ters of cells were removed in the same way as the single cells, but were plucked out several bodies at a time.

After being dissected, the cells were immediately homogenized in 65 μl of cold tris-HCl buffer; two to ten cells were pooled for each sample. After homogenization, ganglia and single cell homogenates were carried through the assay as described elsewhere (*10*). The specificity of the assay was established by identification of the product formed, *N*-methyl octopamine (synephrine), by thin-layer chromatography in three different solvent systems (*10*). More than 95 percent of the radioactivity formed was found to be isographic with synephrine. The catecholamines dopamine and norepinephrine were assayed in ganglia and single cells of *Aplysia californica* by the method of Coyle and Henry (*15*). With this method as little as 25 pg of norepinephrine and 100 pg of dopamine could be measured.

Octopamine was found to be unevenly distributed in the nervous system of *Aplysia californica*. Highest concentrations were found in the buccal ganglion and lowest in the abdominal and pleural ganglia. The cerebral and pedal ganglia showed intermediate concentrations (Table 1). No detectable amounts of octopamine were found in the pleural-abdominal connective, posterior-parapodial nerves, gill, or heart.

Large differences in octopamine concentrations were found in the single neurons examined. Neuron R14 had the highest octopamine concentration (3.66 pmole per cell). Relatively large amounts of octopamine were also found in L2-6, L7, L11, and cell 7 from the buccal ganglion (Table 1). The content of this amine in these cells is of similar magnitude to that of serotonin (*2*) and acetylcholine (*3*) described earlier for single *Aplysia* neurons.

The contents of dopamine and norepinephrine in *Aplysia* ganglion and single cells were also examined. Norepinephrine could not be found in the nervous system of *Aplysia*. Although dopamine was found in *Aplysia* ganglia in concentrations similar to those reported earlier (*4*), this amine was not detected in any of the single cells examined. Although tyrosine hydroxylase and dopamine β-hydroxylase were not directly measured in this study, the absence of dopamine and norepinephrine in neurons that contain high concentrations of octopamine suggests that in *Aplysia* tyrosine hydroxylase and dopamine β-hydroxylase may not coexist in the same cell.

It is likely that octopamine is produced by specific neurons and that this amine has functions of its own in the central nervous system of mollusks and possibly mammals. Recent electrophysiologic studies have shown that at least two receptors exist for octopamine and that these receptors are much more responsive to octopamine than to any other phenylethylamine (*16*). All of these observations indicate that octopamine may function as a neurotransmitter in the nervous system of *Aplysia*.

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25 March 1974

Neuronal Analysis of Wave Form in the Time Domain: Midbrain Units in Electric Fish during Social Behavior

Abstract. *A fish of the genus* Eigenmannia *responds differently to a neighboring conspecific fish with a slightly higher frequency of the electric organ discharge than its own than to one with a slightly lower such frequency than its own. When the two frequencies are beating against each other the special wave shape of the electric organ discharge leads to asymmetries of the beat pattern which are distinct for the two cases. Midbrain neurons, called "ΔF decoders," recognize sign and magnitude of the frequency difference on the basis of these patterns, that is, in the time rather than the frequency domain.*

Certain discoveries of new types of feature-detecting afferent neurons are worthy of wider note when they give insight into the mode of analysis of convergent sensory input. Such a neuron has come to light in fish of the genus *Eigenmannia*, not only solving a problem in how the brain distinguishes between two similar stimulus wave forms, each a combination of two frequencies, but pointing to a mechanism that operates in the time domain, that is, looking at the instantaneous form of the beat rather than the constituent frequencies.

Individuals of "wave species" of fish of the family Gymnotidae emit a regular electric organ discharge (EOD) (*1*). The resulting field is a signal source for high frequency–sensitive electroreceptors (*2*) distributed over the body surface. In 1963 Watanabe and Takeda (*3*) studied the species *Eigenmannia virescens*, a gregarious electric fish whose EOD range extends from 200 to 500 hz fundamental frequency. They found that individuals of this species exhibit tonic frequency shifts in response to slightly higher or lower frequencies emitted by electrodes in the water. I have examined the way the brain analyzes the complex combined fields by recording from single neurons in the peripheral and central nervous systems. A type of neuron found in the midbrain will be described here which responds to a small frequency difference (ΔF) between the two fields, down to less than 0.5 hz, and discriminates whether the neighboring fish is higher or lower ($+\Delta F$ or $-\Delta F$) in EOD frequency.

