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FREQUENCY-RESPONSE ANALYSIS OF CENTRAL VESTIBULAR UNIT ACTIVITY RESULTING FROM ROTATIONAL STIMULATION OF THE SEMICIRCULAR CANALS

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SUMMARY

1. The neural response of semicircular canal-dependent units in the vestibular nuclei of cats has been examined over a frequency range of sinusoidal rotation extending from 0·004 to 0·9 Hz.

2. Frequency–response analysis indicates that, over the range examined, the information contained in the neural signal received by the brain stem was similar to that expected from the mechanical end-organ.

3. Over the experimental frequency range, the relation between neural response and mechanical stimulus was found to be dominated by a single time constant of about 4 sec, such that two response regions can be defined above and below a stimulus frequency of $\frac{1}{4}$ rad/sec ($\simeq \frac{1}{25}$ Hz).

4. Above this frequency the information content of the neural signal tends towards that of angular velocity and below that frequency it tends towards that of angular acceleration.

5. It is inferred (*a*) that the so-called 'long' time constant of the cat's horizontal canal is about 4 sec and (*b*) that during most normal head movements containing frequencies below about 1 Hz the informational mode of neural signals generated in the canal and received in the brain stem probably tends towards that of head angular velocity.

6. This seems appropriate for the generation of vestibulo-ocular reflex compensation for head movement and for reflex damping (negative velocity feed-back) of unintended head and body movements.

7. The average neural gain of central unit responses is estimated at 1264 action potentials/sec, per degree of cupular deflexion. This high value reflects the very small angles of cupular deflexion assessed on the basis of physical characteristics of the canal.

8. The results permit a rough estimate of the elastic restoring coefficient of the cupula in the horizontal canal as $2{\cdot}05 \times 10^{-3}$ dyne.cm.

INTRODUCTION

In previous accounts, it has been reported that during sinusoidal rotation of decerebrate cats at an essentially single frequency (about 0·3 Hz) the firing frequency of neural units in the vestibular nuclei tended to be closely in phase with the imposed stimulus angular velocity (Melvill Jones & Milsum, 1969, 1970). Since at this frequency, the same characteristic is to be expected in the mechanical response of the end-organ (van Egmond, Groen & Jongkees, 1949; Mayne, 1950; Jones & Milsum, 1965), it was inferred that over the narrow range of stimulus frequencies employed, the canal response is carried with generally good integrity through the primary afferent neural pathway into the brain stem.

The present account describes further experiments in which similar observations were made over an extended range of frequencies. The results are examined in terms of a formal analysis of the system's frequency response (Milsum, 1966), since hopefully this might yield numerical parameters descriptive of the system and/or permit detection of significant non-linearities in the transmission path. Preliminary results were briefly reported earlier (Melvill Jones & Milsum, 1969). The present experiments were complemented by an investigation of the system's responses to transient rotational stimuli, the results of which will be reported separately.

METHODS

The methods for diagnosing, stimulating and recording from semicircular canal-dependent units in the vestibular nuclei of cats have already been described in detail, as has the histological method of identifying cell location (Melvill Jones & Milsum, 1970). Briefly, decerebrate preparations were located in a stereotaxic device with the lateral canals in a horizontal plane and the whole assembly mounted on a horizontal platform suspended from the ceiling by four parallel springs. Long extracellular steel micro-electrodes were stereotaxically advanced through a small occipital trephine and through the intact cerebellum, into the vestibular nuclei, whilst rotating the platform by hand in the horizontal plane in an oscillatory fashion. Units showing correlated response were then examined during manually driven oscillation in each of the remaining five degrees of freedom of movement (two rotational and three linear). Only those cells which responded specifically to horizontal rotation were retained for experimental investigation. After location of such a cell, the platform was carefully lowered on to a very smooth servo-driven turntable and the suspensory springs removed so that the whole assembly could be made to rotate through sinusoidal wave forms determined by the output of a low frequency wave form generator. Records of trains of unit spikes and their averaged frequency profile throughout the stimulus cycle were obtained as before.

In the present experiments, the frequency range of sinusoidal stimulation in the plane of the lateral canals extended over more than two decades from 0·9 Hz to 0·004 Hz (i.e. a range of periodic time extending from 1·1 sec to 256 sec), and the stimulus magnitude of these wave forms ranged from 6 to 150°/sec angular velocity amplitude, according to the sensitivity of the cell under investigation.

With the platform secured to the turntable, the usual procedure was to test with sinusoidal stimuli of 4, 16 and 64 sec period, in that order. For cells satisfactorily retained after these procedures, the periodic time was extended to 128 sec (occasionally 256 sec) and then reduced to around 1 sec. In addition, the middle range was sometimes examined at 8 and 32 sec periods. The stimulus amplitude was usually chosen to provide an easily audible modulation of the cell's action potential (AP) frequency during the sinusoidal stimulus.

The results have been analysed for gain and phase in order to present the collected data in a form which permits numerical assessment of relevant parameters. However, it is not intuitively obvious how to choose these parameters. Nor is it possible to evaluate from the results critical parameters which define the systematic response without first attempting the formulation of a model system in which those parameters are defined mathematically. It is, therefore, necessary to preface the description of results with a section on analytical formulation.

ANALYTICAL FORMULATION

Several authors have derived equations to describe the physical response of the semicircular canal to rotational movement in its own plane. Thus van Egmond *et al.* (1949) arrived at a second order linear differential equation of the form:

$$\dot{\Omega} = \left(\ddot{\phi} + \frac{b}{J}\dot{\phi} + \frac{k}{J}\phi\right).\frac{1}{\alpha}$$

in which

Ω = angular velocity of rotational stimulus,
ϕ = angle of cupular deflexion in the ampulla,
J = moment of inertia of endolymphatic fluid,
b = viscous torque per unit rate of relative volume flow,
k = coefficient of spring stiffness in the cupula itself and
α = ratio of cupular angle to angle of fluid volume displacement round the canal circuit.

Jones & Milsum (1965) utilized the corresponding transfer function

$$\frac{\phi}{\Omega}(s) = \frac{\alpha T_1 T_2 . s}{(T_1 s + 1)(T_2 s + 1)} \qquad (1)$$

in which slow and fast time constants $T_1 (\simeq b/k)$ and $T_2 (\simeq J/b)$ appear in the denominator owing to the heavily overdamped characteristics imposed by the small dimensions of the endolymphatic canal. In consequence, the frequency–response of this transfer function naturally falls into three frequency ranges, termed here low (LF), middle (MF) and high (HF) as in Fig. 1. This Figure gives a Bode plot of gain and phase for the

transfer function in eqn. (1) in which the three ranges are separated by frequencies defined numerically as $1/T_1 = k/b$ and $1/T_2 = b/J$ rad/sec (rad/sec = 2πHz).

Next we may derive the gain and phase of the canal model over the three frequency ranges defined in Fig. 1.

