The Relationship between the Firing Rate of a Single Neuron and the Level of Activity in a Population of Neurons

Experimental evidence for resonant enhancement in the population response

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ABSTRACT A quantitative comparison is made between experiment and the theoretically predicted dynamics of a neuron population. The experiment confirms the theoretical prediction that under appropriate conditions an enlarged resonant response should appear in the activity of the neuron population, near the frequency at which there is minimum modulation in the instantaneous rate of a single neuron. These findings bear on the relationship between the firing rate of a single neuron and the firing rate of a population of neurons.

INTRODUCTION AND RESULTS

The experiment reported here was performed with three objectives. The first was to investigate experimentally the relationship between firing rate of a single neuron and the activity level in a whole population of similar neurons. The second objective was to seek evidence of a "resonant" enhancement in the responsiveness of a neuron population, which was theoretically predicted under specified circumstances, and which might be of some direct physiological interest. The third objective was to attempt a quantitative comparison between data gathered from a real neuron, and several theoretical predictions of a general nature which have been presented in a preceding paper (Knight, 1972). This experiment was done with a single neuron: the response of a homogeneous neuron population to a single cycle of stimulus has been inferred by observing the response pattern of the single neuron again and again over many repeated stimulus cycles.

When one deals with a neuron whose firing is both rapid and regular, it is customary to characterize that neuron's activity by its "instantaneous fre-

quency" or "single unit instantaneous firing rate," which is the reciprocal of the interval between impulses. However, if that neuron is firing in response to a periodic input, the period of which is not much longer than the intervals between the impulses with which the neuron responds, then the significance of the single unit instantaneous firing rate becomes less clear. The measure of neural activity which is of direct relevance to physiological effects of that activity not only concerns the time since last firing of a single neuron, but also should be a measure of how many similar neurons are firing at any given phase of the input cycle. If there is convergence upon the next higher order neuron, it will be the momentary level of impulse activity in the entire population which determines the postsynaptic potential in that higher order neuron. In this experiment the single unit rate (measured directly) and the population rate (as inferred from many cycles) in response to a periodic input have been measured simultaneously from a single neuron. Major qualitative differences are found between the two measures of neural activity.

When a neuron fires in response to a periodic input, two important frequencies are involved. One is the frequency of the stimulus input and the other is the "carrier rate" or "center frequency" of the responding neuron, which is the reciprocal of the long-time average of the single unit interpulse interval. The periodic input to the neuron consists of a mean value plus a modulation. In the limit of vanishingly small modulation, the center frequency will be independent of the input modulation frequency, and will depend only on the mean input level and upon the characteristics of the neuron itself. In this limit of small modulation, the response of a homogeneous population of neurons may be analyzed theoretically, in an approximate way that does not require detailed assumptions concerning the underlying machinery of the neurons. A theoretical prediction results: If the input modulation frequency is set close to the center frequency of a single unit, the pooled firing of the neuron population will show an enhanced "resonant" response[1]—a particularly strong modulation in the population's response over the stimulus cycle. This predicted resonant response is specifically a property of the pooled activity of the whole population. The theory predicts that the modulation in the instantaneous rate of a single unit will show the opposite effect: it will drop to a minimum near the point where modulation frequency is equal to center frequency. The experiment bears out these two predictions.

To within experimental error, the frequency responses, of the single unit and population rates, may be measured quantitatively. Theory also predicts what these numbers should be. In its most reduced form, the approximate

[1] The word resonance is used in its technical sense here. In technical terms, there exists a real "resonant" frequency (the center frequency) near which there lies a formal complex frequency which makes a denominator vanish in a frequency response expression.

theory compresses all the characteristics of a neuron to two parameters: the "forgetting rate" (γ), and the "coefficient of variation" (c). The forgetting rate γ essentially defines a time scale for discounting old input, in the determination of when the neuron will fire again. The coefficient of variation c is a measure of the fractional random scatter in interpulse interval times. The phenomenon of population resonance is directly related to the process of forgetting: in the limit of no forgetting ($\gamma = 0$) no resonance should appear. In the theory forgetting is included as a simple exponential discounting of old input with the passage of time. The random variability of interpulse intervals (as characterized by the coefficient of variation) has the effect of limiting the height of the resonance, and of increasing its width. In the present experiment the coefficient of variation was measured directly. The choice of forgetting rate was dictated by the data: an attempt was made to obtain a reasonable fit. The comparison of the experiment and the theory is shown in Fig. 1.

