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THE DISTRIBUTION, ACTIVITY, AND FUNCTION OF THE CILIA IN THE FROG BRAIN

By DEBORAH J. NELSON and ERNEST M. WRIGHT

From the Department of Physiology,
University of California Medical Center,
Los Angeles, California 90024, U.S.A.

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SUMMARY

1. The distribution, activity, and function of the cilia in the brain was studied using *in vitro* preparations of the frog choroid plexuses and ependyma.

2. Scanning electron microscopy revealed that twenty to forty cilia, about 20 μm long, project from the cells of the choroidal epithelium and ependyma into the ventricular system of the brain.

3. These cilia beat at a constant frequency which ranged from 5 to 20 c/s. Ciliary activity was enhanced by ATP, cyclic AMP, theophylline, and acetylcholine, and was depressed by DNP, IAA, Ni^{2+}, La^{3+}, and Co^{2+}.

4. Ciliary motion produced a flow of c.s.f. over the surface of the cells lining the ventricles, and in the choroid plexus this flow reduced the effective thickness of the unstirred layer adjacent to the epithelium by about 100 μm.

5. These results are discussed in relation to the factors that control the frequency of ciliary beating, and the role of the cilia in the circulation of the c.s.f.

INTRODUCTION

Cerebrospinal fluid (c.s.f.) fills the ventricles, canals and subarachnoid spaces of the central nervous system. The major fraction of the c.s.f. is formed within the cerebral ventricles, by the choroid plexuses and possibly other extrachoroidal sites, and flows from the ventricles into the subarachnoid spaces where it is absorbed back into the blood. Although the mechanisms by which the c.s.f. flows along this circulatory pathway are not completely understood, the pressure gradients produced by secretion of the fluid, postural changes, and arterial and respiratory pulsations, are likely to be important. It has been suggested that the cilia lining the ventricles and canals of the brain may also contribute to the circulation

and flow of the c.s.f. within the brain (see the monographs by Davson, 1967, and Milhorat, 1972, for reviews of c.s.f. circulation).

The objective of the present investigation was to determine the distribution and function of the cilia covering the choroid plexuses and ependyma of the frog brain. The motivation for such a study was provided by our observation that ciliary activity generated a flow of solution across the surface of the isolated frog choroid plexus. We have found that cilia are widely distributed in the ventricles of the frog c.n.s.; these cilia beat at frequencies up to 30 c/s, the frequency of the ciliary beat is regulated in a fashion similar to that in other ciliated cells, and the cilia modify the thickness of the unstirred layers adjacent to the ventricular surface of the epithelia. Our conclusion is that cilia play an important role in the physiology of the cerebrospinal fluid in the frog.

METHODS

Adult bullfrogs (*Rana catesbeiana*) were obtained from commercial suppliers and were kept in tap water at room temperature for 1–2 weeks before the experiments. The posterior choroid plexus, which covers the open fourth ventricle (see Text-fig. 7), was removed from the frogs as described previously (Wright, 1972). The third ventricle choroid plexus and ependyma lining the lateral and fourth ventricles were also dissected from the isolated brain.