When two *Eigenmannia* of similar frequency are close enough together in space they both shift their frequencies, without trial and error, in the

respective directions that increase the frequency difference between them (*4*). Because the biological significance is presumably to keep a private frequency for the object-detection function of the electroreceptors, this behavior was called the "jamming avoidance response" (JAR) by Bullock (*1*).

Properties of the JAR were characterized by Bullock, Hamstra, and Scheich (*4*) by stimulating the fish through an electrode dipole with a frequency difference clamp which tracks the EOD intervals of the fish and maintains a stimulus of a constant ΔF. The JAR can be elicited by a ΔF as small as a few tenths of 1 hz. The response has a maximum when the ΔF is 3 to 4 hz. It is graded not only with ΔF but with field intensity of the stimulus; threshold is more than 1000-fold weaker than the fish's own EOD. It has a limited range of about 20 hz at the most effective intensity and ΔF.

The tasks for the electroreceptive system and the central analyzer of the fish in respect to the JAR must include two in particular: (i) to keep track of the ΔF in a mixture of two frequencies, with high precision, whatever the fish's own frequency within its range, and (ii) to recognize the sign of the ΔF, whatever the fish's own frequency within its range.

The neurons to be described here were located in the torus semicircularis of the midbrain, an area which may be homologous to the inferior colliculus (*5–7*), an auditory area. They respond to the ΔF by changing their background discharge rate (Fig. 1). These units may be called "ΔF decoders." When the stimulus is higher in frequency (+ ΔF) the neuron fires more rapidly and when the stimulus is lower (− ΔF) it fires more slowly than its background activity of somewhat less than 10 spikes per second. The discharge rate does not appear to change when the fish is driven down in frequency while maintaining the ΔF; thus the absolute value of the two frequencies cannot be of great importance. Quantitative analysis of the response reveals that the amount of increase or decrease of firing rate is a function of the magnitude of ΔF. The strongest effects, in each direction, are obtained at 3 to 5 hz ΔF (Fig. 2), and thus in the same range as the strongest behavioral response.

