XI. COMMUNICATIONS BIOPHYSICS*

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A. EFFECT OF DIMETHYL SULPHOXIDE (DMSO) ON THE CONDUCTION VELOCITY OF HOMARUS AXONS

The successful preservation of sperm, blood, and bacteria by reversible freezing[1] has sparked interest in the cryobiology of more complex systems. Nervous tissue is particularly interesting, since in it membrance damage may be distinguishable from the disruption of axon metabolism.[2] Attempts to freeze vertebrate nervous tissue have been only partially successful,[3,4] except for the work of Pascoe[5] with rat ganglia.

In most current techniques, preparations are first treated with cryoprotective agents, such as glycerol, ethylene glycol, and dimethyl sulphoxide (DMSO).[1] The effect of these chemicals on nerve conduction is under investigation. Pribor and Nara[4] have exposed frog sciatic nerves to glycerol for 15 min, and found an irreversible reduction in the propagation velocity of the action potential; the effect was

much less with DMSO. Their measurements were made after the nerves had been rinsed for 30 or 60 min in fresh Ringer's solution; there was no attempt to study the nerve during exposure to the cryoprotectant. Pascoe[5] mentions a slowing of nerve conduction in rat ganglia exposed to glycerol.

We have investigated the effect of DMSO on the ventral nerve of the lobster Homarus americanus by measuring changes in the compound action potential of the nerve during progressive exposure to the chemical. Although this may yield information about the impregnated state of DMSO-treated tissue before freezing, it remains to be seen whether the observed changes can be related to cryoprotection.

1. Materials and Methods

Our choice of Homarus americanus was motivated by several factors. As experimental subjects lobsters are inexpensive, readily available, and easily kept. The ventral nerve cord, surrounded by an external sheath of connective tissue, is easy to remove. Our laboratory has previously used this preparation in single-axon experiments.[6,7]

The nerve cord itself is made up of a few giant axons (50-100 μ) and hundreds of small ones.[8] The propagation velocity of these axons increases with their diameter.[9] Although invertebrate axons lack spiral myelin coats, they are often surrounded by glial cells and connective tissue[10]; this is the case in the Homarus ventral nerve.
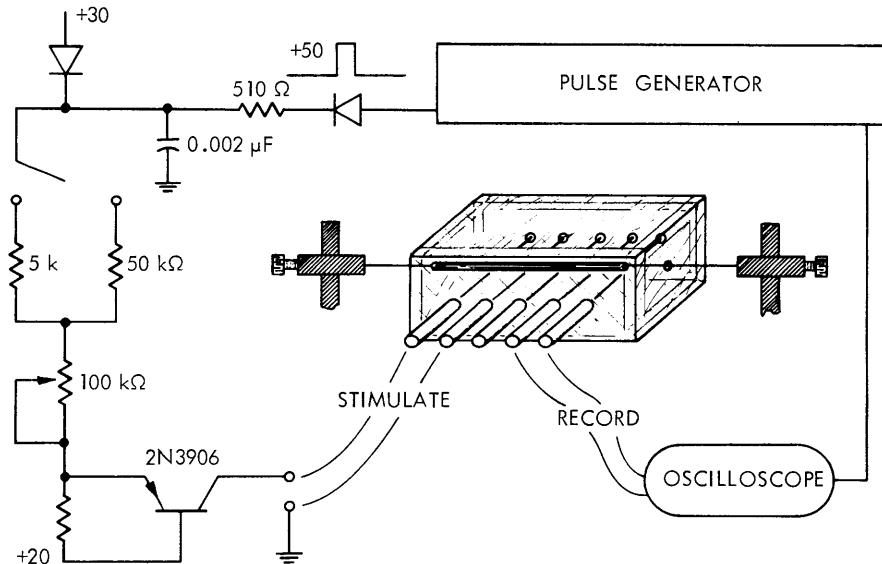

Fig. XI-1. Experimental apparatus. The nerve rests on silver electrodes mounted 0.5 cm apart in a lucite chamber. A pulse generator system (Tektronix 161/162) triggers the oscilloscope, and also provides a +50 V output pulse. A simple circuit converts this to a constant-current stimulus pulse. The recording electrodes are connected differentially to the oscilloscope.