Since the experiment yielded frequency responses for both single unit rate and population rate, the data are sufficient to find an empirical transfer function from population rate to single unit rate. The data points in Fig. 2 give this empirical result. Theory also yields an expression, with the interesting feature that it depends only on the coefficient of variation (which was measured) and *not* on the forgetting rate (which was fitted). Thus the theory makes a prediction which contains no free parameters, and is shown by the curves in Fig. 2.

METHODS AND DISCUSSION OF METHODS

A visual neuron, the eccentric cell in the compound lateral eye of the horseshoe crab *Limulus polyphemus*, was chosen for this experiment. The choice was dictated by the sizable body of quantitative information available concerning this neuron (Dodge et al., 1970; Hartline and Ratliff, 1972) and by personal familiarity with the preparation procedures.

The general setup was usual: the excised eye served as the fourth side of a covered moist chamber. Light was led to a single facet of the eye by a narrow (0.4 mm) glass fiber optic bundle, mounted on a micromanipulator. The light came from a glow modulator system. The moist chamber was filled with previously filtered and aerated *Limulus* blood, and a small bundle of nerve fibers, dissected from the optic nerve, was lifted through the air/blood interface and mounted on a cotton wick recording electrode. A single unit was isolated optically, by using the micromanipulator. The signal from the wick electrode went to an AC preamplifier and thence to the amplifier of an oscilloscope. The oscilloscope output drove an audio monitor system and also fed a pulse-height discriminator which interfaced with a small computer (CDC-160A). The glow modulator tube was pulsed at a high frequency