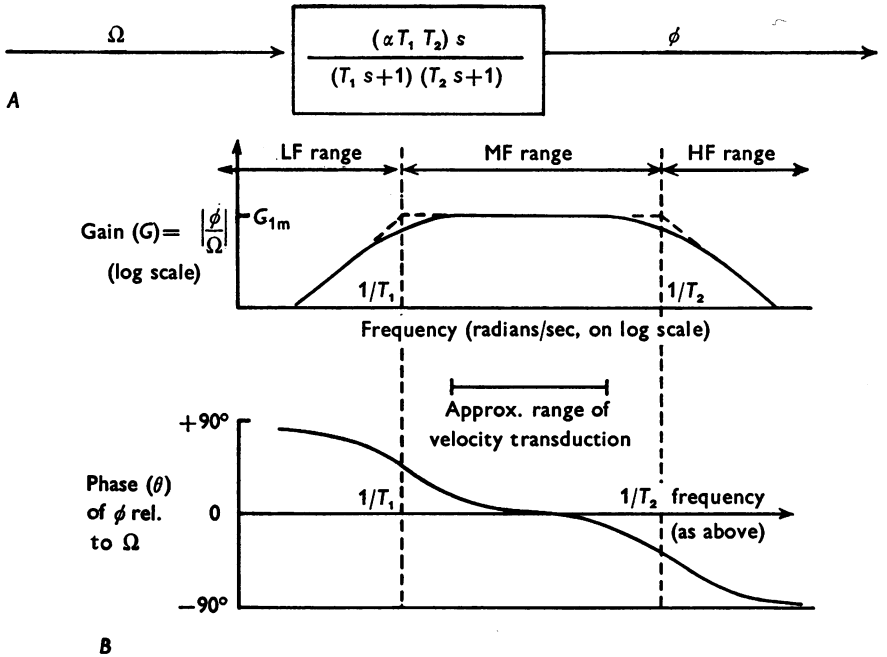

Fig. 1. The semicircular canal model and frequency response. (*A*) Linear model of the canal according to eqn. (1): Ω = stimulus angular velocity; ϕ = cupular response angle. (*B*) Model's frequency response (Bode plot) of cupular angle (ϕ) to sinusoidal angular velocity input (Ω): LF, MF, HF ranges = low, medium, high frequency ranges. G_{1m} = middle frequency gain in the mechanical components of the canal.

Middle frequency gain and phase of the canal

Replacing s by $j\omega$ in eqn. (1), $j = \sqrt{-1}$, and noting that in the middle frequency range $\omega T_1 \gg 1$ and $\omega T_2 \ll 1$, then

$$\frac{\phi}{\Omega}(j\omega) = \alpha T_2 = \alpha(J/b),$$

whence the gain of the canal (G_1) in the middle frequency range (G_{1m}) and the phase, θ_m, of cupular angle relative to rotational stimulus angular velocity are given by

$$G_{1m} = \alpha T_2 = \alpha(J/b)$$

and

$$\theta_m = 0. \qquad (2)$$

Thus G_{1m} proves a particular useful measure since it emerges as the product of the two system parameters α and T_2, the latter being not only numerically equal to the ratio of inertial to viscous terms in the real physical system, but also determining the frequency which separates the MF and HF ranges. It is noteworthy that G_{1m} is independent of cupular stiffness (k) and hence the response in the centre part of the MF range is not affected by cupular elasticity.

Low frequency gain and phase

In the low frequency range both $\omega T_1 \ll 1$ and $\omega T_2 \ll 1$, so that eqn. (1) becomes

$$\frac{\phi}{\Omega}(j\omega) = \alpha T_1 T_2 . (j\omega),$$

or

$$\frac{\phi}{\dot{\Omega}}(j\omega) = \alpha T_1 T_2 = G_{1m} . T_1 = \alpha(J/k).$$

This implies that in the LF range the canal acts as a sluggish angular accelerometer for which the gain (G_{1l}) and phase (θ_1), *expressed in terms of stimulus angular acceleration* ($\dot{\Omega}$), are given by

$$G_{1l} = G_{1m} . T_1$$

and

$$\theta_1 = 0. \tag{3}$$

Thus, G_{1l} also proves a useful measure, since if G_{1m} is known, it provides one of several means of determining the important parameter $T_1 = b/k$, which in turn defines the frequency which separates the MF and LF ranges. Furthermore, if G_{1m} and b are known, G_{1l} provides a means of assessing k, the spring stiffness term, since

$$k = (G_{1m}/G_{1l})b$$

High frequency gain and phase

The HF range is experimentally inaccessible with the present methods, since in the cat T_2 is probably around 0·0015 sec (Fernández & Valentinuzzi, 1968) and hence the lower end of the HF range would be around 650 rad/sec or about 100 Hz. However, for the sake of completeness, it may be noted that in the HF range since $\omega T_1 \gg 1$ and $\omega T_2 \gg 1$, eqn. (1) becomes

$$\frac{\phi}{\Omega}(j\omega) = \frac{\alpha}{j\omega} \quad \text{or} \quad \frac{\phi}{\psi}(j\omega) = \alpha,$$

where

ψ = stimulus angular displacement.

This implies that in the HF range the canal would theoretically act as an angular displacement (Ψ) transducer in which the gain (G_{1h}) and phase (θ_h), *expressed in terms of stimulus angle* (Ψ), are given by

$$G_{1h} = \alpha = G_{1m}/T_2$$

and

$$\theta_h = 0. \tag{4}$$

Thus in the HF range the inertial forces dominate and the fluid tends to remain stationary in space, with the constant, α, representing the geometric relation between fluid and cupular displacement. If such frequencies could be made experimentally accessible, the ratio of G_{1m} and G_{1h} could possibly be used to estimate the fast time constant, T_2.

Neural transmission from canal to brain stem

The above analyses indicate that G_{1m}, G_{1l}, θ_m and θ_l provide potentially accessible measures which could yield important information concerning the basic parameters characterizing the physical response of the canal end-organ. The present experimental approach, however, measures the responses of neural units in the brain stem rather than the cupular angle. It is therefore necessary to examine how the above conclusions may be applied to the measurement of the neural response.

First, it will be assumed that the information content of the neural signal is characterized by the instantaneous frequency (f) of APs. While this assumption may not be valid for all neural information transmission, the experimental evidence presented below allows one to adopt this assumption with some confidence in the present receptor system. Next, it has been shown that many units in the vestibular nuclei are sufficiently close to threshold that during a sinusoidal cycle they may be suppressed below their threshold of firing through part of each cycle (Melvill Jones & Milsum, 1970). Indeed, 26% of all cells examined were spontaneously inactive and hence silent for more than half of each cycle. In addition, it was found that in general the neural signal varied as a power function of stimulus magnitude. When these considerations are taken into account we may write the following relationship;

$$f = \beta(\phi - \phi_{th})^n, \tag{5}$$

where f = cell firing frequency (AP/sec),

β = AP/sec per (cupular angle above threshold)^n,

ϕ = cupular angle,

ϕ_{th} = cupular angle at which cell starts firing.

The gain (G_2) of the neural stage can now be expressed as a function of the cupular angle above threshold

$$G_2 \triangleq \frac{f}{\phi - \phi_{th}}$$
$$= \beta(\phi - \phi_{th})^{n-1}. \quad (6)$$

It would now be helpful to find an expression for the over-all gain of the system $G = f/\Omega$, since this must be what actually emerges from the experimental results. But unfortunately the cupular angle ϕ cannot be eliminated from eqns. (1) and (6) to yield an overall transfer function for $f/\Omega(s)$, because of the non-linearities described above. In practice, however, a value of 0·72 was found for n (Melvill Jones & Milsum, 1970), which is sufficiently close to unity that assumption of a linearized transfer function is not very inaccurate, especially for fairly constant magnitudes of cupular deflexion. Experimentally, this constancy could often be approximated by suitably adjusting the stimulus magnitude as described in Methods. In any case, it is easy to make specific corrections for experimental data in which large variations in cupular angle could be predicted, as has been done in the presentation of results below. Thus, bearing these points in mind, eqn. (5) may be simplified to

$$f = \beta(\phi - \phi_{th}). \quad (7)$$

The next necessary step is to eliminate the threshold non-linearity, and this may be achieved by introducing delta variables as indicated in Fig. 2. Let Δf represent the maximum difference in neural response in AP/sec, and let the corresponding differences in stimulatory angular velocity and in cupular angle be $\Delta\Omega$ and $\Delta\phi$ respectively. Then if maximum and minimum firing frequencies of the response are f_1 and f_2 respectively, and from eqn. (7),

$$\Delta f = f_1 - f_2 = \beta(\phi_1 - \phi_{th}) - \beta(\phi_2 - \phi_{th})$$
$$= \beta(\phi_1 - \phi_2) = \beta.\Delta\phi \quad (8)$$

whence by analogy with eqn. (6)

$$G_2 = \frac{\Delta f}{\Delta \phi} = \beta. \quad (9)$$

It is now possible to utilize directly the concept of over-all gain and phase in the system. Specifically the over-all gain is

$$G = G_1.G_2, \quad (10)$$

where G_1 is defined for the three frequency ranges in eqns. (2), (3) and (4), and G_2 is defined as β in eqns. (8) and (9) for the linear case. Note that G_2

as here defined is independent of frequency, even in the non-linear case, while G_1 is frequency dependent but linear. Thus specifically for the middle frequency range and from eqns. (2) and (10), the over-all gain, G_m is given by

$$G_m = \alpha\beta T_2. \tag{11}$$

The over-all phase θ is given simply by the θ of eqns. (2), (3) and (4), since as modelled the neural stage introduces no change in phase during