The electrical apparatus is shown in Fig. XI-1.   It produces 0.3 ms constant-current pulses of 20-900 µA.   The center electrode was used only to support the fiber; thus there was 1 cm between stimulating and recording electrodes.

2.   Experimental Procedure

i.   The lobster was anesthetized by immersion in an MS-222/sea water solution. The tail, extremities, upper shell, and internal organs were removed to expose the two ventral nerves.

ii.   The circumesophageal connectives were cut out with the brain and first ganglion included to facilitate handling.   They were impaled on rubber pads immersed in sea water and desheathed.

iii.   The desheathed nerve was placed on a rack of silver electrodes in a lucite chamber (capacity 27 ml) which was temporarily flooded with artificial sea water. The nerve was tied to manipulators with thread to reduce movement during the experiment.

iv.   The chamber was emptied of sea water through a hole in its bottom; a residual layer of water and a lucite cover insured a moist, saturated atmosphere. Recording was postponed for at least a minute; otherwise, the threshold was anomalously high.

v.   The stimulus was slowly increased until several peaks appeared in the compound action potential.   Oscillographs were obtained for several levels of stimulus current.   Recording lasted for 5-6 min, during which the response was rather stable.   The chamber was then refilled with artificial sea water.

vi.   Procedures 4 and 5 were repeated after 3 min to verify that the response was reproducible.

vii.   The chamber was refilled with a solution of DMSO and sea water.   After several minutes, the chamber was again emptied for recording.   This procedure was repeated several times for a variety of exposure intervals.

3.   Data and Results

Our results were based on oscilloscope data taken from 8 ventral nerves exposed to 0, 5, 10, and 15 percent solutions of DMSO in artificial sea water.   (These concentrations correspond to 0, 0.58, 1.16, and 1.74 $\underline{M}$.) Two nerves from different lobsters were used for each concentration; one nerve was fresh, the other had been stored for several hours at 10°C.   This did not significantly affect the data.

We display typical oscilloscope data in Fig. XI-2.   Figure XI-2a and XI-2b demonstrates the effect of increasing the stimulus current (from 47 µA to 120 µA) during a recording session.   Usually a three-peak pattern emerged.   The peaks grew with increased stimulation until nearly stable sizes were reached.   The introduction of still other peaks and the diphasic nature of the response sometimes complicated
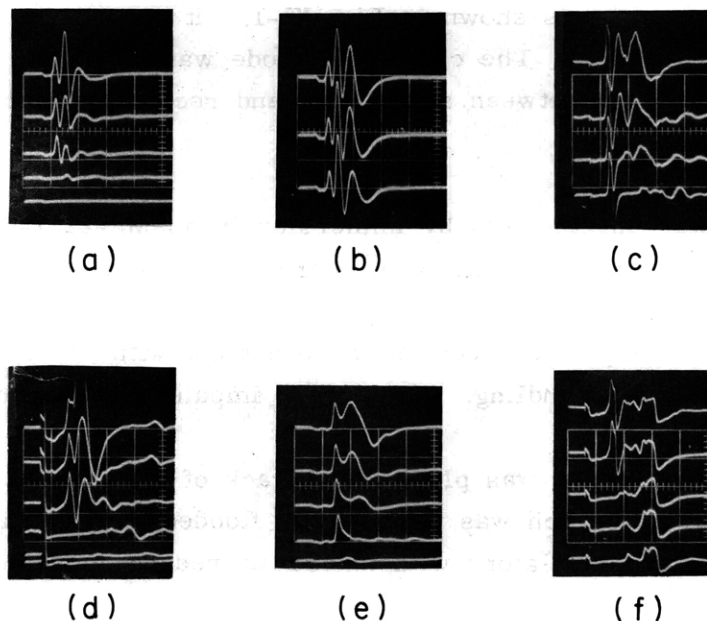
Fig. XI-2. Typical oscilloscope data. Scale 2 mV/cm and 2 ms/cm. Numbers in parenthesis label each nerve according to DMSO concentration (first number) and order of use. (1 = used at once; 2 = temporarily stored.)

