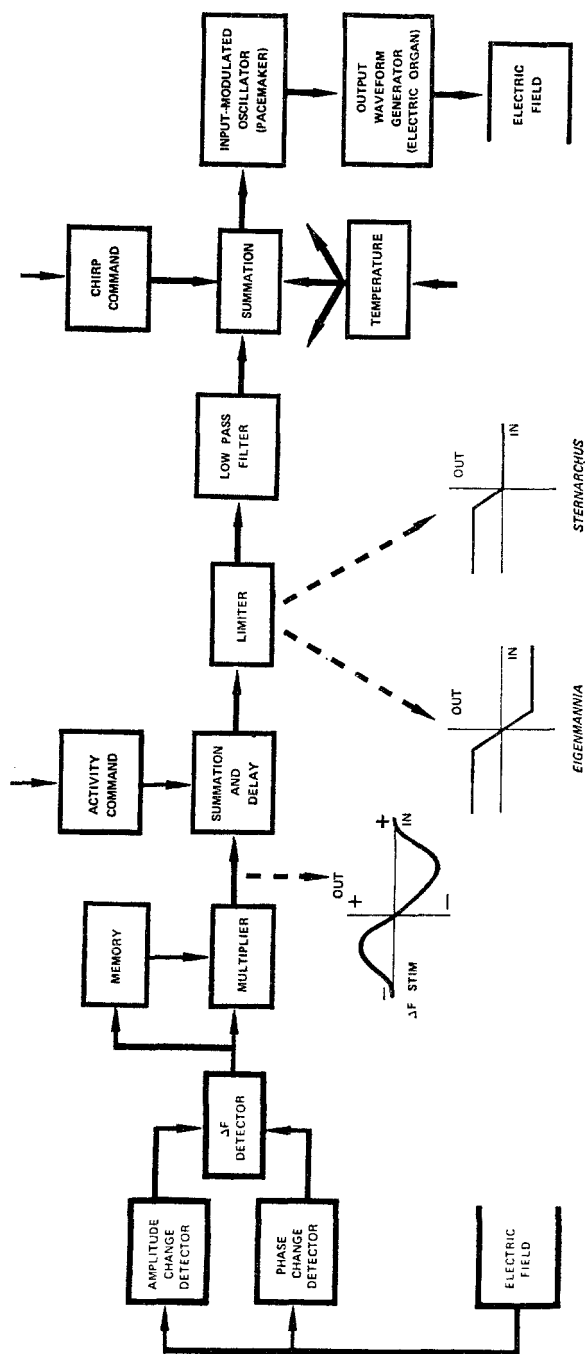
Fig. 11. Block diagram of inferred formal equivalent components in the J.A.R. system

measures both the difference frequency and its sign and we lump this function for the present into the "ΔF detector". The ΔF detector is supposed to receive signals of at least two kinds. One represents changes in amplitude, as in the envelope of beat frequency, and operating in the time domain, preserves information on the direction of change, up or down, at any time. The other represents changes in the phase of the combined a. c. field relative to one of the constituent rhythms, normally the fish's own and also preserves information on the direction of change and the sign of the phase shift at any time. We offer no evidence in this paper that such extractions actually take place but propose them not only because the parameters mentioned are obviously available in the relevant environment but also because together they offer the most plausible way to extract both ΔF and its sign. Another possibility, an array of extremely narrow filters, one of which the fish takes as his own frequency seems physiologically unlikely. In a subsequent paper on single units in the brain we will discuss the proposition that ΔF and its sign are extracted by the fish by comparing the direction of amplitude change and the sign of the phase change at the same time.

The ΔF detector is followed by a multiplicative operator and the result of all the operations so far is the input-output function shown in the inset, with a best frequency on each side of $\Delta F = 0$. A multiplicative operator is indicated rather than a summation because the best frequency is independent of amplitude (over a wide range). The ΔF detector and multiplicative operator must have properties at least as sensitive (for small ΔF's), as fast (for large ΔF's), and as stable as the input-output behavior shown in the insets later in the diagram.