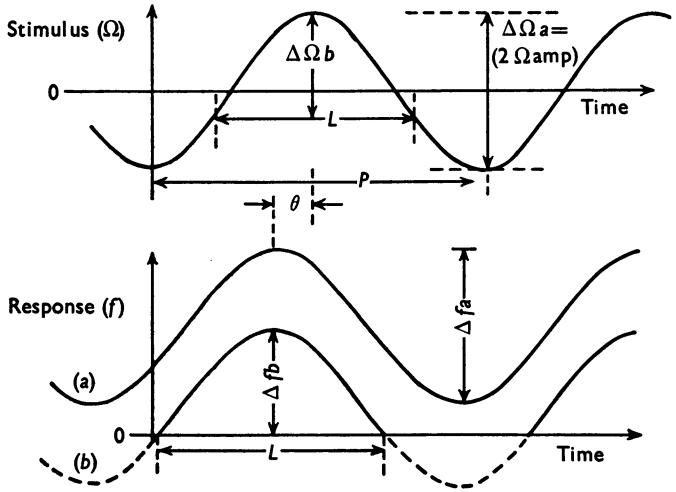

Fig. 2. Idealized stimulus response relations to illustrate variables employed in Analytical Formulation: (*a*) response of a cell firing all round the cycle without threshold cut-off; (*b*) response of a cell firing over only part of the cycle (L) due to threshold cut-off. P = period of all sine waves. f and Δf = response (firing frequency) and max. change of response. Ω and $\Delta\Omega$ = stimulus (angular velocity) and change of stimulus during the part of the cycle (L) which corresponds to the duration of active firing. Ω amp = stimulus amplitude. θ = phase difference between stimulus and response.

sinusoidal stimulation. This latter assumption is undoubtedly a simplification of the real case. Indeed, as previously reported, cells which are cut-off during part of a stimulatory sinusoidal cycle tend to manifest a steeper rising part of their response than the falling part. This dynamic asymmetry, or skewing, of the cut-off response curve effectively constitutes a form of phase-lead (Milsum & Melvill Jones, 1969). However, a mathematical description of this phenomenon was not established by these authors, apart from the simple assessment of the phase effect already mentioned. Hence, rather than adding further terms to described G_2, strictly empirical corrections for phase will be made as appropriate in the presentation of results.

We thus arrive at the conclusion that reasonably valid data relating to the description of end-organ characteristics would be determined by measuring G as a function of $\Delta f/\Delta\Omega$ and $\Delta f/\Delta\dot{\Omega}$ in the MF and LF frequency ranges respectively.

In practice, the actual calculation of MF gain of individual records was performed as previously described (Melvill Jones & Milsum, 1970), using the relation (see Fig. 2),

$$G_{\mathrm{m}} = \frac{\Delta f}{\Delta\Omega} = \frac{\Delta f}{\Omega \mathrm{amp}(1-\cos\pi L/P)}. \tag{12}$$

The corresponding relation for the low-frequency gain, G_{l}, follows directly by comparison of eqns. (2) and (3) as

$$G_{\mathrm{l}} = \frac{\Delta f}{\Delta\dot{\Omega}} = \frac{\Delta f}{\dot{\Omega} \mathrm{amp}(1-\cos\pi L/P)}. \tag{13}$$

An important point emerges here, from the facts that the present neurophysiological approach necessitates elimination of cupular angle from the transfer function determining the over-all gain and that this requires a conversion to 'delta' variables. However, this procedure necessarily restricts the assessment of gain to that part of a cycle over which a cell is actively firing. As pointed out in the previous article (Melvill Jones & Milsum, 1970), this implies that we are measuring the characteristics of the signal *arriving* at the cell in question, rather than the signal fed forward by that cell. Nevertheless, in that article reasons are adduced which suggest that the signal characterized by the parameters measured in the manner discussed above probably does reflect the nature of the departing, as well as the arriving signal when the ensemble of neurones is considered as a whole; stated otherwise, one effect of an ensemble of variable threshold neurones is to linearize their over-all response.

The above analytical study suggests that a considerable body of quantitative information could be derived from a relatively simple experimental procedure in which the responses of specifically canal-dependent cells in the brain stem are recorded simultaneously with rotational stimulus angular velocity during sinusoidal oscillations over an extended frequency range. In summary

1. The Bode plot should permit assessment of whether the whole system is operating approximately in the manner suggested by eqn. (1), at least over the low and middle frequency ranges.

2. If so, then the actual form of the plots should permit an assessment of the value of the slow time constant, T_1, and hence the value (b/k).

3. The value of G_l/G_m provides an alternative estimate of $T_1 = b/k$ from those cells successfully examined at very low frequencies.

4. If $G_m = \alpha\beta.T_2$, and if $T_2(= J/b)$ and α can be calculated from a knowledge of fluid density and viscosity and the physical dimensions of the canal, then it should be possible to estimate β, the neural gain.

5. With $G_l = G_m.b/k$, and since the damping term b should be calculable on the basis of known physical parameters, the experimentally determined ratio G_l/G_m should permit an estimate of the value of k.

RESULTS

Figs. 3 and 4 show averaged records as they emerged from on-line computation during an actual experimental run. The second of the two cycles in each Figure is only a repetition of the first, and is shown for convenience of visual interpretation. In Fig. 3, the successive traces from the top give averaged stimulus expressed as *angular velocity*, averaged AP frequency throughout the cycle and zero AP frequency. Thus the AP frequency of the cell recorded in Fig. 3 was sinusoidally modulated above and below the cell's spontaneous firing frequency (approximately 35 AP/sec) as a result of the sinusoidally modulated rotational stimulus to the horizontal canals. The stimulation frequency in this instance was 1/16 Hz, which turns out to be close to that separating the middle and low frequency regimes as defined in the section on Analytical Formulation and in Fig. 1. The response in Fig. 3 is about 40° phase advanced relative to the stimulus angular velocity, and it should be noted that this phase value is close to the 45° phase advancement which would be predicted at the 'break' or separation frequency on theoretical grounds. The middle frequency gain G_m expressed as change in AP frequency/change in angular velocity ($\Delta f/\Delta\Omega$) can be directly calculated from the record in Fig. 3 without the use of eqn. (12), since it clearly is the ratio of amplitude of response (14 AP/sec) to amplitude of stimulus (32°/sec) which thus equals 0·44 AP/sec per degree/sec.

Fig. 4 shows the averaged response of another cell (lower trace) which had a spontaneous firing frequency (7 AP/sec) sufficiently low that its active response was cut-off during part of the inhibitory half of the stimulus cycle. In this Figure, the top trace showing stimulus velocity has been divided into two halves for technical reasons but in reality represents a continuous sine wave. The peak of the neural response is about 65° phase advanced relative to the imposed angular velocity, which is consistent with the stimulus frequency (0·016 Hz) lying well within the low frequency regime defined in Fig. 1. The calculation of G_m is not quite so simple as in Fig. 3, but is readily performed according to eqn (12).

Thus, from this record, the middle frequency gain G_m, was 0·50 AP/sec per degree/sec.