(a) Control (0-1). Peaks are added one at a time by gradually increasing the stimulus current. Near threshold, peaks move slightly toward the left as current increases. Stimuli: 47, 53, 60, 68, 77 µA.

(b) Control (0-1). Same nerve with more intense stimulation. Number, height, and position of peaks becomes almost constant. Stimuli: 88, 100, 115 µA.

(c) 10% DMSO (10-1, 3-min exposure). Basic pattern is still evident although peaks are wider, and somewhat delayed. Stimuli: 26, 30, 34, 38 µA.

(d) 15% DMSO (15-2, 3-min exposure). Peaks are further broadened and delayed. Stimuli: 30, 34, 38, 42, 47, 53 µA.

(e) 15% DMSO (15-1, 5-min exposure). An example of considerable broadening. Stimuli: 44, 47, 50, 54, 58 µA.

(f) 10% DMSO (10-1, 16-min exposure). In some cases peaks become doubled; this made useful measurements difficult or impossible to obtain. Stimuli: 18, 21, 24, 27, 30 µA.

this simple behavior.

We measured the time between the onset of the stimulus pulse and the appearance of a given peak. Measurements were made with stimuli considerably above threshold; for lower values the observed delay was slightly longer (Fig. XI-2a, XI-2c). This resulted partly from the finite stimulus pulse width (0.3 ms). We labeled the three peaks that we observed α, β, γ in order of increasing delay. The α peak represented the axon population of largest conduction velocity, that is, the largest diameter and smallest surface/volume ratio. Thresholds of these peaks were also measured.

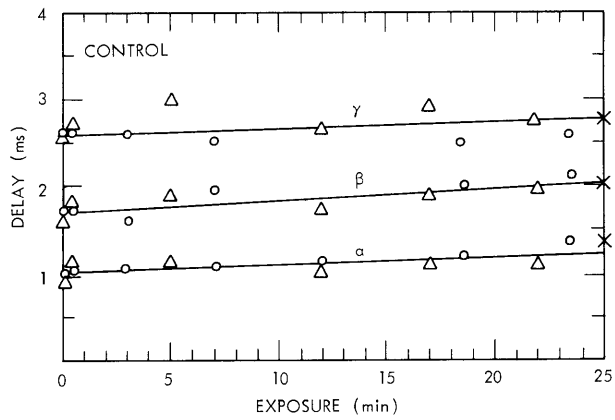Exposure to 10 or 15% DMSO delayed and broadened the peaks (Fig. XI-2c and

Fig. XI-3. Delay vs duration of exposure in control experiment. Triangles represent nerves used at once; open circles, those stored temporarily. Points (x) are for 71 min exposure to artificial sea water.
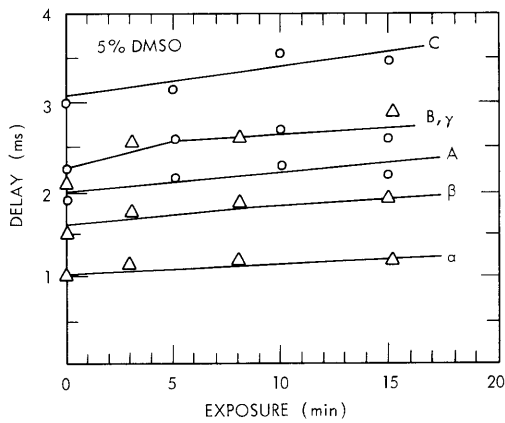

Fig. XI-4.

Delay vs duration of exposure in 5% DMSO. Curves A, B, C represent the nerve 5-2 in which the a, β, γ pattern, though familiar, was shifted to higher delays.
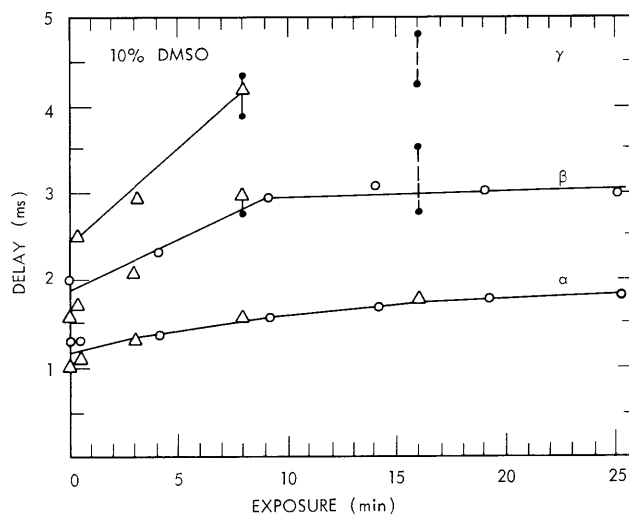

Fig. XI-5. Delay vs duration of exposure in 10% DMSO.