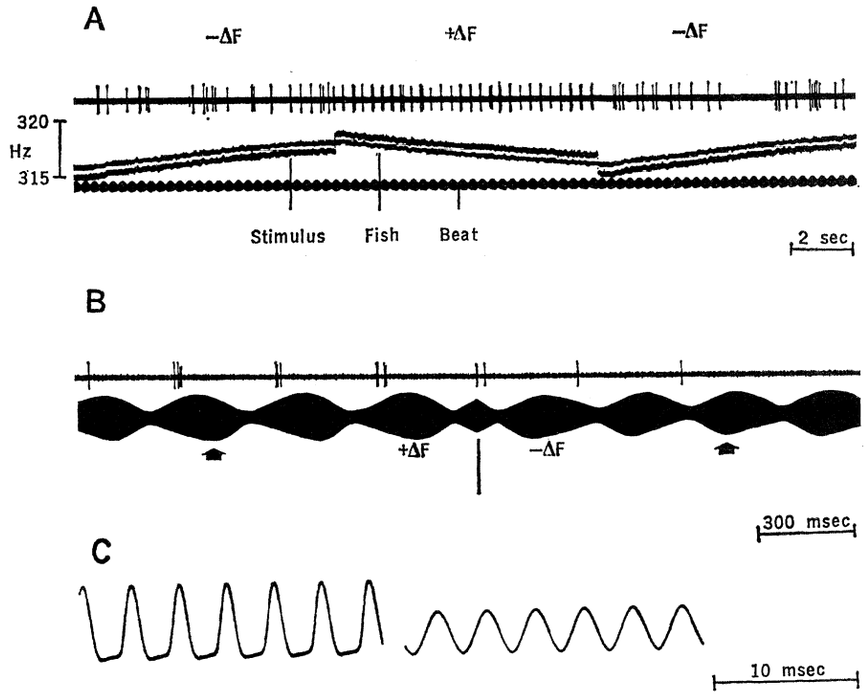

Fig. 1. (A) Simultaneous recording of the electric organ frequency and of a "ΔF decoder" neuron in the midbrain during a ΔF-clamped jamming stimulus. Upper trace: neuronal discharge mostly in bursts. Middle trace: the fish's EOD and the stimulus frequency. The stimulus changes every 8 seconds from 3 hz below (−3 ΔF) to the same amount above the fish's frequency (+3 ΔF) and again below. Lower trace: beat envelope of the two signals as mixed in the water. The neuron fires only occasionally during − ΔF but gives regular bursts of activity synchronous with the beat at + ΔF. (B) Illustration of the fixed phase relationships of the spike burst relative to the beat cycle in the same neuron. Notice the asymmetry in the lower (head negative) relative to the upper outline of the beat envelope. The maxima on the lower envelope (arrows) occur at different times during the beat cycle for + ΔF and − ΔF. The spike bursts in the "ΔF decoder" do not necessarily coincide with these maxima as would be the case in a large proportion of P units. (C) (Left) Electric organ discharge of *Eigenmannia*; head positivity upward as in (A) and (B). (Right) ΔF stimulus. The linear superposition of the two signals generates beats with the characteristic envelope shown in (B).

The response described in this report could place this ΔF decoder neuron as an important link between the electroreceptive system and the motor control of the electric organ. During the JAR the reciprocal behavior of the decoder neuron is just what is needed as an input to the pacemaker nucleus in the medulla that drives the EOD (*8*), providing the neuron is coupled with an inhibitory sign to the pacemaker. Stimuli with + ΔF, causing increase of firing rate in the unit, would inhibit the pacemaker, and − ΔF, causing decrease of firing below the spontaneous level, would diminish tonic inhibition and thus speed the pacemaker up. Additional evidence indicates a close functional relationship to the pacemaker. I have obtained frequency shifts from the pacemaker by electrical stimulation in the same torus layers where the ΔF decoders are found. The relevance for acoustic analysis and sensorimotor control of output frequency is clear by recalling that Brown (*7*) could elicit vocalization in a songbird by stimulation in torus areas (inferior colliculus) where Biederman-Thorson (*9*) found complex auditory neurons. It is then a significant point that the ΔF decoder might have a dual function within the neuronal loop controlling the JAR, that of a feature detector and that of an efferent controller.

Besides the immediate importance for the control of a behavior (JAR), this neuron is of special interest because it illustrates some new aspects of afferent analysis of mixtures of wave form and because its feature detection can be explained on the basis of known properties of neurons of lower order in the lateral line nerve and in central nuclei.

The physical basis of the input analysis is the beating of the two signals in the electric field which occurs at the frequency of the ΔF. The envelope of the beat looks quite different for + ΔF and − ΔF (Fig. 1B), the maximum on the circumference of the lower envelope being early in the beat cycle

for $-\Delta F$ and late for $+\Delta F$. This is due to the asymmetry of the wave shape of the EOD, which is rich in harmonics and resembles a sine wave clipped in one polarity (Fig. 1C). The superposed stimulus can, for simplicity, be a sine wave, since it is normally 40 db or more weaker and the influence of a fishlike distortion is correspondingly weak in determining the asymmetry of the beat envelope. The importance of the asymmetry is shown by substituting a pure sine wave for the fish's own EOD. This can be done by curarizing, which stops the electric organ but not the medullary pacemaker. One substitute sine wave can be driven by a trigger derived from recording in the pacemaker nucleus. The other one is weaker and at a few hertz difference. Even at the optimum ΔF and intensity, the fish can no longer discriminate between $+\Delta F$ and $-\Delta F$, in the JAR. Merely clipping the stronger sine wave, in the correct polarity, restores normal behavior.

Among high frequency–sensitive electroreceptors, P units follow the outline of the head negative half of the beat envelope by varying discharge rate (*2*, *10–12*). More specifically, the probability of firing each EOD cycle is increased at the beat maximum. Similar effects have been described for auditory nerve fibers during presentation of combination tones (*13*).