Fig. 5 shows a series of tracings from records such as that in Fig. 4. The tracings in this Figure were all obtained from a single cell whilst the animal was exposed to sinusoidal rotational stimuli of progressively increasing periodic time. The top trace represents the wave form of the stimulus

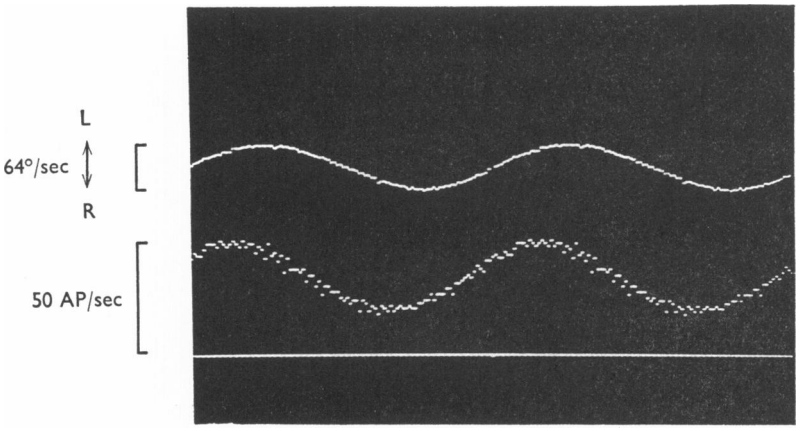

Fig. 3. Average stimulus (angular velocity, upper trace) and response (AP frequency, middle trace) from a canal-dependent unit in the left medial vestibular nucleus which fired all round the cycle. Periodic time, 16 sec; average of 14 cycles. Response phase advanced by approx. 40° relative to stimulus. The straight line gives zero AP frequency. The averaged data for the cycle are written out twice to aid visual interpretation. Symmetrical response is characteristic of cells firing all round the cycle.

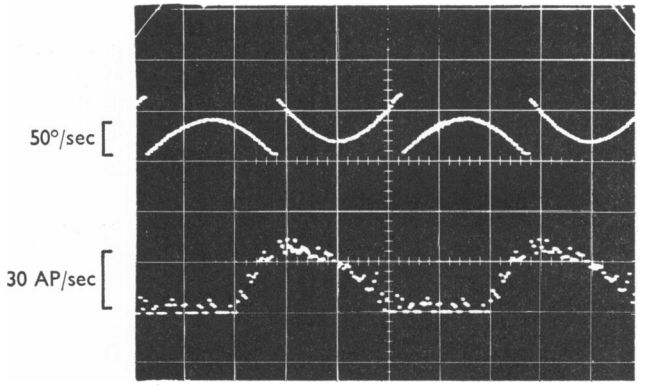

Fig. 4. Average stimulus–response relation from a cell in the right medial vestibular nucleus showing threshold cut-off during part of the cycle. Period, 64 sec; average of 2 cycles. Response phase advanced by approx. 65°; it shows the asymmetry which is characteristic of cut-off patterns of response.

angular velocity for all records, although of course the time scale of each record must be adjusted according to its stimulus period. The vertical dashed line gives the phase of maximum leftward angular velocity, and it can be seen that the phase of response became progressively more advanced relative to this line as the period of oscillation increased, until the response was nearly 90° phase advanced at the longest period employed.

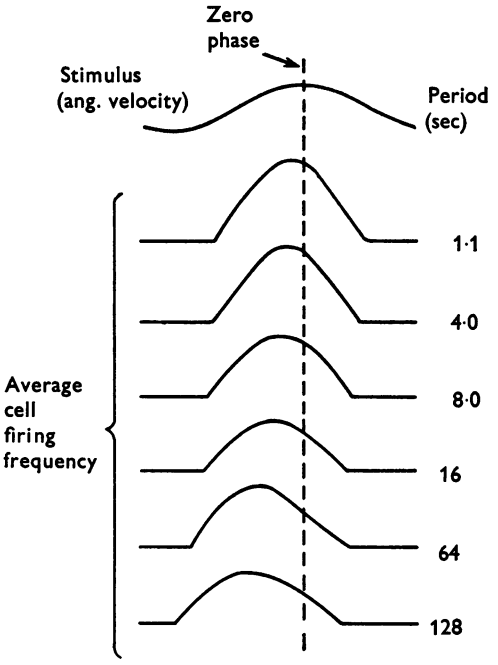

Fig. 5. Tracings of averaged stimulus–response relations obtained from one cell on exposure to sinusoidal rotational stimuli over a wide range of frequencies. The top trace represents the sinusoidal stimulus. The progressive phase advancement and gain attenuation with decreasing frequency of stimulus are given numerically in Table 1.

TABLE 1. Changes of phase and gain (G_m = AP/sec per degree/sec), see eqns. (1) and (12), with stimulus frequency, obtained from the original records of Fig. 5. Phase gives advancement of peak response relative to peak stimulus

Period (sec)	Gain (G_m)	Phase (degrees)
1·1	0·91	14
4·0	0·67	23
8·0	0·58	33
16	0·49	41
64	0·18	71
128	0·12	85

The middle frequency gain, G_m, cannot be assessed directly from this figure, since the stimulus amplitude was progressively increased as the frequency decreased, in order to offset the reduction in G_m to be expected from the end-organ's dynamic response. However, the measured values of phase and gain obtained from this cell are given in Table 1 in which both the progressive phase advancement and gain attenuation, with decreasing stimulus frequency, are clearly evident. It should be noted that while the frequencies extend over both MF and LF ranges, nevertheless, it is appropriate to compute the gain first in terms of G_m, in order to plot the data as a continuous Bode diagram (Figs. 6 and 7). Later, it will be appropriate to compute G_1 at a very low frequency in order to estimate T_1, by means of eqn. (3).

Bode diagram

The prolonged and somewhat violent movement stimuli required in these experiments made it difficult to retain identifiable single units sufficiently long to cover the low-middle frequency range needed for the present frequency response analysis testing. Indeed, a typical sequence with a single cell occupied from 2 to 5 hr during most of which time the recording platform was being rotated more or less vigorously. Nevertheless, from experiments on twenty-four cats, twelve single units were each successfully examined over ranges extending between one and two decades. The results from these units are presented graphically in the Bode diagram of Fig. 6, in which the upper and lower parts give the logarithmic gain and the phase data respectively. The gain values have been normalized with respect to their average value, G_f, in the narrow frequency range 0·25–1·0 Hz (1·6–6·3 rad/sec) which is presumed to lie within the middle frequency domain of the end-organ hydrodynamics (Melvill Jones & Milsum, 1970). On occasions, especially in the region of normalization, points tend to become superimposed and such points have been slightly separated in order to retain their identities. The curved dashed lines show the theoretical Bode plots of the middle and low frequency regions defined by $T_1 = 2{\cdot}0$ and $6{\cdot}0$ sec in eqn. (1), these values being chosen to outline the approximate range of the observed data. The straight dashed lines show the corresponding asymptotic curves (see Fig. 1), with the special feature that each intersection of horizontal and sloped asymptotes occurs at a frequency defined by

$$\omega = 1/T_1 = 2\pi F,$$

where

ω, F = angular frequency of stimulus in rad/sec and Hz respectively.

T_1 = the corresponding time constant, in eqn. (1).

Evidently, there was a tendency for the data to fall within a general pattern which accords with the theoretical response of the linear model proposed in Fig. 1. The validity of this conclusion is strengthened by the fact that in a given set of response data, gain and phase must be fitted simultaneously since they are not independent measures of the system. Thus, the over-all pattern seen in Fig. 6 comprises changes in both gain and phase which are generally appropriate to eqn. (1).

