EXPERIMENTAL MEASUREMENT OF THE STIFFNESS OF THE CUPULA

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ABSTRACT An experimental procedure is described which consists of cutting the canal duct, inserting a micropipette and administering known volumetric displacements to the cupula. The cupula is made visible by dying the endolymph. Known displacements are administered to the cupula, and the time constant of the return to its equilibrium position is measured. With this information, the stiffness of the cupula is calculated. The experiment was successfully carried out on five White King pigeons. The mean stiffness found is somewhat less than other results reported in the literature, and reasons for this discrepancy are noted.

INTRODUCTION

The response of the semicircular canals to angular motion is best understood from a mechanical point of view. For that reason, physical scientists and engineers have been called on for some time to explain the physical phenomema occuring in this organ. These scientists, using their traditional "tools," developed analytical models for the response of the canals to angular acceleration.

The presently accepted lumped-parameter model is due to Steinhausen (1933). He suggested that the canals respond to angular acceleration in the same manner as would a heavily-damped torsion pendulum. The "torsion pendulum equation" he proposed may be written in the form:

$$J\ddot{\xi} + B\dot{\xi} + K\xi = -J\alpha, \tag{1}$$

where ξ, $\dot{\xi}$, and $\ddot{\xi}$ represent the mean angular displacement, mean angular velocity, and mean angular acceleration, respectively, of the endolymph with respect to the skull. The term α is the inertial angular acceleration of the skull in the plane of the canal. J is the moment of inertia defined by the mass distribution of the endolymph. The damping coefficient B denotes the ratio of the torque resulting from viscous forces to the mean angular velocity of the endolymph. K represents the torsional stiffness, or restoring torque coefficient, of the cupula.

Since the response is highly overdamped, this equation can be written in terms of two time constants (Van Buskirk, 1976):

$$\ddot{\xi} + (1/T_2)\dot{\xi} + (1/T_1T_2)\xi = -\alpha. \tag{2}$$

$T_1 = B/K$ is the "long" time constant and $T_2 = J/B$ is the "short" time constant. Due to the heavy damping, $T_2 \ll T_1$.

It is apparent from Eq. 2 that the response of the canals to any input is governed by the two time constants. Therefore, it has been natural for researchers to seek the values for these time constants experimentally. They were able to do this by assuming that the sensation of angular velocity and the slow phase velocity of nystagmus are proportional to the displacement of the endolymph ξ. Typical of these experiments is the work of van Egmond et al. (1949) and Niven and Hixson (1961). The time constants they obtained are open to question, however, since the signal initiated at the canal is subjected to neural processing.

Other investigators have sought to determine the time constants for the semicircular canal system analytically. The torsion pendulum coefficients can be written in terms of certain basic canal parameters, i.e.

$$J = \rho\pi a^2 R^2 l, \tag{3}$$

$$B = 8\pi\mu R^2 l, \tag{4}$$

$$K = \pi^2 a^4 R^2 k_c, \tag{5}$$

where μ is the viscosity of the endolymph, R is the radius of curvature of the membranous duct, k_c is the pressure-volume modulus of the cupula, a is the radius of the membranous duct, l is the length of the membranous duct, and ρ is the density of the endolymph. Each of these parameters has been measured directly with the exception of the pressure-volume modulus of the cupula, which, to date, could only be inferred from the indirect experiments mentioned in the preceding paragraph. In this paper, we describe a procedure we used to directly measure the quantity k_c and the torsional stiffness of the cupula.

THEORETICAL BACKGROUND

The stiffness of the cupula is defined in terms of its pressure-volume modulus; that is, $k_c = \Delta p/\Delta V$ where ΔV is the volumetric displacement undergone by the cupula when subjected to a pressure difference Δp. Thus, if a known volumetric displacement were applied to the cupula and the pressure difference measured, the stiffness could be ascertained. A distinct problem arises in this approach from the fact that the cupula is a very weak structure. The maximum pressure difference across the cupula, for reasonable values of cupula displacement, is in the range of 10^{-4} cm of water, as reported by Oman and Young (1972). This pressure is much too small to be measured with conventional equipment or techniques.

The method described here involves displacing the cupula and observing the time constant associated with the return of the cupula to its equilibrium position. If the cupula is displaced volumetrically by an amount V_o, and then allowed to return to its equilibrium position by virtue of its stiffness, the volumetric displacement of the

cupula is given by

$$V = V_o e^{-t/T_1}, \tag{6}$$

where V is the displacement at any time t and T_1 is the time constant for the return, given by

$$T_1 = B/K. \tag{7}$$

(Note: $B/K = 8\mu l/\pi a^4 k_c$). Thus, if the time constant T_1 were measured, the cupula stiffness, either K or k_c, is easily found, since all of the other parameters are known.