The firing pattern of the ΔF decoder neuron could be explained by assuming that it requires the convergence of two inputs, coinciding within about 100 msec, from two different subtypes of P units. The first must be one with little or no difference in the timing of its maximum firing rate during the beat cycle, depending on the sign of ΔF. The second must be one that shifts its peak corresponding to the different occurrence of the maximum in the lower beat envelope during $+\Delta F$ and $-\Delta F$. The peak activity of the second unit caused by $+\Delta F$ will coincide with that of the first unit, whereas during $-\Delta F$ the two units will be so out of phase that they cannot fire the ΔF decoder. Peripheral and higher order neurons with the postulated properties and an outline of a model have been described (*10*).

The findings illustrate that the analysis of mixtures of frequencies in these electric fish is not accomplished by an array of tuned filters as has been one of the suggestions for auditory performance. There is only one filter area common to the electroreceptors which correspond approximately to the frequency range of the species (*2*). The analysis instead relies on the occurrence of events in the fine structure of waves and the extraction of patterns in the time domain. The temporal pattern comes about by the special wave shape of the electric organ discharge. This scheme is more compatible with the rival set of explanations known as the "periodicity theory" of hearing (*14*). However, wave form analysis as discussed here might be developed to higher standards in the electrosensory system. A comparison with the auditory system confronted with the same problem may emphasize this point. The fish's way of measuring absolute frequency differences in mixtures compares to the solution of the same problem in auditory psychophysics. The frequency difference corresponds to the number of audible beats per second, whose period increases with the closeness of the two tones. The limit of absolute sensitivity to the beat phenomenon is reached when the ear fails to resolve the intensity change over time. Thus in comparing the ability to discriminate absolute ΔF's the two systems are very similar; both take advantage of a time domain phenomenon.

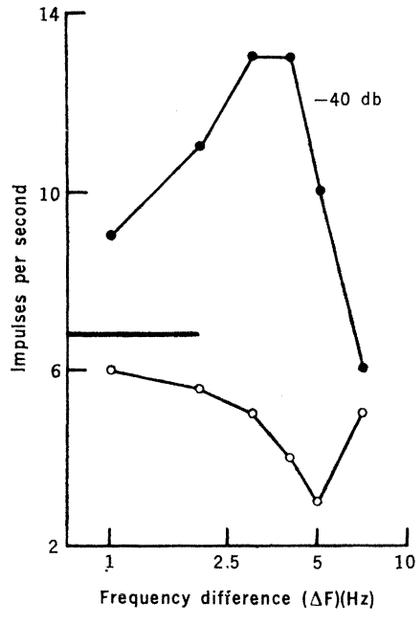

Fig. 2. Activity of a ΔF decoder unit plotted against the frequency difference between the electric organ and the stimulus. The neuron is tuned to a ΔF of 3 to 5 hz. The response is differential relative to the background activity (heavy bar) during $+\Delta F$ (closed circles) and $-\Delta F$ (open circles). Values were averaged over 40 seconds. The stimulus intensity (−40 db, arbitrary units) is about 20 db below the EOD and 40 db above behavioral threshold (*4*).

The power of the beat analysis in the fish is evident upon comparison with the ability of the auditory system to discriminate the sign of the ΔF. Helmholtz (*15*) first reported that in mixtures of two frequencies close to each other only one pitch is heard, which is modulated in intensity at the frequency of the ΔF. The pitch of the combination tone lies in the middle between the primaries when the latter tones have equal intensity (*16*). The perceived pitch in the mixture 300 + 303 hz is therefore 3 hz above that for the mixture 300 + 297 hz; this is the just noticeable difference. Thus the ear could only distinguish +3 from a −3 hz ΔF, to use our terminology, and this only if the two tones have approximately equal intensities. *Eigenmannia* can tell a fraction of +1 from a fraction of −1 hz ΔF, and even when the foreign signal is 60 db weaker than its own EOD (*2*, *4*).

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17. Supported by grants to T. H. Bullock from NIH, NSF, and ONR. I thank Prof. Bullock for constant support and encouragement throughout the course of this study.
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23 October 1973; revised 4 March 1974