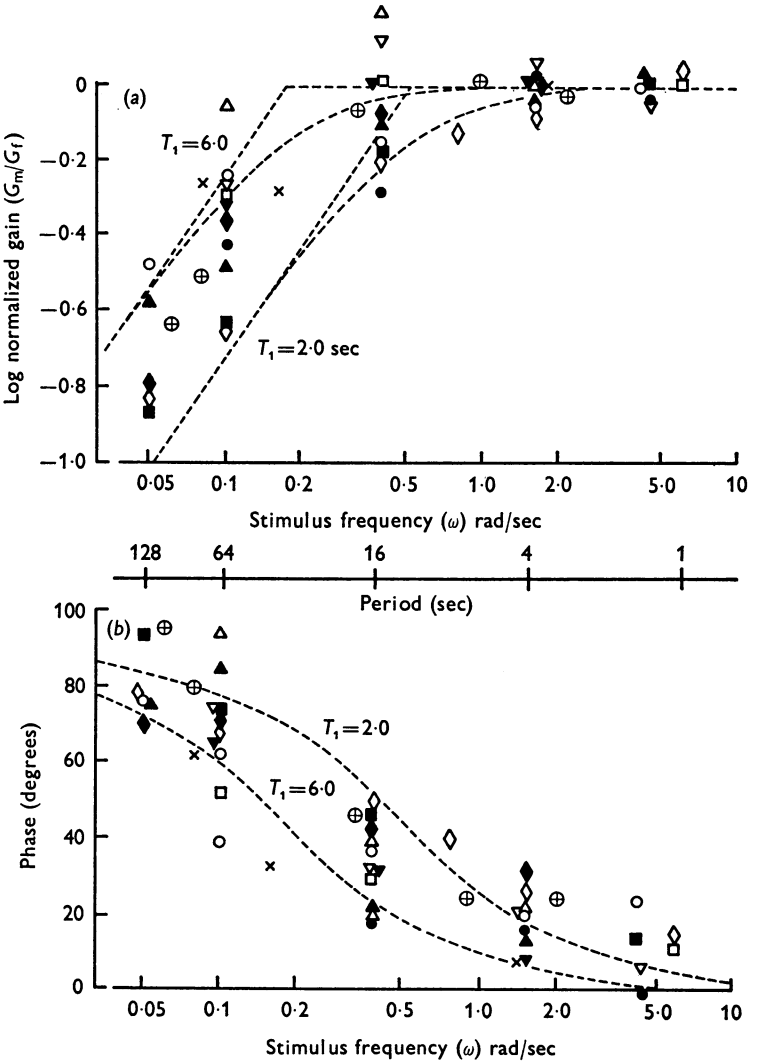

Fig. 6. Bode plot of gain and phase for the collective data from twelve single units before correction for non-linearities. G_f = average value of G_m determined in the frequency range 0·25–1·0 Hz.

In the analytical formulation, it was pointed out that there appears to be a power relation between gain and stimulus amplitudes, and it is therefore desirable to make specific corrections for the gain data according to the procedure described in an earlier paper (Melvill Jones & Milsum, 1970). Moreover, it has also been pointed out (Milsum & Melvill Jones, 1969) that there is a statistically reliable tendency for cells cut-off during part of the stimulus cycle by their threshold of firing, to exhibit an asymmetric response which *of itself* tends to phase advance the peak response.

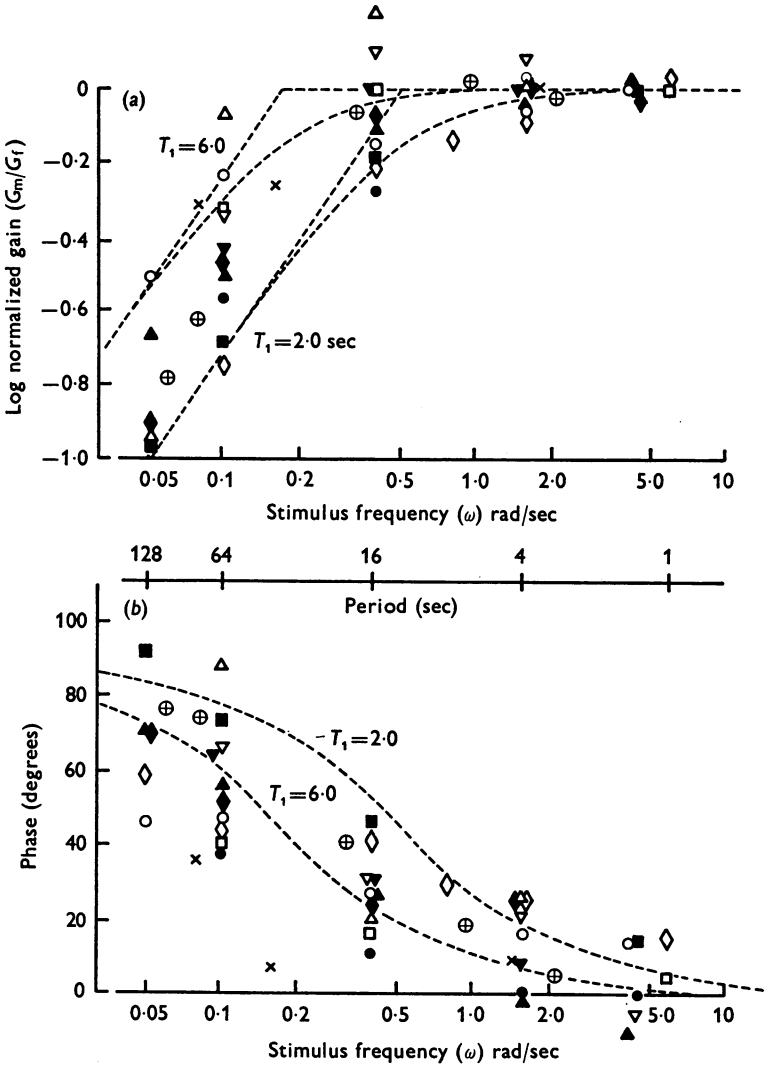

Fig. 7. Bode plot of gain and phase for the same data as in Fig. 6, after correction for non-linearities described in the text.

Fig. 7 shows the data of Fig. 6 after correction for these two effects. In both figures the results from each separate unit are identified by a separate symbol.

At the low end of the frequency range, the corrected gain data fit between the lines describing responses with time constants of 2 and 6 sec, rather more closely than in Fig. 6. The corrected phase data lie closer to the drawn curves at the high end of the frequency range, but have dropped somewhat below them at the low end.

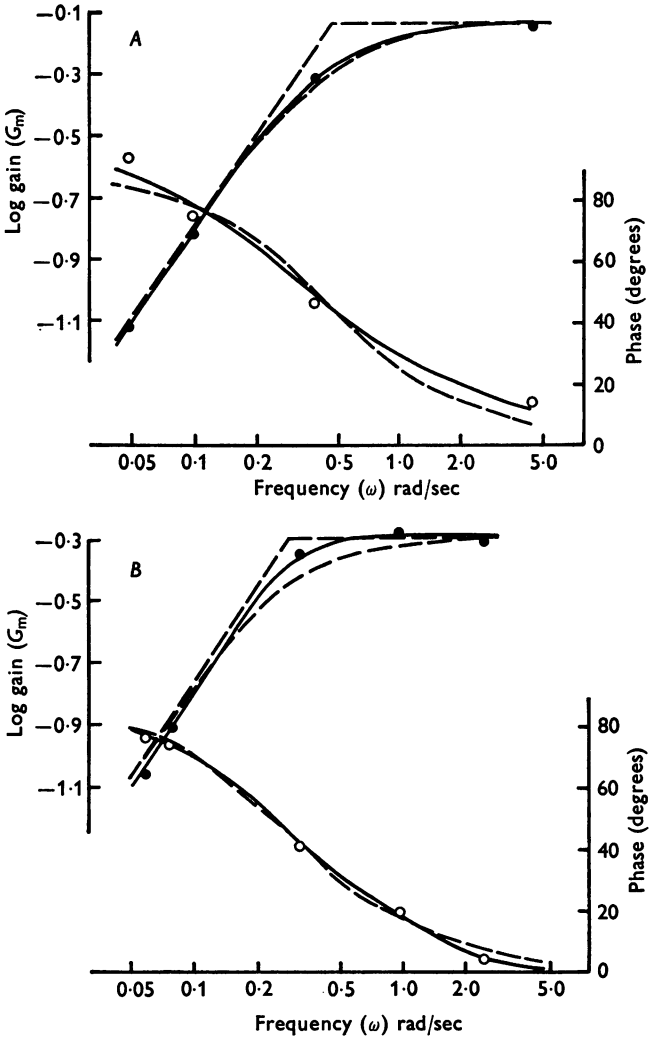

Fig. 8. Bode plots of results from two cells in two different cats. All data have been corrected for non-linearities as in Fig. 7. Time constants (*A*) T_1 = 2·3 sec. (*B*) T_1 = 3·5 sec.