METHODS

A White King pigeon was first anesthetized; the skull was exposed and the rectus muscles of the neck reflected. The bird's head was placed in a specially developed apparatus for positioning and holding the head. A stereomicroscope was used from this point on in the procedure. An air-driven dental drill was used to expose the bony horizontal canal and ampulla. The bony canal and ampulla were thinned with the drill. A cavity was formed from vinyl putty and continuously filled with isotonic saline solution from an infusion bottle. Thus, a saline pool was created with the canal system completely submerged. This solution helps maintain the membranous labyrinth when it is exposed. The membranous duct and ampulla were exposed by re-

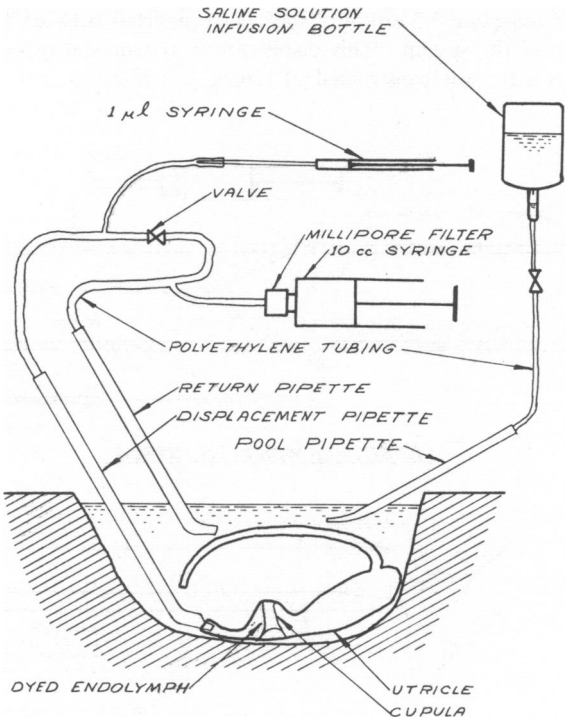

FIGURE 1 Experimental apparatus schematic.

moving the remaining thin shell of bony labyrinth with microforceps. The membranous duct was cut with a pair of no. 8 micro-scissors near the ampulla.

The cupula under normal circumstances is transparent and thus invisible. We circumvented this difficulty by dyeing the endolymph. This made one edge of the cupula visible. The dye was a blue translucent "artificial endolymph"; i.e. it had the same osmolality (300–310 mosmol; Aldred et al., 1940) and milliequivalents of potassium (140–160 meq/liter; Wolfson, 1966) as endolymph. To dye the endolymph, we gently perfused the cut end of the duct with a 40 μm diameter pipette. Care was taken not to produce any large deflections of the endolymph. The dye reached the edge of the cupula by diffusion.

In order to measure the time constant T_1 in Eq. 6, a known volumetric displacement must be administered to the endolymph, and the time measured for its return to equilibrium. This displacement was administered to the endolymph and, thus, to the cupula by inserting a fluid-filled micropipette, and displacing the fluid in the pipette through tubing connected to a microsyringe.

One must not introduce any extraneous pressures throughout the experiment, especially in carrying out the positioning of the micropipette. Undue pressures will dislodge the cupula from its crista. Therefore, the pipette and displacement system contained a return pipette that was submerged in the saline pool. This arrangement eliminated any hydrostatic heads that could develop. Fig. 1 is a schematic of the experimental apparatus, showing how the two pipettes were connected. A valve placed in the tubing, connecting the two pipettes, was shut when the cupula was to be displaced. A 1 μl syringe (Hamilton 7101NCH, Hamilton Co., Reno, Nev.), which was connected between the valve and displacement pipette, could produce volumetric displacements of 0.02 μl. When the valve was opened, the cupula was free to return to its equilibrium position. A 10 cc syringe and 1.2 μm filter was also connected between the valve and return pipette, in order to fill the system. This displacement system and pipette are illustrated in Fig. 2. The entire system was constructed of tubing and material that was large enough in