XI-2d). This effect is consistent with a decrease in the propagation velocity of the individual axons. Considerable broadening and splitting of the peaks was not uncommon (Fig. XI-3e and XI-3f). Once the pattern became unrecognizable the nerve was discarded.

In Figs. XI-3 through XI-7, we plot delay against duration of exposure to DMSO. The time spent in recording (i.e., in air) is not included. The control nerves
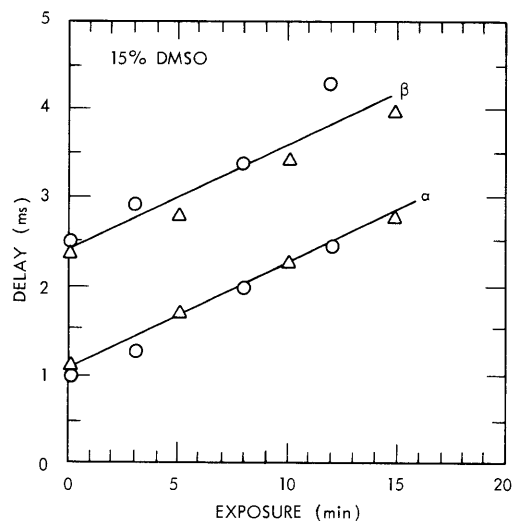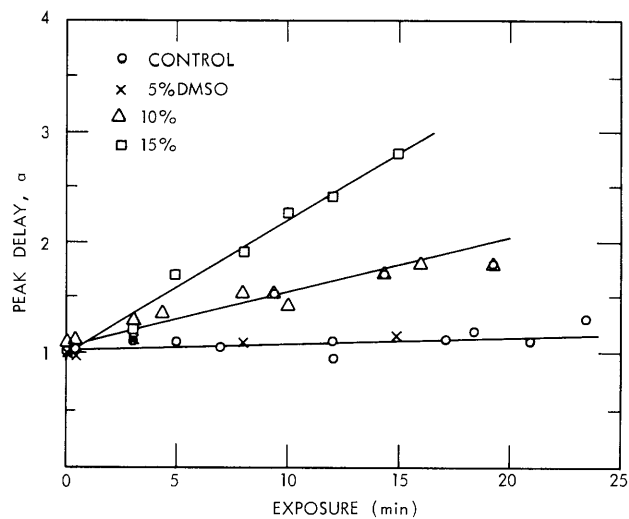
Fig. XI-6. Delay vs duration of exposure in 15% DMSO.

Fig. XI-7. Comparison of delays (a peak). The delay effect varied markedly with DMSO concentration. The initial slopes for the a peak are .007, .052, .193 ms delay per minute exposure.

(Fig. XI-3) were exposed to artificial sea water instead of DMSO, but both initial recording sessions were held as usual. There was only a small increase in delay. Even after 71-min exposure the electrical response (x) was basically unchanged. Surprisingly, 15-min exposure to 5% DMSO (Fig. XI-4) had no observable effect on the delays, although there was an effect on thresholds.

Exposure to 10% DMSO (Fig. XI-5) gave a cumulative delay far in excess of control values. At first, the β-peak delay increased linearly with exposure; saturation began after 10-min exposure. This also seemed to be the tendency with the γ peak. The joined dots in Fig. XI-5 represent the two horns of a peak that doubled (see Fig. XI-2f). The range of the a peak delay is too small to discern saturation.

Exposure to 15% DMSO (Fig. XI-6) gave a marked linear increase in delay up to 15-min exposure. The pattern of peaks became too distorted for reliable measurements at longer exposures. The effect of DMSO concentration on the a-peak delay is summarized in Fig. XI-7.