Although the data in Figs. 6 and 7 follow the general pattern outlined by $2 < T_1 < 6$ sec in eqn. (1), a problem arises as to whether the data from different cells should be lumped together in order to obtain a better fit, since the value of T_1 may not necessarily be the same for all animals. Thus Fig. 8*a* and *b* show the individual plots of results, corrected in the same way as those in Fig. 7, from single cells in two different cats. Here the continuous lines show the best visual fitting obtainable for a single value each of $T_1 = 2{\cdot}3$ and $T_1 = 3{\cdot}5$ sec for *a* and *b* respectively. In each part of the Figure, the simultaneous close fits of gain and phase data strongly suggest on the one hand that over the frequency range tested close conformity obtained with the mathematical model and on the other hand that statistically valid differences may obtain between individual values of the definitive parameter, T_1.

Comparison of middle and low frequency gains

In eqn. (3) of the analytical formulation, it was shown that an alternative estimate of T_1 is available from the ratio G_l/G_m. The characteristics of the experimental data suggest that G_l may be satisfactorily estimated from responses at periodic times exceeding about 100 sec. Table 2 gives

TABLE 2. Estimation of the time constant T_1 from the ratio G_l/G_m as in eqn. (3). The neural units are identified with those in Figs. 6 and 7 by their symbols. G_m and G_l are expressed respectively in terms of AP/sec per degree/sec and AP/sec per degree/sec^2. The last value in column 7 is the mean of the values in that column and may be compared with mean G_l/mean G_m = 3·8 sec. Original values of low frequency gain G_m have been corrected for the power relation between mechanical and neural response as in eqn. (6) and subsequent text

Cell symbol	Mean G_m between 1·0 and 0·25 Hz	G_m at frequency < 0·01 Hz		Period (sec)	G_l = column $4 \times \frac{\text{period}}{2\pi}$	T_1 (sec) = $\frac{\text{column 6}}{\text{column 2}}$
		Original	Corrected			
⊕	0·52	0·12	0·09	110	1·58	3·0
◆	1·00	0·16	0·13	128	2·65	2·7
■	0·75	0·10	0·08	128	1·63	2·2
▲	1·44	0·38	0·32	128	6·53	4·5
○	0·70	0·23	0·22	128	4·49	6·4
◇	0·78	0·12	0·10	128	2·04	2·6
Means	0·87	0·19	0·16	—	3·31	3·6

the relevant values obtained from those cells for which response data were obtained at a stimulus frequency less than 0·01 Hz. The original values of G_m at frequencies less than 0·01 Hz have been corrected for the power relation between stimulus and response as described above.

The values of T_1 obtained in this way are rather variable, but essentially

they lie in the bracket of $2 < T_1 < 6$ sec suggested by the frequency response plots of Figs. 6 and 7, with a mean value of 3·6 sec and a range from 2·2 to 6·4 sec. This value may be compared with the value of mean G_1/mean G_m = 3·8 sec. The mean value of G_m in the MF range for the units in Table 2 was 0·87 AP/sec per degree/sec. This value is fairly close to the corresponding value of 0·76, S.E. ± 0·08 reported for thirty-nine units (Melvill Jones & Milsum, 1970) which suggests that the present group of six units in Table 2 was a reasonably representative one.

Neural gain

It was suggested in the section on Analytical Formulation that the neural gain, β, might be assessed by quantifying the value of change in AP frequency per unit change in cupular angle. As pointed out, the values of both α and T_2 ($\simeq J/b$) must first be assessed, where these define respectively the ratio of angle of cupular deflexion per unit angle of fluid displacement round the endolymphatic canal and the upper cut-off frequency of Fig. 1. Fortunately, both these terms should be available from anatomical and physical data alone.

Thus, as pointed out by Jones & Spells (1963), an approximate value of α can be calculated from the expression $\alpha = \pi^2 r^2 R/V$, where V is the ampulla volume, r the internal radius of the endolymphatic canal and R the radius of curvature of the canal. These authors found a mean value for r^2R/V of 0·0509 (S.D. 0·0264) determined from measurements on forty-four different species and hence their data indicate a mean value of $\alpha = \pi^2 \times 0{\cdot}0509 = 0{\cdot}50$. Fernández & Valentinuzzi (1968) measured relevant dimensions specifically in the domestic cat and give values of $V = 546\ \text{cm}^3 \times 10^{-6}$, $R = 1{\cdot}7$ mm and $r = 0{\cdot}115$ mm. From these data a corresponding value of $\alpha = 0{\cdot}41$ emerges. Moreover, the latter authors arrive at a specific value for J/b ($\simeq T_2$) = 1/657 sec in the cat. Thus, adopting the values of α and T_2 from the data of Fernández & Valentinuzzi, the value of β can be assessed from eqn. (11) as

$$\beta = G_m/\alpha T_2$$

$$= G_m \times 1600 \text{ AP/sec per unit change of cupular angle.}$$

Now since the mean value of G_m obtained from thirty-nine units in the MF range was 0·79 AP/sec per degree/sec, the calculated value of β becomes

$$\beta = 0{\cdot}79 \times 1600$$

$$= 1264 \text{ AP/sec per degree of cupular deflexion.}$$

Implications of this surprisingly large value for what is here termed the neural gain are discussed below.

Estimation of spring constant, k

Inserting the mean values of G_m and G_1 obtained from Table 2, eqn (3) becomes

$$G_1/G_m = 3{\cdot}31/0{\cdot}87 = T_1.$$

But $$T_1 = b/k$$

and hence $$k = 0{\cdot}26b.$$

But Fernández & Valentinuzzi (1968) calculated a value for b of $7{\cdot}89 \times 10^{-3}$ dyne.cm.sec and hence a numerical value for k may be assessed as

$$k = 2{\cdot}05 \times 10^{-3} \text{ dyne.cm.}$$

DISCUSSION

In an investigation of semicircular canal dimensions as a function of animal size, Jones & Spells (1963) found consistent, but minute, changes in critical measurements from one animal species to another. These changes were shown, by dimensional analysis, to adjust the hydrodynamic response of the end-organ to match the likely changes in speed of head movement to be expected from changes in animal weight. More specifically it has since been shown that the changes are just such as to shift the middle frequency range (i.e. range of velocity transduction) of the canal response (Fig. 1) in proportion to the likely change in frequency content of head movement with animal size (Mayne, 1965; Melvill Jones, 1971*a*). With the physical characteristics of the end-organ apparently so nicely matched to likely patterns of head movement, it seemed important to enquire how faithfully its *mechanical* response is transmitted by the afferent *neural* chain to the brain stem, and the main purpose of the present investigation was to attempt an answer to this question.

Various authors have examined unit neural responses to rotational canal stimulation at the level of the primary afferent neurones (Ross, 1936; Lowenstein & Sand, 1940; Groen, Lowenstein & Vendrik, 1952; Rupert, Moushegian & Galambos, 1962; Sala, 1965; Klinke, 1970; Goldberg & Fernández, 1971; Fernández & Goldberg, 1971) and in the brain stem (Adrian, 1943; Gernandt, 1949; Duensing & Schaeffer, 1958; Shimazu & Precht, 1966; Wilson, Wylie & Marco, 1968; Wilson, Kato, Peterson & Wylie, 1965; Ryu, McCabe & Funasaka, 1969; Melvill Jones & Milsum, 1970).

However, the most meaningful way to approach the present question is by means of a formal frequency–response analysis made over a wide

range of rotational stimulus frequencies, since it is in these terms that the above deductions were made.