We cannot yet explain the form of the characteristic stimulus response curve (Fig. 7) with its best frequency peak at $\Delta F = $ ca. 3 Hz and positive and negative slopes on each side of about 6 db/octave. The positive slope (low ΔF) acts like a simple high pass filter; it is thus formally equivalent to or would be caused by a suitably fast, exponential, sensory adaptation, though there is reason to doubt that this is the cause in the present case. Before comparing time constants and ΔF values, we should recall that the ΔF or beat frequency must be extracted from the signal. The receptors can only extract this frequency by some nonlinear operation such as a threshould detection. A corner frequency of 3 Hz in a simple filter means a time constant of 53 msec. The only receptor adaptation reported thus far in these fish has a time constant of about one second (Hagiwara and Morita, 1963). The right hand (negative), high ΔF slope acts like a low pass filter and is therefore formally equivalent to a flicker fusion property. A receptor time constant of 53 msec for the rise of response to a step stimulus could account for this, given the assumption that makes beat frequencies available, but this seems several times too long for familiar

receptors. We tentatively ascribe both slopes and the peak of the "best frequency" curve to central properties; perhaps the low ΔF values are difficult for the detector and the high values are weighted as less important because less jamming.

We show the ΔF detector going to a memory which can influence the multiplicative operator. This is required by the "settling down" behavior. After a long rest the J.A.R. may be larger than following several stimuli. With adequate stimuli the lower or plateau response shows the effect of this experience for up to 60 min.

The next block introduces the delay necessary to explain the response latency. There is of course delay at many points in the living system in the fish due to conduction time, synaptic dalays and the like, so this is a distributed function lumped here for convenience. Very likely most of the response latency in the fish is attributable to one or a few sites in the system and probably between the second order afferent neuron and the pacemaker nucleus. But we cannot say at present more precisely where it is.

At this point we also show the summation of the input from a component we will call activity command. This is required by the evidence especially from *Eigenmannia* of alteration of the ongoing pacemaker frequency, generally, and in many experiments exclusively, in the direction of depression during certain kinds of mechanical stimulation, during probing activity and during some instances of turning head for tail. The activity command is probably a heterogeneous category of both spontaneous and environmentally induced alterations in pacemaker frequency of different rates and duration. We place it here somewhat arbitrarily but primarily to precede the low pass filter.

The block entitled limiter is included for three reasons in *Sternarchus* and two in *Eigenmannia*. The common reason is that the response does show a maximum as shown in the insets. This could be allowed for in several ways; we choose to call the equivalent operator a limiter. The additional reason for *Sternarchus* is that it exhibits no output for a positive ΔF input, as shown in the input-output curve (inset). Although we place the *Sternarchus* characteristic of not shifting its frequency below the resting frequency in response to stimuli, at this point in the scheme, it cannot be excluded that this property inheres in the ΔF or amplitude detector. We are only guessing that the output of the ΔF detector is probably the same for *Eigenmannia* and *Sternarchus*. This guess is largely based on the fact that *Sternarchus* is clearly capable of discriminating between positive and negative ΔF's and therefore presumably of detecting positive ΔF's although it does not choose to respond to them (Granath *et al.*, 1967). Not only does *Sternarchus* fail to shift its frequency below the resting value for a positive ΔF, it also returns to the

resting frequency from a higher level achieved as the result of a negative ΔF stimulus, at exactly the same rate whether the negative ΔF stimulus is simply removed or a positive stimulus is substituted.

The output of the limiter is passed through a low pass filter because the J.A.R. takes many seconds to attain a new value. The filter must be complex in nature and most likely can only be characterized with non-linear properties. The first approximation to it would be two low pass filters in parallel, one with a considerably longer time constant than the other. The shorter time constant is on the order of 15 sec and the longer is on the order of two minutes. These would take care of the common and simplest form of the response to a step function stimulus. However, many responses are not of the same form for the shift in frequency upwards and the shift in frequency downwards; the former in particular may be complex, with several phases.

The output of the low pass filter is then summed with the output of a chirp command block which like the activity command may be a higher function of the brain mixing spontaneity and input from the environment. Bullock gave some evidence (1969) and Cleworth (1969) has additional evidence that chirping is an intention sign of aggression elicited by the presence of other fish of the same or closely related species. The chirp command is introduced after the low pass filter because the rate of change of frequency during a chirp is very fast compared to that of the J.A.R. or of the activity response and the recovery to the resting frequency is much faster than would be expected from the known dynamics of the J.A.R.

At this point we also sum the effect of temperature since this influences the resting frequency of the fish especially. This block like those of the top row is not essential to the minimum scheme for a J.A.R. but it is introduced because temperature has such a strong influence on experimental procedures. The difference frequencies important to these fish as judged by the J.A.R. are small compared to the effects of a change of temperature of $1°C$. Of course temperature has in reality a distributed effect; presumably each step in the system is separately influenced by temperature but from the experimental point of view its effect on the resting frequency is apparently the most important.

The summed output of these components is then used to alter the frequency of an ongoing rhythm in the pacemaker center of the medulla. This acts like a voltage-controlled oscillator where the input is of low frequency compared to the oscillator. The pacemaker in turn drives the output wave form generator located in the fish's electric organ which faithfully preserves the fundamental frequency of the oscillator with normally a fixed delay for conduction and transmission time.