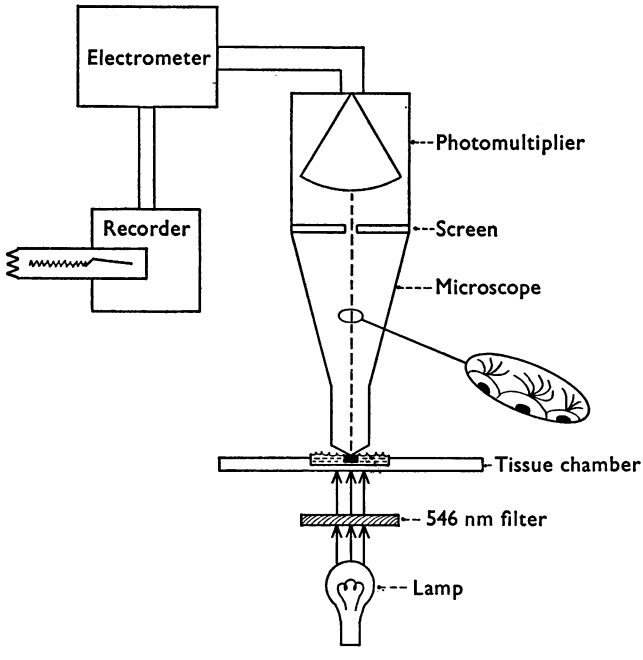
Method used to monitor ciliary motion

The activity of the cilia on the choroid plexus was monitored photometrically as described by Eckert & Murakami (1971, 1972) for cilia in the oviduct of the mud puppy. Briefly, a 1·5 mm^2 piece of tissue from the central region of the triangular shaped plexus (see Pl. 1) was mounted in a slide chamber by wrapping the tissue, ventricular side up, around a thin glass plate (0·12 mm thick), and securing it in position with a bi-pronged metal hook. The slide chamber was placed on the stage of a microscope, and a 40 × water immersion objective was focused on the cilia of the cells passing over the edge of the glass plate so that the cilia were observed to beat in profile (see Text-fig. 1). The field viewed through the objective was projected onto a screen placed before the photocathode of a photomultiplier tube (EMI 9524B). The screen contained a pinhole that was equivalent to a field of 0·5 μm in diameter at the plane of focus. This aperture was positioned over the group of cilia projecting from one cell. Fluctuations in the light intensity produced by ciliary movement across the aperture were detected by the photomultiplier. The photocurrent was amplified using an electrometer (Keithley, 610B) and recorded on a high speed pen recorder (Brush, Mark II). The frequency response of the recorder was maximal at 100 c/s. Each cycle of ciliary motion produced one biphasic wave on the pen recorder (see Text-figs. 2–5). Photomultiplier noise was recorded by moving the preparation out of the field of view. A similar procedure was employed to record the cilia on the ependyma.

Saline was perfused through the slide chamber (volume 0·5 ml.) at a rate of 0·5 ml./min. The composition of the solution flowing over the tissue was changed by means of a Chromatronix sample injection valve (no. R605V) which was placed close to the inlet port of the slide chamber.

Measurement of unstirred layer thickness

A quantitative measurement of the thickness of the unstirred layer adjacent to the ventricular surface of the choroidal epithelium was obtained from the time course of the build up and decay of 'streaming potentials' (see Diamond, 1966; Wright & Prather, 1970; and Smulders & Wright, 1971). Streaming potentials are those electrical potentials produced by the osmotic flow of water across ion selective membranes. The electrical potential difference across the choroid plexus was recorded as described previously (Wright & Prather, 1970; Wright, 1972), and



Text-fig. 1. Apparatus for photometric monitoring of the beating frequency of choroid plexus cilia. The photomultiplier sampled small areas of the image (area seen in oval disk). The image was projected to the photomultiplier through a hole in the screen equivalent to a field of 0·5 μm in diameter at the plane of focus. Changes in light intensity due to ciliary movement across the aperture of the pinhole were detected by the photomultiplier, amplified and displayed on a high speed chart recorder.

streaming potentials were generated by the addition of sucrose to the ventricular fluid. The time course of the build up of these streaming potentials, 1–2 mV/100 mM sucrose, is related to the thickness of the ventricular unstirred layer (δ) and to the diffusion coefficient of the solute (D) used to generate the flow by the relation $t_{\frac{1}{2}} = 0{\cdot}38\,\delta^2/D$ (see Diamond, 1966). In the gall-bladder we have established the validity of this method by comparing the results to those obtained by two independent procedures (see Smulders & Wright, 1971).

The distribution of cilia

Scanning electron microscopy was used to obtain information concerning the distribution of cilia on the choroid plexuses and ependyma. The isolated tissues were pinned on a thin cork disk to minimize surface damage during the subsequent fixation procedure. The tissue was washed in bicarbonate buffered saline for 45 min and fixation was initiated in 1 % glutaraldehyde for one hour and completed in 3 % glutaraldehyde for another hour. The tissues were washed and stored overnight at 4° C in 115 mM sodium cacodylate buffer. They were post-fixed in 1 % osmium tetroxide for 90 min, washed in distilled water, dehydrated in a graded series of ethanol, washed in acetone and dried in a critical point drying apparatus using liquid carbon dioxide. The ventricular surface of the tissues was then coated with carbon and gold in a vacuum evaporator, and examined using a 'Steroscan' S4 scanning electron microscope (Cambridge Instrument Co.) at an accelerating voltage of 8 kV.