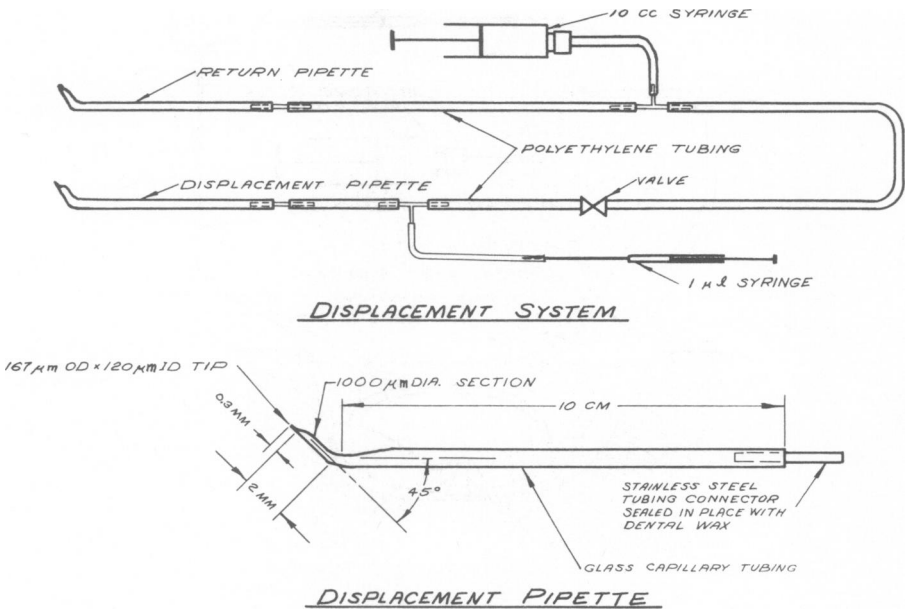

FIGURE 2 Displacement system and pipette.

diameter to assure negligible contribution to the system damping. Extrapolation of flow rate data indicates that the pipette tip also contributed a negligible amount to the system damping.

The displacement pipette was placed in the micromanipulator and connected to the remainder of the displacement system. The system was completely filled with a solution possessing the same osmolality and milliequivalents of potassium as endolymph. The return pipette was positioned in the saline pool, along with the displacement pipette. We then checked the system to see if any thermal density gradient-induced flow existed by introducing a small amount of dye into the displacement pipette tip and observing to see if there was any movement of the dye. Care was taken throughout the entire procedure to prevent thermal gradients in the displacement system and saline pool.

The displacement pipette was positioned carefully in the end of the duct, with the displacement system valve open to prevent any pressure buildup. When the pipette was as far into the duct as it could be placed by the manipulator, a pair of microforceps was used to pull the duct onto the pipette tip a small, additional amount. This formed a seal between the pipette and duct.

At this point, the preparation could be checked to see if the cupula would respond to displacements and return to its equilibrium position. If the cupula was functioning, and had not been destroyed by the lengthy procedures to this point, data recording was initiated. With the valve closed, several small displacements were given, and the accumulated total volumetric displacement at each point was recorded. The points for these incremental displacements were chosen where the position of the cupula could be ascertained using stationary landmarks, such as blood vessels on the ampulla wall or the microscope eyepiece scale. The valve in the dis-

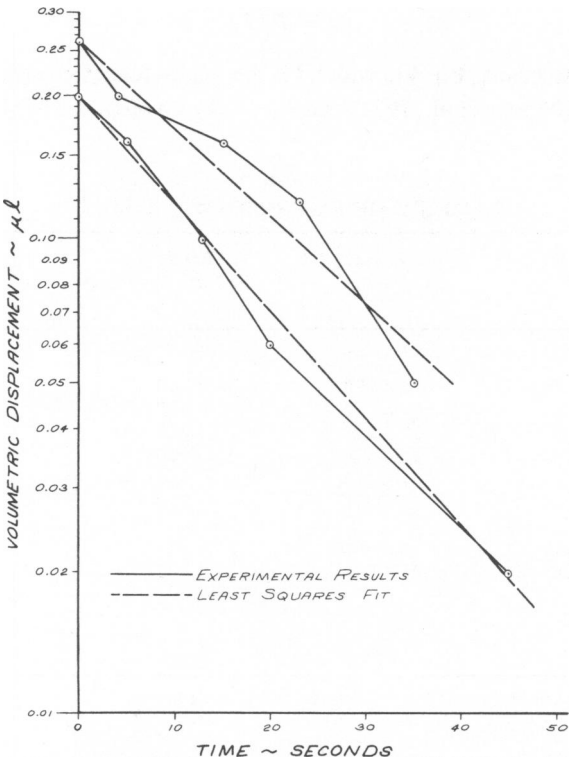

FIGURE 3 Volumetric displacement vs. time for the two returns of experiment 56.

placement system was then opened, and the times for the cupula to return to these landmarks were recorded. This procedure of displacements and time recording was continued until the preparation was destroyed, or the dye diffused into the cupula to the extent that the edge was no longer visible. At this point, the duct was dissected out, and its length measured.