An especially important point is the absence of a feedback connection from the pacemaker in the brain to the frequency difference detector except through the external electric field in the water; that is, the absence of a connection within the brain. This conclusion comes from the experiments on compartmentalization and on curarized fish both of which indicate that although the pacemaker is generating its rhythm this does not provide useable information to combine with sensory input of a ΔF stimulus adequate for a ΔF detector to initiate a J.A.R. Both the stronger and the weaker frequency must be in the external field received by the electroreceptors and moreover by the same part of the body; it does not suffice for one set of the receptors to receive one rhythm and another set the other rhythm. This strongly suggests that at least a crucial step in the ΔF detection block occurs in the receptors themselves. Note that we do not propose that these blocks are located in a certain part of the fish; indeed the phase and amplitude detector blocks may each involve both peripheral and central nervous system with nonidentical but overlapping populations of receptors and central convergence for noise reduction and integration.

The early blocks, through the ΔF detector and multiplier, are particularly marked by the wide dynamic range over which they work, a range of 60 to 80 db, and also by the accuracy and the speed of response to a change in ΔF. There must be some delay in these components but even taken together with the major delay which we place formally in the next block, the total latency to a change in pacemaker frequency is typically a fraction of a second and often as short as 150 msec. This is particularly interesting in connection with the problem of determining the sign of ΔF, which is done without error and without hunting. Even if we do not assume that most of the 150 msec is further on in the signal chain after this determination but especially if we do, then at the highly effective ΔF of 3 Hz the discrimination has been made within a fraction of a cycle of the beat frequency. This fraction may be very small as we can see in another way, instead of trying to partition the 150 msec delay within and subsequent to the discrimination. If we stimulate with a smaller ΔF, say 0.1 Hz, even though this is not as effective in J.A.R. amplitude, the latency is not greatly prolonged. Because of the uncertainty of designating a take-off point for a feeble response we should be conservative and call the delay anything up to 1 sec but this is still only 1/10th of a cycle of the ΔF. In other words, the detector cannot be of the cycle counting type.

The voltage sensitivity for an increment to the fish's own field, given above (0.25 μv, peak to peak/cm, longitudinally; probably < 0.1 in optimum salinity) is of interest. It is significantly below the figure of 0.2μv, presumably r.m.s. reported by Howe et al. (1966) for a conditioned be-

havioral response to food in *Sternarchus* and well below their figure of 15 μv/cm, r.m.s. for the J.A.R. It is close to the value of 0.15 μv/cm (presumably peak to peak) found by Machin and Lissmann (1960) for *Gymnarchus*, quite a different fish, in an unrelated family. It is approaching the order of magnitude of the conditioned response sensitivity of 0.01 μv/cm (peak to zero) reported by Kalmijn (1966) and Dijkgraaf and Kalmijn (1966) for *Raja*, on ordinary ray not an electric fish; therefore this sensitivity is not an increment but an absolute threshold. The most sensitive J.A.R. heretofore noted required 3–10 μv/cm (Watanabe and Takeda, 1963; *Eigenmannia*). In contrast to these values obtained with behavioral end points, increment thresholds for single units in the active electrosensorysystem are much higher, e.g. 10—15 μV (peak to peak)/cm (Scheich *et al.*, 1972), 150 μv/cm (Hagiwara *et al.*, 1962). As Bennett and others have pointed out, comparisons like this require the qualification that possible differences in the method of applying current could influence the effective stimulus. One of these factors we have shown to be salinity of the medium.

The sensitivity in gymnotids must have its significance in the need for detecting small differences in considerable voltages in the active object-detecting mechanism since the signal, according to Lissmann's (1958) well accepted suggestion, is a small distortion of the animal's own electric organ field. This contrasts with the passive, DC and low frequency sensitive electroreceptor system of the ampullary sense organs, like those in sharks and rays (Kalmijn, 1966), where the need extends to small absolute voltages.

Gymnotids have receptors with this latter type of (DC) response (Bullock and Chichibu, 1965) and the histologic category of ampullary sense organs (Szabo, 1965; Szamier and Wachtel, 1969; Bennett, 1971). It seems possible that they participate in the low ΔF detection by following the amplitude envelope of low beat frequency voltages, which are clearly within their range.

Prof. Dr. Th. H. Bullock
Robert H. Hamstra, Jr.
Dr. Henning Scheich
Dept. of Neuroscience of the School of
Medicine and Neurobiology Unit of
Scripps Inst. of Oceanography
University of California
San Diego, California, U.S.A.