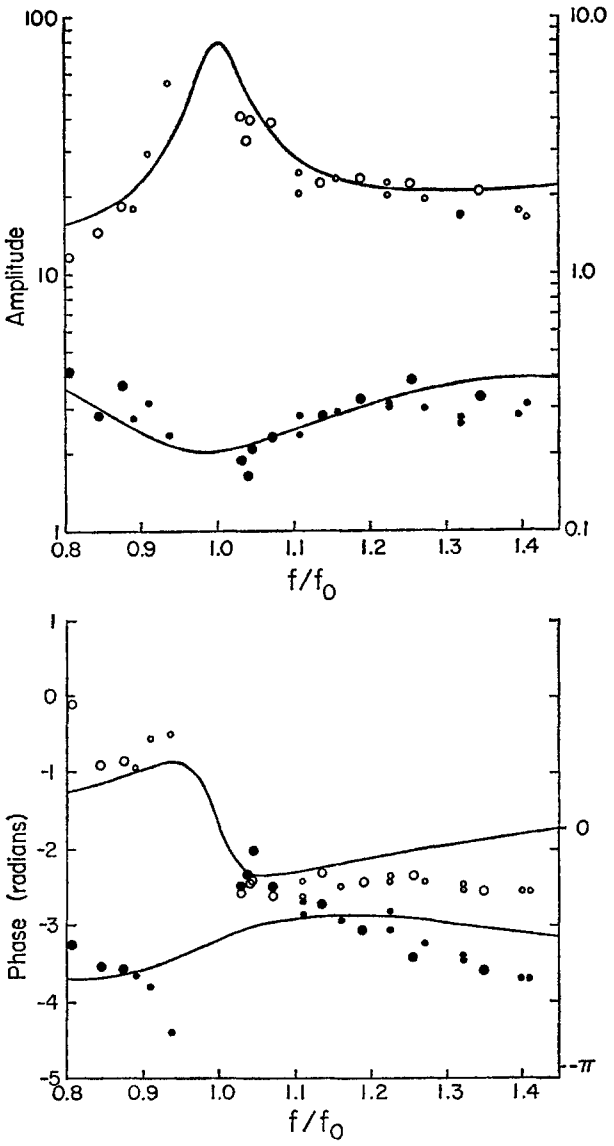

FIGURE 1. Amplitude and phase of the frequency response of a single unit (filled circles) and of the population (open circles). The left-hand ordinate is for the experimental measurements. The solid curves are from the theory, and correspond to the right-hand ordinate. The difference between left-hand and right-hand ordinates reflects the unknown amplitude and phase in the generator potential (see text).

(center frequency, 400/sec). This pulse rate was frequency modulated by a voltage input, which was a constant voltage, plus a sine wave of variable amplitude drawn from a function generator. The function generator also put out a phase mark on another channel at the top of the sine wave, and

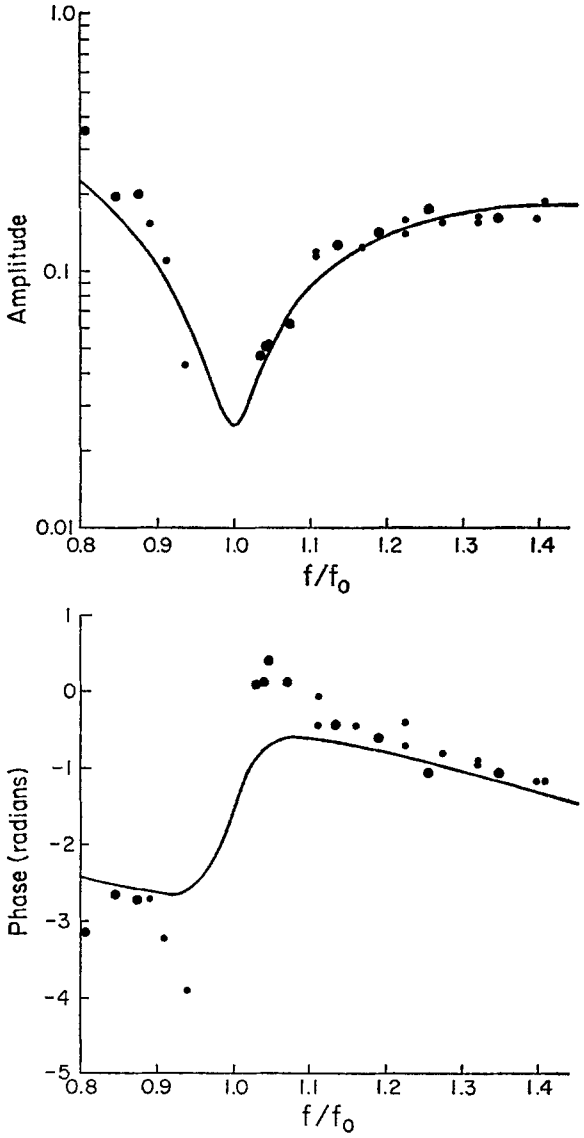

FIGURE 2. Amplitude and phase of the transduction from population response to single unit response. The solid lines are theoretical and the filled circles are from the experiment.

this phase mark was delivered to a second discriminator. To monitor modulation depth, the pulses delivered to the glow modulator tube were also fed to a factor of 16 downcount scaling circuit, the output pulses of which were in turn sent to a third discriminator which interfaced with the computer. At the end of each run, the nerve impulse times were classified according to

which consecutive 20 msec time interval in the stimulus cycle they occupied. In this way the computer generated a cycle histogram of the population rate, which it returned through a digital-to-analogue converter, to a second monitoring oscilloscope at the experimental setup. The histogram and the three channels of impulse time information were also stored on magnetic tape by the computer for later processing.