RESULTS

The results can best be explained by examining one experiment in detail. Fig. 3 shows a semilogrithmic plot of two volumetric displacements (in microliters) vs. time (in seconds) for experiment 56. These results are typical of the five individual experiments in which data were obtained.

Each data point is indicated by a dot and successive points are jointed by straight lines. The slope of the lines, joining each successive point, represents the value of $1/T_1$ between the two data points. The theory predicts that, for a linearly stiff cupula, these points should lie on a straight line in a semilog plot. The scatter in Fig. 3 is assumed to be due to experimental error. Therefore, for each return, a least-squares fit was applied, where only the slope was allowed to change and the zero time intercept, or initial volumetric displacement, was fixed. This produced an overall time constant for each individual return.

The torsional stiffness was then determined from

$$K = B/T_1, \tag{8}$$

where B is calculated using Eq. 4 in which l is the actual length measured in the experiment, μ = 1.0 cp (Money et al., 1971) and R = 2.4 mm (Grant, 1973).

TABLE I

SUMMARY OF EXPERIMENTAL RESULTS

Experiment Number	Torsional Stiffness K	Pressure Volume Modulus k_c	Time Constant T_1
	dyn-cm/rad	10^4 dyn/cm^5	s
56	0.00070	7.0	20
60	0.00062	6.2	23
61	0.00080	8.0	18
63	0.00070	7.0	20
65	0.00072	7.2	19
Mean	0.00071	7.1	20
Standard Deviation	0.00015		
Standard Error	0.00003		

Calculations Based on the Following Constants:

Endolymph Viscosity	1.0	cp
Duct Radius	0.115	mm
Canal Radius of Curvature	2.4	mm
Duct Length	1.3πR	

Table I gives the mean torsional stiffness for each experiment. Also listed is the pressure-volume modulus calculated from Eq. 5, and the long-time constant of the canal calculated using Eq. 7. (Note: The time constant T_1 in Table I is the approximate value for the intact canal; the time constants measured in the experiments are different because the cut duct length varies. The value of l used to calculate the terms in Table I is $l = 1.3\pi R$. R and μ are as above).

COMPARISON WITH OTHER RESULTS

Three previous attempts to measure the slow decay time constant of the pigeon horizontal canal are to be found in the literature. These were reported by Schierbeck (1953), Mayne (1950), and Outerbridge (1969). A fourth determination on the anterior canal was recently reported by Correia and Landolt (1973).

Schierbeck's (1953) measurement of the slow decay time constant was based on the method of cupulometry, developed by van Egmond et al. (1949). He applied this method by timing the duration of head and eye nystagmus after administering step changes in angular velocity. Although he did not calculate a time constant, this is easily done from the data he presents on one normal pigeon. For head nystagmus duration the time constant is 7.6 s, and from eye nystagmus duration the time constant is 6.1 s.

Mayne (1950) estimated the slow decay time constant from experimental measurements made by Mowrer (1935). Mowrer measured the period of latency for head movements in pigeons for various step changes in angular accelerations. Mayne, using the concept that there must be a threshold cupula deviation before any reactions would occur, equated the latency period to the magnitude of the acceleration. He then fitted this relation to Mowrer's data, and determined a slow decay time constant of 8.3 s.

Outerbridge (1969) conducted an involved set of experiments in which he measured the slow decay time constant for head and eye nystagmus. His measurements, in both cases, were made using step changes in angular velocity and sinusoidal excitation. For the head nystagmus movements, he obtained a dominant time constant of approximately 9.0 s. There was an overshoot present in the step response case, which was similar to secondary nystagmus observed in eye movements. Outerbridge, however, claims that this time constant may not reflect the slow decay time constant for the canal system, since the canals are part of a feedback loop. The canals are contained in the head and are thus being stimulated by the movements of the head. He could not confirm his speculation, since most of the parameters in the feedback loop are unknowns.

For eye nystagmus, Outerbridge obtained a time constant of approximately 2.5 s. In this determination, the bird's head was fixed in a head holder, thus preventing head nystagmus and avoiding canal stimulation. Because this time constant was obtained in the open loop fashion of conventional cupulometry, he feels that this is the correct value, and speculates that the head movement time constant reflects the head and neck dynamics, not the canal time constant.

Correia and Landolt (1973) obtained a slow decay time constant of 3.3 s for the anterior canal of the pigeon. They recorded the nervous activity of primary nerves and

calculated the time constant using phase shift measurements during sinusoidal stimulation. The anterior canal crista of the pigeon contains a structure called the eminentia cruciatae, which consists of two projections from the face of the crista forming a small septum. This structure may perform a stiffening function for the cupula. With a stiffer cupula, the time constant would shorten. The horizontal crista of the pigeon does not contain this structure.