4. Conclusion

Treating Homarus ventral nerves with DMSO resulted in slowed propagation, at least for the axon populations that were measured. This effect was initially cumulative and linear in time and increased for increasing concentrations of DMSO. No appreciable effect was seen with 5% DMSO; in 10% DMSO the effect seemed to saturate after 10 min; in 15% DMSO there was no saturation within 15 min. The threshold for excitation usually dropped after exposure to DMSO, although it often rose again by the end of the experiment.

We have found that other cryoprotective agents (glycerol, ethylene glycol) also affect electrical response. This reemphasizes that quantitative measurements like the present ones must precede meaningful cryobiological study of a given preparation.

<div align="right">I. M. Asher</div>

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B. EFFECTS OF REFRACTORINESS ON AUDITORY-NERVE STATISTICS

This report summarizes a study[1] based on investigations of Siebert[2] and Colburn,[3] who first presented hearing models that specify performance by the optimum use of a set of decision statistics on auditory-nerve activity. In their investigations the activities of the individual nerve fibers, which can be described by sample functions from random point processes, are modeled as Poisson processes. In contrast to the exponential distribution of inter-event times in a Poisson process, however, interval histograms of auditory-nerve data show a period of low initial firing probability.[4] This discrepancy is called "refractoriness." In the report summarized here[1] the effects of various kinds of refractory models on firing patterns and on the decision statistics of Siebert and Colburn were studied.

One class of processes showing refractoriness can be described as nonstationary renewal processes[5] with conditional probability (hazard function) of the form $R(t|t') = h(t) G(t, t')$, where t' is the time of the most recent firing, and $h(t)$, the driving function, is dependent only on stimulus and time. The function $G(t, t')$, the refractory function, increases monotonically in t (with t' fixed) from zero when $t \leqslant t'$ to an asymptote of 1 in the order of a few milliseconds. Two different families (models) of $G(t, t')$ are studied.

The first, which was suggested originally by Siebert and Gray[6] and is called here the "rate-dependent model," assumed that $G(t, t') = g\left[\int_{t'}^{t} h(\zeta)\, d\zeta\right]$. This model enables some analytic simplicity, and it is consistent with the observation that the refractory period is often shorter when the fiber receives more stimulation.[6] The major drawback of this rate-dependent model is that it predicts that the unconditional rate of firing $R(t)$ is proportional to $h(t)$. Gray[7] has shown that this proportionality is not always consistent with auditory-nerve data. Gray devised the recovered probability histogram, a PST histogram of firings preceded by an interval with no firings, as an estimate of $h(t)$ in refractory models of the type that we are considering. He compared these histograms with regular PST histograms, which are estimates of $R(t)$. Figures XI-8 and XI-9 show the lack of proportionality between the two types of histograms.

The second model studied here is called the "rate-independent model" because
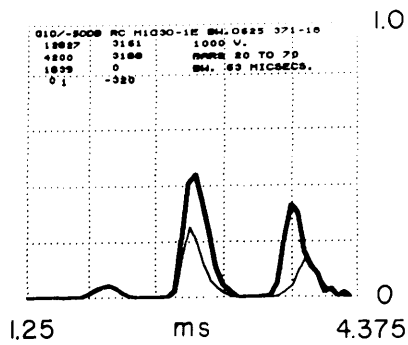
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Fig. XI-8.

Unit 371-18. PST (light line) and recovered probability histogram (heavy line) from activity of nerve fibers of cat. Bin width, 0.0625 ms. Stimulus, 10/s, -50 dB rarefaction clicks (from P. R. Gray[7]).
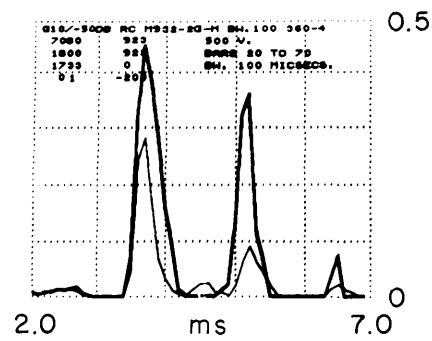
Fig. XI-9.