In stimulating the eye it was necessary to abandon the established procedure of light on for about 20 sec and off for about 100 sec per run, which fosters the long-term stability of the preparation but leads to a continuous downward drift in the neuron's center frequency throughout the 20 sec. In this experiment it was necessary to hold the neuron's center frequency at a known level to within a per cent or two during a run, and this demanded that the light be left on continuously. As a result the cell's center frequency gradually declined as the experiment progressed, steadily but slowly, and not appreciably during a 20 sec run. This slow decline proved valuable, and was exploited in performing the experiment: in the theory which this experiment was designed to examine, the sensitive dependence upon frequency is in fact a dependence upon the *ratio* between modulation frequency and center frequency, so it was natural to set a fixed modulation frequency and allow the center frequency to creep past it during the course of the experiment. Proceeding in this way has a particular advantage: the conversion of flickering light to modulated intracellular voltage (generator potential) is frequency dependent, and that frequency dependence is believed to be one of the more stable aspects of this preparation. By working at a single modulation frequency we confine our ignorance of what voltage the impulse encoder sees to a single unknown amplitude and a single unknown phase. The center frequency of the neuron was brought close to 4 impulses/sec by inserting an appropriate neutral density filter in the light path. A fine setting to 4/sec was made by adjusting the duration of the glow modulator pulses. The function generator was set at 3 cps. The neuron's center frequency gradually declined to 2/sec, at which point the experiment was terminated. These firing rates, which are an order of magnitude lower than what is usual for *Limulus* experiments, were chosen for the following reasons. (*a*) The decline in center frequency is gradual at these modest rates. (*b*) The simple theory assumes that irregularities in interpulse interval are uncorrelated, and experiments have shown that eccentric cell impulses become uncorrelated when they are separated by more than about 0.3 sec (Shapley, 1971). (*c*) Since the experiment relies on a stable generator potential, a frequency should be chosen where the generator frequency response is insensitive to parametric changes; the generator amplitude is sensitive to frequency changes above 3 cps (Knight et al., 1970). (*d*) In the usual regime the eccentric cell behaves much like a "simple integrate-and-fire" encoder. That is,

it shows only slight memory loss between impulses, as indicated by its lack of any pronounced tendency to phase lock to periodic stimuli. Without memory loss, no resonance should be anticipated. The theory indicates that a lengthened interval would lead to greater memory loss, and a more pronounced resonance; the theoretical resonance height depends on the *ratio* of the forgetting rate to the center frequency. These four considerations set an upper bound on the center frequency and the following one sets a lower bound. (*e*) Near the anticipated resonance, the center frequency must be determined without much more than 1% error, and the computer program is limited to 20 sec of data on any run. Eccentric cell interpulse intervals vary about $\pm 10\%$ (Ratliff et al., 1968); the coefficient of variation of this cell was 0.09. It takes about 60 impulses, or 3/sec, to achieve the necessary error bound.

Because there is substantial random variability in interpulse intervals, the measurement of output modulation faces a serious signal-to-noise problem. For the single unit rate, the problem is at its worst near resonance, where the output modulation drops to small values. It might seem that the problem could be solved by means of a large input modulation. However, the theory (which is a linearized theory) only claims to work in the limit of small modulation. How large a modulation may be used, without causing drastic changes in response, may be estimated from the nonlinear theory without noise, which is given in section 6 of the previous paper (Knight, 1972). The conclusion there is that a result of finite modulation is phase locking, and that this condition is particularly encouraged if (*a*) there is much forgetting between impulses or (*b*) if the modulation frequency is close to center frequency. Of course both are preconditions of this experiment.