Presented in this way as Bode plots of frequency–response, we find that the results in Figs. 6, 7 and 8 fall into a general pattern of gain and phase changes with frequency, which are similar to those to be expected from the hydrodynamic characteristics of the end-organ (Steinhausen, 1933; van Egmond *et al.* 1949; Mayne, 1950 and 1965; Groen *et al.* 1952; Jones & Milsum, 1965). Thus in the MF range of frequencies, i.e. on the right-hand side of the figures, the normalized gain G_m, defined as change in AP frequency per unit change in rotational angular velocity, approximates a flat characteristic and the phase of the neural response approaches that of the stimulus angular velocity. However, marked but systematic changes occur in both these parameters as the frequency of stimulation is reduced, i.e. towards the left of the Figures. Bearing in mind that the ordinate of the gain curve is logarithmic, it can be seen that very large changes in G_m occurred over the frequency range 0·5–0·05 rad/sec (about 0·08–0·008 Hz).

Eventually, the rate of change of gain with frequency seems to settle at around a tenfold reduction of gain per decade of frequency change. Over the same lower frequency range the neural response became nearly 90° phase advanced relative to the stimulus angular velocity. Both these changes are to be expected from the same simple physical system which would result from interactions between mechanical forces due to inertia and viscosity of the endolymph in the canal, and elasticity of the cupula, as modelled in eqn. (1). It seems reasonable to infer therefore that over the frequency range tested, the neural signal generated in the brain stem by adequate stimulation of the canal represents a fairly faithful replica of the mechanical end-organ response over the whole range of frequencies tested. The very close fits of both gain and phase data for two individual cells successfully examined over the whole range and seen in Fig. 8*a* and *b* add weight to this view.

Of course, no inference can be drawn from these experiments about the neural response to rotational stimuli in the range of frequencies above about 1 Hz. It may be noted, however, that if the response were to conform with eqn. (1) and Fig. 1, then the HF regime would formally begin at a frequency defined by $1/T_2 \simeq b/J$ radians per second. As already noted, this value has been calculated from known physical characteristics of the cat canal as 657 rad/sec, or $657/2\pi \simeq 100$ Hz (Fernández & Valentinuzzi, 1968) and this asymptotic break frequency (see Fig. 1) is well above the range of accurate velocity transduction depicted in the Figure. But even frequencies considerably below this value would seem unlikely to occur in natural movement, since as has been pointed out previously (Melvill Jones

& Milsum, 1970), Walsh (1966) has shown that jolting movements applied to the body tend to be substantially filtered by body and neck dynamics. Moreover, at frequencies on the order of 100 Hz and above, the pulse code modulation method employed by these brain-stem neurones could hardly transmit the information efficiently (Lee & Milsum, 1971) since firing frequencies above about 80/sec have seldom been observed in them. Thus it seems unlikely that the mechanical factors implicit in T_2 of eqn. (1) should in practice contribute significantly to divergence from the MF range of response during natural head movement.

These considerations do not, of course, eliminate the possibility of other factors coming into play. Indeed, Goldberg & Fernández (1971) and Fernández & Goldberg (1971) have demonstrated the introduction of a 'lead' term into the primary afferent neural response of the monkey to rotational oscillations between about 1 and 8 Hz. Furthermore, the increased gain associated with this phenomenon may well be responsible for the increase of gain in the vestibulo-ocular reflex of man observed by Benson (1970) in the range 1–6 Hz of passively induced head oscillations.

In their study of primary afferent neurone responses in the monkey, Fernández and Golberg noted significant differences in the response characteristics of regularly and irregularly firing nerve fibres. Whereas the regular ones generally appeared to follow the expected mechanical end-organ response of eqn. (2), the irregular ones tended to show both an adapting characteristic at the low frequency end of the spectrum and the above mentioned lead term at the high end. Again, Malcolm & Melvill Jones (1970) have demonstrated an adaptive term which would become effective at very low frequencies in the vestibulo-ocular reflex of man. Although the degree of regularity in the present records of action potentials varied somewhat, the differences were not sufficiently defined to attempt meaningful separation. It may well be therefore that, particularly at the low frequency end, our results have been to some extent influenced by an adaptive term. However, such influence does not appear to have been great in view of the generally monotonic pattern of data points in Figs. 6, 7 and 8.

A rather surprising outcome of the results was the very high value of neural gain β, defined in eqns. (7) and (9) as the AP/sec per cupular angle above that associated with a given cell's threshold. The value of 1264 AP/sec per degree of cupular deflexion depends of course upon theoretical reasoning as well as experimental findings. In particular, it reflects the very small angles of cupular deflexion per unit angular velocity of rotational stimulus calculated from the dimensionsal data of Fernández

& Valentinuzzi (1968). Thus with the value of $J/b = 1/657$ sec and $\alpha = 0{\cdot}41$ for the cat horizontal canal, eqn. (2) gives

$$G_{1m} = \alpha\,(J/b)$$
$$= \frac{0{\cdot}41}{657} = 0{\cdot}62 \times 10^{-3}$$

angle of cupular deflexion per unit angular velocity of rotation.

This would imply very small angular deflexions of the cupula during head movements. For example, in one sensitive cell the threshold angular velocity amplitude for audible detection of firing frequency modulation at a sinusoidal frequency around 1 Hz was about 2°/sec and this would imply a corresponding angular amplitude of cupular movement around $1{\cdot}2 \times 10^{-3}$ degrees. In view of strong histological similarities with the cochlear sensory mechanism (Wersäll & Lundqvist, 1966), it is of interest to compare this small value with corresponding sensory hair deflexions in the organ of Corti. From the data of Johnstone & Johnstone (1966), we may calculate a maximum angle of hair deflexion at 74 db SPL and 1000 Hz of approximately 10^{-3} degrees which is on the same order of magnitude as the value arrived at above. Nevertheless, if this assessment is realistic, one cannot help questioning the functional significance of the apparently large available angle of cupular movement in the ampulla (Dohlman, 1935).

Reviewing the results as a whole in the context of the introduction to this discussion, it is inferred that the neural signal transmitted to cell bodies in the cat brain stem as a result of rotational canal stimulation probably approximates fairly closely the mechanical response of the end-organ over the frequency range of this experiment. Broadly speaking, the whole system appears to operate over this range as though it were dominated by one time constant of around 4 sec. This implies that above about $\frac{1}{4}$ rad/sec $\simeq \frac{1}{25}$ Hz, the system generates a message tied to angular velocity of the head whilst below this frequency it becomes tied to angular acceleration. Of course, in reality the 'asymptotic' transition from one regime to the other must be replaced by curved lines such as the dashed ones in the upper diagrams of Figs. 6 and 7. In view of this low value of the lower transition due to T_1 and the high value of the upper transition due to T_2 in eqn. (1) and Fig. 1, one may guess that the normal signal content in the message received by the brain will approximate that of angular velocity.

It is interesting to speculate on the physiological significance of this velocity feed-back signal derived from head rotation. First, the vestibulo-ocular reflex responsible for automatic stabilization of the retinal image during head movement, requires an eye angular velocity relative to the

skull which is always equal but opposite to the instantaneous head velocity. A velocity modulated input would seem appropriate for driving such a system (Melvill Jones, 1971*b*). Secondly, such feed-back information would be phase advanced relative to the head displacement incurred and, as indicated by Jones & Milsum (1965) and elaborated by Outerbridge (1969), this signal would therefore be appropriate for the generation of effective damping of unintended head movements by use of the neck muscles. The plausibility of such a mechanism is enhanced by the recent observation that neck muscle motoneurones receive an extensive monosynaptic innervation, both excitatory and inhibitory, from the vestibular nuclei (Wilson & Yoshida, 1969*a*, *b*). Presumably, this source of damping or negative velocity feed-back could also act through vestibulo-spinal pathways (Wilson & Yoshida, 1969*a*; Lund & Pompeiano, 1968; Erulka, Sprague, Whitsel, Dogan & Janetta, 1966; Nyberg-Hansen, 1964) on postural muscles to aid body stabilization.