Unit 360-4. PST (light line) and recovered probability histogram (heavy line) from activity of nerve fibers of cat. Bin width, 0.10 ms. Stimulus 10/s, -50 dB rarefaction clicks (from P. R. Gray[7]).
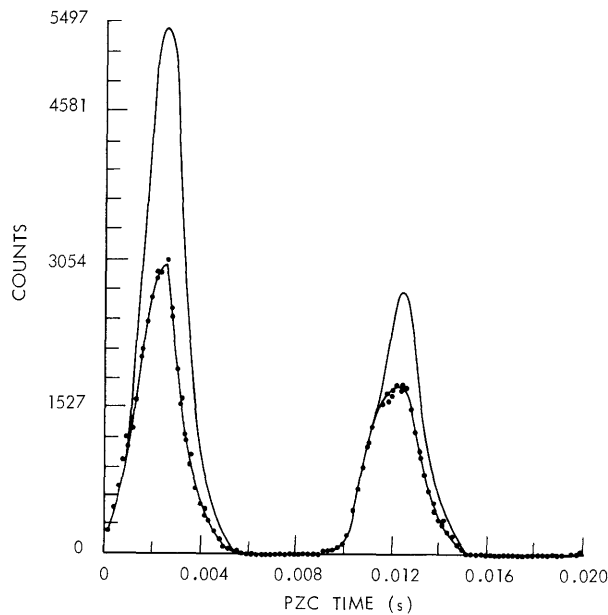

Fig. XI-10. Simulated PZC histograms (points), rough estimate of theoretical curve. Model is rate-independent with deadtime refractoriness. Note the skewness and mode shifting in the histograms compared with the driving functions.

it assumes a refractory function of the form $G(t, t') = f(t-t')$.

A third model, called the "threshold model," was also studied. The threshold model, first formulated as a model for auditory-nerve firings by Weiss,[8] is not a renewal process like the others; it stipulates that a firing occurs when a Gaussian excitation function crosses a threshold function. The rate-independent and threshold models are more difficult to analyze than the rate-dependent model, but simulations (see Figs. XI-10 and XI-11) for appropriate parameters are qualitatively more consistent with Gray's data.

In their studies of auditory decisions, Siebert[2] and Colburn[3] found that the appropriate decision statistics are $J = \sum_m D_m J_m$ for certain monaural discriminations and
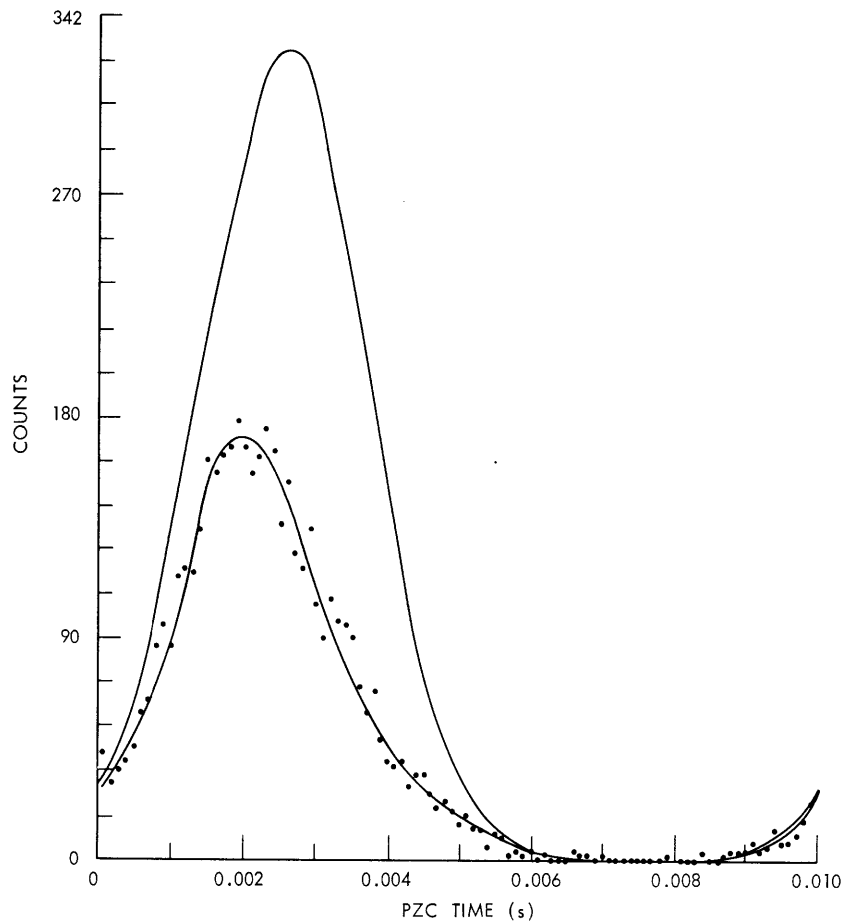