Except for the eye nystagmus long time constant of 2.5 s obtained by Outerbridge, the remaining data in the literature on the horizontal canal would be in approximate agreement.

These values are still a factor of approximately 40% of those obtained in the experiment described in this paper. Outerbridge's data contained some secondary nystagmus, which indicated an adaptive effect or overshoot that may account for the discrepancy. Young and Oman (1968) and Malcolm (1968) developed analytic models to explain overshoot based on a neural shift of the zero displacement reference level of the cupula. Thus, when the cupula returned to its equilibrium position, there was still an error signal, which causes the subjective overshoot. The adaptive effect is strictly of nervous system origin, and does not reflect the canal dynamics.

All of the previous work was based on data which were processed by the nervous system, and subject to an adaptive effect, as indicated by Outerbridge's data. The long time constant in this experiment was obtained directly from the canal itself and thus was not subject to conditioning by the nervous system. An adaptive time constant of approximately three times the long time constant could almost account for the error between determinations of approximately 8 s, and the value obtained here of 20 s.

DISCUSSION AND CONCLUSIONS

In order to detect movement of the cupula under the microscope, displacements an order of magnitude larger than normal physiological displacements had to be used. The nominal physiological displacement that could be expected would be in the neighborhood of 0.002 μl (Grant, 1973). The minimum detectable displacement, for practical observation under the microscope, was approximately 0.01 μl, and displacements as large as 0.2 μl were used. These gross displacements may have created some structural damage to the cupula, which could reduce its apparent stiffness. If this was the case, then it might explain the larger time constant measured in this experiment.

There are some experimental observations to support the possibility of structural damage. In many of the unsuccessful preparations, in which the cupula would not respond and return to its equilibrium position, further staining with dye would reveal that part of the cupula had actually been torn away from its base, the crista. The tear ran from the base of the crista, up its face to the crest, as shown in Fig. 4. When this damage was visible, the cupula exhibited essentially zero stiffness, and would usually remain in any position in which it was placed. Occasionally, however, when deflected to the damaged side, with the opposite side intact, it would return slightly toward its centered position. This observation suggests the cupular material in the damaged configuration responds to tensile forces on its lateral face, and not to compressive forces.

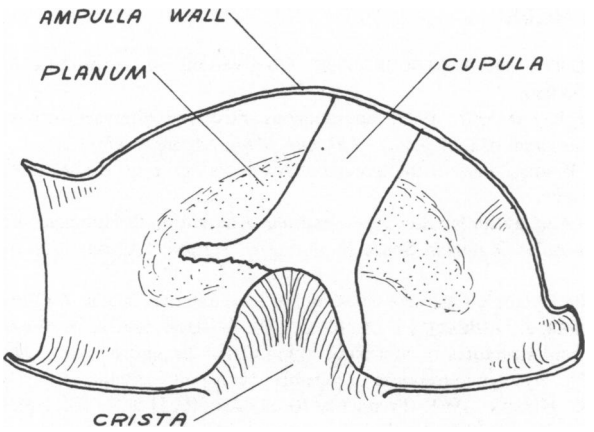

FIGURE 4 Longitudinal cross section of membranous ampulla, showing cupula torn from the face of the crista.

Oman and Young (1972) suggest that any procedure that subjects the cupula to pressures which exceed the maximum physiological pressure may traumatize the cupula, or damage the cupula-crista attachment. This speculation may, indeed, be true, but the observations made (with a low-power light microscope) during this set of experiments do not substantiate observable damage to the cupula-crista attachment. However, internal cupular material structural damage or other unobservable damage may have occurred.

Another possible source of error in the experiment may have been caused by the introduction of the dye into the duct and ampulla. The presence of the dye may have changed the elastic properties of the cupula. Malcolm (personal communication, 1972) speculates that there could be a gel present on either side of the cupula. If this gel is present, it could possibly add to the stiffness of the cupula. With the introduction of the dye, this gel may have been washed out, or mechanically agitated and transformed into a solution. The absence of the gel may explain the longer time constants and low cupular stiffness we observed.

The value of cupula stiffness obtained in these experiments was through direct observation of the cupula rather than through the measure of some neural-processed variable. To effect this observation, however, large, nonphysiological displacements of the cupula were necessary. We trust, however, that the results, not having been conditioned by the nervous system, reflect the true physical characteristics of the end organ itself.

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