These considerations dictated the cycle of operations in the experiment. First, a 20 sec run was taken with no modulation, to determine the center frequency. Then the light modulation was turned up while the sound of the impulse train was monitored on a loudspeaker. Either the unmodulated or the phase-locked response is a monotonous beat on the audio monitor. In between, the beat has a notable nonperiodic texture. When this condition was achieved another run was taken, at the end of which the population cycle histogram was displayed on the monitor oscilloscope. From this display a judgment was made about how to change the modulation. If signal-to-noise was poor the modulation was turned up. If the wave form seen was distorted from sinusoidal, the modulation was turned down. Sometimes no readjustment was necessary. Another run was taken. This pair of modulated runs was followed by an unmodulated run to start the next cycle. The center frequency, which was later related to each modulated run, was the average of the two values obtained before and after.

Subsequently modulation values were extracted from the data by a least-

squares fitting procedure described elsewhere (Knight et al., 1970).[2] The modulation in population response was determined in a similar way from the record of the cycle histogram. The frequency responses were calculated in the form (output modulation/mean output)/(input modulation/mean input), which is the form convenient to compare with theory.

The experimental design anticipated discarding the earlier of each pair of modulated runs. This was not always necessary, and conversely sometimes both runs were discarded. The first criterion for discarding came from notes made immediately after each run. The second criterion came from data processing: if harmonic content was excessive, or if the center frequency of the single unit rate was much deviated by the presence of modulation, then that run was rejected. The least-squares procedure also gave a "uniqueness of fit" estimate. The fit to the single unit rate becomes nonunique when phase locking occurs, and some runs were rejected on that basis. Two runs were discarded simply because they were in bad disagreement with all the rest of the data. From a span of 50 modulated runs, 16 runs were discarded and the rest are represented in Figs. 1 and 2. The oversize data points indicate the averages of two consecutive runs in those cases where no modulation readjustment was made. The averaging was done on the fitting coefficients, before reduction to amplitude and phase.

For the theoretical curves of Fig. 1, the value $\gamma/f_0 = 0.75$ (f_0 is center frequency) was chosen because agreement with the experimental points looked reasonable. Higher values give less agreement, but values of γ/f_0 down to 0.5 give agreement comparable to what is shown. In the upper frame of Fig. 1 the theoretical amplitude has been shifted, as shown on the right-hand vertical scale. In the lower frame the phase has been similarly shifted. These two operations adjust for the unknown amplitude and phase in the generator potential modulation. In Fig. 2 there are no adjustable parameters.

THEORETICAL

A detailed theory was developed in the previous paper. In brief outline, here is how the theory may be put into a form to compare with the present experiment. According to the simplest neuron model (integrate-and-fire model) the single unit modulation response is given by the running average of the input modulation:

$$B = \frac{1}{T_0} \int_{-T_0}^{0} dt\, s(t) \qquad (1)$$

[2] To determine the modulation in instantaneous frequency, a record was kept of the times at which impulses occurred. To each occurrence time was assigned an instantaneous frequency which was the reciprocal of the time since the previous impulse. An assumed output modulation form was adjusted to give the best possible fit, in the least-squares sense, to the measured instantaneous frequencies at all the impulse occurrence times.

(equation 3.12)[3] where $s(t)$ is the stimulus modulation, B is the response modulation at the moment $t = 0$, and T_0 is the period between impulses in the absence of modulation. If for the stimulus modulation $s(t)$ we substitute the sinusoid $\exp(i\omega t)$ the value of which is unity at the moment $t = 0$, we find

$$B = \frac{1}{T_0}\int_{-T_0}^{0} e^{i\omega t}\,dt = \frac{1 - e^{-i\omega T_0}}{i\omega T_0} \tag{2}$$

(equations 3.14 and 3.15). This is the transfer function from the stimulus to the single unit rate. For different reasons it is also *the transfer function from the population rate to the single unit rate* (equation 4.3). If, in the absence of modulation, the interpulse interval T_0 shows random variations, then equation 2 must be replaced by its reasonable generalization

$$B = \frac{1 - \langle e^{-i\omega T_0}\rangle}{i\omega\langle T_0\rangle} \tag{3}$$