Solutions

The majority of experiments were carried out in a solution containing 85 mM-NaCl, 2 mM-KCl, 1 mM-$MgSO_4$, 1 mM-$CaCl_2$ and 25 mM-$NaHCO_3$ equilibrated with 95 % O_2/5 % CO_2. Some experiments were also carried out in a solution containing 105·4 mM-NaCl, 2 mM-KCl, 1 mM-$MgSO_4$ and 1 mM-$CaCl_2$ buffered with either 2·125 mM-Na_2HPO_4/0·375 mM-NaH_2PO_4 or 3 mM Tris Chloride. These latter solutions were equilibrated with 100 % O_2. We observed that the cilia beat equally well in all three buffered solutions. Glutaraldehyde solutions were prepared in solutions of 115 mM Na cacodylate buffered to pH 7·2.

All experiments were carried out at room temperature (22–24° C). Errors are expressed as standard errors of the mean with the number of estimates added in parentheses.

RESULTS

Scanning electron microscopy

The distribution of cilia on the fourth ventricle choroid plexus, the third ventricle choroid plexus, and the ependyma lining the fourth ventricle was obtained from scanning electron micrographs. Pls. 1–2 illustrate the results obtained for the posterior choroid plexus. A low power micrograph of the ventricular surface of the plexus is shown in plate 1*a*. The highly folded surface of the plexus has a uniform granular appearance, and the micrographs made at higher magnifications (Pls. 1*b* and 2*a*) reveal that this is due to the cilia projecting from the epithelium into the ventricle. About thirty to forty cilia emerge from the central region of each cell to form the tufts seen at lower magnifications. Each cilium is about 20 μm long and about 0·25 μm in diameter. At the highest magnification used (Pl. 2*b*) the microvilli that make up the brush border of the choroidal epithelium are readily distinguishable.

The cilia are distributed on the third ventricle choroid plexus and on the ependyma of the fourth ventricle in a similar fashion to the posterior choroid plexus. In each case a group of thirty to forty cilia about 20 μm in length project from the centre of practically every cell.

Ciliary activity

The cilia of the frog choroid plexus beat at a remarkably constant rate for periods lasting up to 3 hr, for example, in one experiment where the frequency was recorded every 5 min for three hours the average frequency was $6{\cdot}8 \pm 0{\cdot}1$ c/s. Although the beat frequency was maintained at a constant level, the basal rate varied widely from one plexus to another. In fifty-six experiments the average basal rate was $13{\cdot}1 \pm 0{\cdot}4$ c/s. The results obtained in four experiments are illustrated in Text-figs. 2–5, where the basal beating rate varied from 8 to 16 c/s. These Figures show segments of the actual records obtained during each phase of the experiment in addition to a graphical summary of the results.

In the choroid plexus the behaviour of the local population of cilia on any given cell reflects the behaviour of the total population, i.e. at any phase of any given experiment the rate of beating was constant, within a few cycles per second, from one cell to another. These observations are in marked contrast to those reported for the *Necturus* oviduct where at any given time only about half the ciliated cells are spontaneously beating, and where the activity of the cilia on a given cell slowly waxes and wanes (see Fig. 1, Eckert & Murakami, 1972).

Experiments were also performed on ependymal cilia of the lateral ventricle to determine frequency and stability of beating. In two experiments readings were recorded at approximately 10 min intervals for a period of at least 100 min. Basal beat frequency remained stable throughout the experimental periods at 21 c/s. Thus ependymal cilia, like choroidal cilia, maintain extremely stable beat frequencies, but unlike choroidal cilia appear to beat at much higher basal rhythms. The bulk of remaining experimental work is concerned with choroidal cilia owing to the ease of accessibility of the plexus.