Fig. XI-11. Simulated PZC histogram (points), rough estimate of theoretical curve (lower curve) and expected histogram for the equivalent process without refractory effects (upper curve). Threshold model. Note the skewness and mode shifting in the histogram compared with the upper (nonrefractory) curve.

$L = \sum_m C_m L_m$ for binaural tasks, where J_m is the number of counts (or firings) of the mth fiber, L_m is the number of "coincidences" of the mth fiber pair, and C_m and D_m are weighting constants. L_m is defined by $L_m = \sum_i \sum_j d\left(t_i^{ml} - t_j^{mr}\right)$, where t_i^{mr} and t_j^{ml} are the times of ith and j^{th} firings of the left and right fibers of the mth fiber pair, and

$$d(x) = 1 \qquad |x| \leq \sigma$$

$$ = 0 \qquad \text{otherwise}$$

where σ is a constant of the order of 10^{-3} seconds. Because of the applicability of the Central Limit theorem, J and L are distributed normally, and we can describe the statistics of J and L in terms of the mean and variance of J_m and L_m (under the assumption that J_m and L_m are statistically independent random variables for different m). The major part of the study reported here is a set of expressions obtained analytically or through simulation for mean variance of counts J_m and coincidences L_m for the refractory nerve models described here.

For any point process it can be shown that

$$E(\text{counts}) = \int_{T_i}^{T_f} R(t)\, dt,$$

$$E(\text{coincidences}) = \int_{T_i}^{T_f} R_\ell(t) \int_{t-\sigma}^{t+\sigma} R_r(t+\ell)\, d\ell dt,$$

and

$$\text{Var (counts)} = \int_{T_i}^{T_f} \int_{T_i}^{T_f} R(t_1, t_2)\, dt_2 dt_1 + \int_{T_i}^{T_f} R(t)\, dt - \left[\int_{T_i}^{T_f} R(t)\, dt\right]^2,$$

where (T_i, T_f) is the stimulus interval, ℓ and r denote "left" and "right" ears, and $R(t_1, t_2)$ is the joint unconditional rate of firing. By using the properties of the rate-dependent model and Laplace transform techniques, we can further evaluate these expressions for the rate-dependent model to show that

$$E(\text{counts}) = \frac{\int_{T_i}^{T_f} h(u)\, du}{m_o}$$

$$E(\text{coincidences}) = \frac{1}{m_o^2} \int_{T_i}^{T_f} h_\ell(t) \int_{t-\sigma}^{t+\sigma} h_r(u) \, du dt$$

and

$$\text{Var (counts)} = \frac{\int_{T_i}^{T_f} h(u) \, du}{m_o^3} \left(2m_1 - m_o^2\right) + \frac{1}{m_o^4} \left(2m_1^2 - m_2 m_o\right),$$

where $m_i = \int_0^\infty y^i \exp\left(-\int_0^y g(u) \, du\right) dy$. Even for the rate-dependent model we are unable to evaluate exactly the variance of coincidences. The approximation that we derive, however, is shown by simulations to be accurate for the cases in which we are interested.

For the two other refractory models analytical expressions are difficult to obtain. Consequently, we studied the mean and variance of L_m and J_m through simulations. We concluded that the variances of counts and coincidences can be substantially different from those derived from Poisson processes with the same rate functions $R(t)$. Moreover, for appropriate parameter choices the behavior of the different refractory models is similar.

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