(equation 8.12), which is very nearly the theoretical expression plotted in Fig. 2. If the fluctuations of T_0 from its mean $\bar{T}_0$ are small[4] then we may write

$$\begin{aligned}\langle e^{-i\omega T_0}\rangle &= \langle e^{-i\omega\bar{T}_0}e^{-i\omega(T_0-\bar{T}_0)}\rangle \\ &= e^{-i\omega\bar{T}_0}\langle 1 - i\omega(T_0 - \bar{T}_0) + \frac{1}{2!}(-i\omega)^2(T_0 - \bar{T}_0)^2 + \cdots\rangle\end{aligned} \tag{4}$$

(equation 8.19). The means of the first two terms in the pointed brackets are evidently unity and zero, whence

$$\langle e^{-i\omega T_0}\rangle \cong e^{-i\omega\bar{T}_0}\left\{1 - \frac{\omega^2}{2}\langle(T_0 - \bar{T}_0)^2\rangle\right\} \tag{5}$$

(equation 8.20), and equation 3 becomes approximately

$$B = \frac{1 - e^{-i\omega\bar{T}_0}\left\{1 - \frac{\omega^2}{2}\langle(T_0 - \bar{T}_0)^2\rangle\right\}}{i\omega\bar{T}} \tag{6}$$

the amplitude and phase of which are the curves plotted in Fig. 2. The fluctuation term in equation 6 is related to the coefficient of variation c by

$$\langle(T_0 - \bar{T}_0)^2\rangle = c\bar{T}_0^2 \tag{7}$$

[3] Equation numbers with decimal points will refer to corresponding equations in the previous paper (Knight, 1972).

[4] We indicate mean either by pointed brackets or by an overhead bar depending on typographical convenience.

(equation 8.12). The value $c = 0.09$, which persisted throughout the experiment, was used in the calculation. (Fig. 4 of the previous paper [Knight, 1972] shows the analogous result when $c = 0$.)

On rather general theoretical grounds, the behavior of a broad class of real neurons should be typified by the "forgetful integrate-and-fire neuron model." As in equation 2 the single unit frequency response in this model has a numerator containing two terms which represent the present and past limits of an averaging integral. However, in the forgetful model the numerator term representing the present is favored by a weighting factor exp (γT_0). The full expression is

$$F = \frac{e^{\gamma T_0} - e^{-i\omega T_0}}{(i\omega + \gamma)T_0} \tag{8}$$

(equation 5.10). If the interpulse interval has random scatter, this generalizes to

$$F = \frac{\langle e^{\gamma T_0}\rangle - \langle e^{-i\omega T_0}\rangle}{(i\omega + \gamma)\langle T_0\rangle} \tag{9}$$

(equation 8.17). If again the scatter about the mean is small, this may be approximated by

$$F = \frac{e^{\gamma \bar{T}_0}\left\{1 + \frac{\gamma^2}{2}\langle (T_0 - \bar{T}_0)^2\rangle\right\} - e^{-i\omega \bar{T}_0}\left\{1 - \frac{\omega^2}{2}\langle (T_0 - \bar{T}_0)^2\rangle\right\}}{(i\omega + \gamma)\bar{T}_0} \tag{10}$$

in exactly the same way that equation 6 was derived. The value $\gamma\ \bar{T}_0 = 0.75$ was substituted into equation 10, which then yielded the theoretical amplitude and phase that are plotted as curves along with the single-unit data in Fig. 1. Finally, the theoretical population response was obtained from

$$P = F/B \tag{11}$$

(equation 8.18), the amplitude and phase of which are plotted along with the population data in Fig. 1. The result should also be compared with Fig. 6 of the previous paper (Knight, 1972) (in that figure the coefficient of variation is given by $\tau f_0 = c$).