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REFERENCES

ADRIAN, E. D. (1943). Discharges from vestibular receptors in the cat. *J. Physiol.* **101**, 389–407.

BENSON, A. J. (1970). Interactions between semicircular canals and gravireceptors. In *Proceedings XVIII International Congress of Aviation and Space Medicine*, pp. 249–261, ed. BUSHBY, D. E. Dordrecht-Holland: Reidel Publishing Co.

DOHLMAN, G. (1935). Some practical and theoretical points in labyrinthology. *Proc. R. Soc. Med.* **28**, 1371–1380.

DUENSING, F. & SCHAEFER, K. P. (1958). Die Aktivität einzelner Neuron im Bereich der Vestibulariskerne bei Horizontalbeschleunigungen unter besonderer Berürksichtigung de vestibulären Nystagmus. *Arch. Psychiat. Nervenkrankh.* **198**, 225–252.

ERULKA, S. D., SPRAGUE, J. M., WHITSEL, B. L., DOGAN, S. & JANETTA, P. J. (1966). Organization of the vestibular projection to the spinal cord of the cat. *J. Neurophysiol.* **29**, 626–664.

FERNÁNDEZ, C. & GOLDBERG, J. M. (1971). Physiology of peripheral neurons innervating the semicircular canals of the squirrel monkey. II. The response to sinusoidal stimulation and the dynamics of the peripheral vestibular system. *J. Neurophysiol.* **34**, 661–675.

FERNÁNDEZ, C. & VALENTINUZZI, M. (1968). A study on the biophysical characteristics of the cat labyrinth. *Acta oto-lar.* **65**, 293–310.

GERNANDT, B. E. (1949). Response of mammalian vestibular neurones to horizontal rotation and caloric stimulation. *J. Neurophysiol.* **12**, 173–184.

GOLDBERG, J. M. & FERNÁNDEZ, C. (1971). Physiology of peripheral neurons innervating the semicircular canals of the squirrel monkey. I. The resting discharge and the response to constant angular accelerations. *J. Neurophysiol.* **34**, 635–663.

GROEN, J. J., LOWENSTEIN, O. & VENDRIK, A. J. H. (1952). The mechanical analysis of the responses from the end-organs of the horizontal semicircular canal in the isolated elasmobranch labyrinth. *J. Physiol.* **117**, 54–62.

JOHNSTONE, J. R. & JOHNSTONE, B. M. (1966). Origin of summatory potential. *J. acoust. Soc. Am.* **40**, 1405–1419

JONES, G. M. & MILSUM, J. H. (1965). Spatial and dynamic aspects of visual fixation. *IEEE Trans. bio-med. Engng* **BME-12**, 54–62.

JONES, G. M. & SPELLS, K. E. (1963). A theoretical and comparative study of the functional dependence of the semicircular canal upon its physical dimensions. *Proc. R. Soc.* B **157**, 403–419.

KLINKE, R. (1970). Efferent influence on the vestibular organ during active movements of the body. *Pflügers Arch. ges. Physiol.* **318**, 325–332.

LEE, H. C. & MILSUM, J. H. (1971). Statistical analysis of multi-unit multipath neural communication. *Math. Biosci.* **11**, 181–202.

LOWENSTEIN, O. & SAND, A. (1940). The mechanism of the semicircular canal. A study of responses of single fibre preparations to angular accelerations and to rotation at constant speed. *Proc. R. Soc.* B **129**, 256–275.

LUND, S. & POMPEIANO, O. (1968). Monosynaptic excitation of alpha motoneurones from supraspinal structures in the cat. *Acta physiol. scand.* **73**, 1–21.

MALCOLM, R. & MELVILL JONES, G. (1970). A quantitative study of vestibular adaptation in humans. *Acta oto-lar.* **70**, 126–135.

MAYNE, R. (1950). The dynamic characteristics of the semicircular canals. *J. comp. physiol. Psychol* **43**, 304–319.

MAYNE, R. (1965). The functional parameters of the semicircular canals. Tech. Report GERA-1056, Goodyear Aerospace Corp., Arizona. *NASA tech. Rep.* no. NAS 9-4460.

MELVILL JONES, G. (1971*a*). Functional significance of size in the semicircular canals. In *Handbook of Sensory Physiology*, VI, pp. 22. Berlin: Springer-Verlag. (in the Press).

MELVILL JONES, G. (1971*b*). On the organization of neural control of the vestibulo-ocular reflex arc. In *The Control of Eye Movements*, ed. BACH-Y-RITA, P. & COLLINS, C. C. New York: Academic Press.

MELVILL JONES, G. & MILSUM, J. H. (1969). Fidelity of information transfer in a vestibular afferent pathway. *J. Physiol.* **205**, 29*P*.

MELVILL JONES, G. & MILSUM, J. H. (1970). Characteristics of neural transmission from the semicircular canal to the vestibular nuclei of cats. *J. Physiol.* **209**, 295–316.

MILSUM, J. H. (1966). *Biological Control Systems Analysis*, pp. 142. New York: McGraw-Hill.

MILSUM, J. H. & MELVILL JONES, G. (1969). Dynamic asymmetry in neural components of the vestibular system. *Ann. N.Y. Acad. Sci.* **156**, 851–871.

NYBERG-HANSEN, R. (1964). Origin and termination of fibres from the vestibular nuclei descending in the medial longitudinal fasciculus. *J. comp. Neurol.* **122**, 355–367.

OUTERBRIDGE, J. S. (1969). Experimental and theoretical investigation of vestibularly-driven head and eye movements. Ph.D. Thesis, Dept. of Physiology, McGill University, Montreal, Canada.

ROSS, D. A. (1936). Electrical studies on the frog's labyrinth. *J. Physiol.* **86**, 117–146.

RUPERT, A., MOUSHEGIAN, G. & GALAMBOS, R. (1962). Microelectrode studies of primary vestibular neurones in cat. *Expl Neurol.* **5**, 100–109.

RYU, J. H., MCCABE, B. F. & FUNASAKA, S. (1969). Types of neuronal activity in the medial vestibular nucleus. *Acta oto-lar.* **68**, 137–141.

SALA, O. (1965). The efferent vestibular system. *Acta oto-lar.* suppl. **197**, 1–34.

SHIMAZU, H. & PRECHT, W. (1966). Inhibition of central vestibular neurons from the contralateral labyrinth and its mediating pathway. *J. Neurophysiol.* **29**, 467–492.

STEINHAUSEN, W. (1933). Über die Beobachtun der Cupula in den Bogengangsampullen des Labyrinths des lebenden Hechts. *Pflügers. Arch. ges. Physiol.* **232**, 500–512.

VAN EGMOND, A. A. J., GROEN, J. J. & JONGKEES, L. B. W. (1949). The mechanics of the semi-circular canal. *J. Physiol.* **110**, 1–17.

WALSH, E. G. (1966). Head movements during rail travel. *Bio-med. Engng* **1**, 402–407.

WERSÄLL, J. & LUNDQVIST, PER.-G. (1966). Morphological polarization of the mechano receptors of the vestibular and acoustic systems. *NASA Symposium SP*-115, 57–72.

WILSON, V. J., KATO, M., PETERSON, B. W. & WYLIE, R. M. (1965). A single unit analysis of the organization of Dieters' nucleus. *J. Neurophysiol.* **30**, 603–619.

WILSON, V. J., WYLIE, R. M. & MARCO, L. A. (1968). Synaptic inputs to cells in the medial vestibular nucleus. *J. Neurophysiol.* **31**, 176–185.

WILSON, V. J. & YOSHIDA, M. (1969*a*). Comparison of effects of stimulation on Deiters' nucleus and medial longitudinal fasciculus on neck, forelimb, and hindlimb motoneurones. *J. Neurophysiol.* **32**, 743–758.

WILSON, V. J. & YOSHIDA, M. (1969*b*). Monosynaptic inhibition of neck motoneurons by the medial vestibular nucleus. *Expl Brain Res.* **9**, 365–380.