In conclusion, here is how B and F are expressed in terms of the modulation frequency f, the center frequency f_0, the forgetting rate γ, and the coefficient of variation c. The expressions are

$$B = \frac{1 - e^{-2\pi i f/f_0}\ (1 - \frac{1}{2}(c \cdot 2\pi f/f_0)^2)}{2\pi i f/f_0} \tag{12}$$

and

$$F = \frac{e^{\gamma/f_0}\,(1 + \frac{1}{2}(c\cdot\gamma/f_0)^2) - e^{-2\pi i f/f_0}\,(1 - \frac{1}{2}(c\cdot 2\pi f/f_0)^2)}{2\pi i(f/f_0) + (\gamma/f_0)}. \qquad (13)$$

The fact that f and γ appear only in the combinations f/f_0 and γ/f_0 was of course crucial in the design of the experiment.

DISCUSSION

Because of the noise problem inherent in this experiment, there is considerable scatter in the data, and no conclusions should be based on any single point. Nonetheless, there is a substantial similarity between the results of theory and of experiment. In Fig. 1 the most obvious disagreement is in the phase at the upper end of the relative frequency range, where the phase of both single unit and population rates systematically falls behind the predictions of theory. At its greatest this phase lag goes to about a tenth of a cycle (the total height of the phase graph is 6 radians, not quite a full cycle).

The phase lag may be an artifact of the experimental procedure. Between the resonant point at $f/f_0 = 1$ and the last data point on the right, the encoding neuron's time scale, as measured by the center frequency, had become stretched out by some 40%. If the generator potential were likewise "running down" it would introduce the observed phase trend into the data. On the other hand, a real departure of this magnitude from the very simplified theory would be no surprise, and could arise, for example, if the real encoder were to discount past input in a way different from the simple exponential assumed in the model. The fitted value $\gamma/f_0 = 0.75$ corresponds to a characteristic forgetting time of about 0.45 sec, which is quite comparable to the interpulse times in the experiment. Under this circumstance a nonexponential profile for forgetting could yield a substantial departure.

In Fig. 2 the one systematic discrepancy is in the phase near resonance. The abrupt change predicted by theory is exaggerated in the experimental result. This probably is not a breakdown in the theory but rather a result of overmodulation. If the theory were in error we would also expect that the population rate data of Fig. 1 would show a systematic departure of phase near the resonance, since the logical relation among the curves is from the single unit response through the transduction of Fig. 2 (or equation 11) to the population response. This reasoning casts suspicion on the phase data near resonance of the single unit response. There is a second good reason for this suspicion: section 3 of the previous paper (Knight, 1972) indicates that overmodulation will be more severe in the single unit response than in the population response. The severity of the overmodulation problem in the immediate neighborhood of the resonance led to the discarding of all runs that fell within the gap that the figures show there.

Presumably the population resonance does not play a significant role in the normal functioning of the *Limulus* eye. Under normal conditions the eccentric cell center frequency is not far below the flicker-fusion threshold frequency.[5] However, one may speculate how evolution may have exploited the effect in other systems. The population resonance is uniquely suited to the task of frequency discrimination, and one might look for its application in auditory systems, or in the frequency-sensitive electrical sense of certain fish. If the effect were utilized at more than one neuronal level, sharper frequency tuning should be found the farther one went along the sensory pathway.

The data of Fig. 1 show that a population of neurons may carry signals which contain frequencies well beyond the center frequency of any single neuron. These data also show that, as the center frequency is approached or exceeded, the single unit instantaneous frequency if it is used naively becomes an altogether misleading indicator of what the population are doing. Where the population show a maximum response the single unit rate shows a minimum. The results of the experiment were reasonably fit by a simple encoder model: the stochastic and forgetful integrate-and-fire model. This indicates that more detailed knowledge of neuronal impulse encoding may be unnecessary in the further exploration of some aspects of the dynamics of nerve populations.

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[5] Unpublished observations of F. A. Dodge, recording from nerve fiber bundles of intact animals in the field, give a center frequency of 6/sec or more. The flicker-fusion frequency is at about 10 cps.