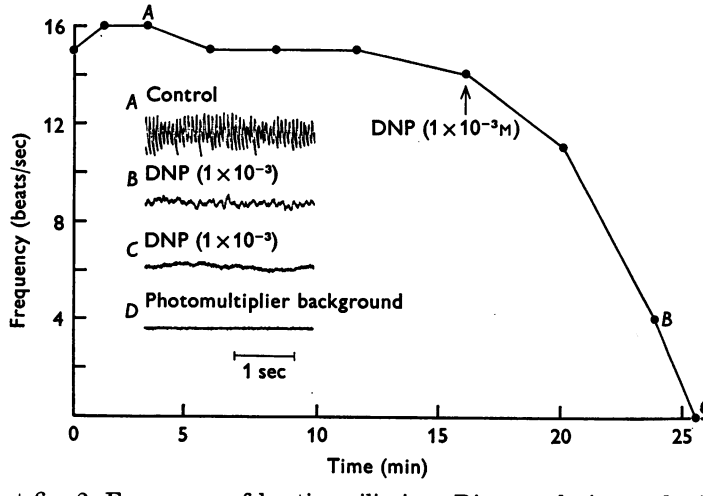
Inhibition of ciliary activity

(1) Metabolic inhibitors: 2,4-dinitrophenol (DNP), a potent uncoupler of oxidative phosphorylation, irreversibly stopped ciliary activity in $7 \pm 2(4)$ min. Text-fig. 2 shows the time course of the effect in one of these experiments. The basal frequency in this plexus was 16 c/s and 10 min after the addition of 1 mM-DNP the ciliary activity was indistinguishable from the photomultiplier noise (record *C vs. D*).

Iodoacetic acid (IAA), which inhibits anaerobic glycolysis, also stopped ciliary activity, but in this case it took $28 \pm 4(3)$ min. This inhibition was also irreversible even when the plexus was washed with saline containing cysteine (1 mM). Previously it had been reported that cysteine reversed the action of this inhibitor on ciliary activity in *Mytilus* gill preparations

(Usaki, 1956). Cyanide (0·01–1 mM) also blocked ciliary activity in the choroid plexus, but azide (1 mM) even after 2 hr did not.

(2) Ions: Ni (5 mM) reversibly inhibits ciliary motility in *Paramecium* (Naitoh, 1966). Text-fig. 3 shows the effect of this ion on the activity of the cilia on the choroid plexus. In the absence of Ni^{2+} the basal rate of beating was about 12 c/s. and on addition of Ni^{2+} (1 mM) to the saline the rate



Text-fig. 2. Frequency of beating cilia in a Ringer solution and with the addition of 1 mM-DNP. Cilia which showed a stable maintenance of beating frequency, were irreversibly inhibited by the addition of DNP. DNP perfusion through the chamber during the period indicated produced eventual complete cessation of all spontaneous activity. *A*: pen-recording of frequency monitored by photomultiplier during perfusion with Ringer solution. *B*: recording of partial inhibition of ciliary beat frequency produced 8 min after DNP addition *C*: complete cessation of ciliary activity after 10 min of DNP perfusion. *D*: pen-tracing of noise produced by photomultiplier with the preparation out of the field of view.

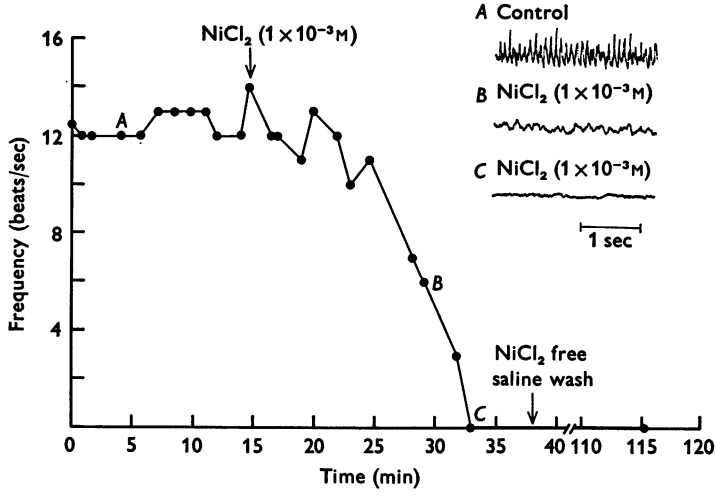
decreased slowly and stopped altogether about 15 min later. Washing the tissue with Ni^{2+} free saline for an hour failed to reverse the inhibition.

La (5×10^{-4} M) added to saline buffered with Tris chloride also produced irreversible inhibition of the ciliary beat within $12 \pm 1(3)$ min. Co (1 mM), on the other hand, did not eliminate ciliary beating but reduced the rate to 30 % of the normal base line value. Neither Sr (1 mM) nor Mn (1 mM) proved to be effective in modifying the rate of ciliary beating in this preparation. Likewise, neither calcium free (Ca-free or Ca-free + 1 mM-EGTA), nor magnesium free solutions produced any alteration in the beating pattern. Thus, the sequence of inhibitory ions is La^{3+}, Ni^{2+} > Co^{2+} > Sr^{2+}, Mn^{2+}, Ca^{2+}, Mg^{2+}.

Finally, in one experiment where we lowered the pH of the saline to 4·4 ciliary activity ceased within 14 min and beating was completely restored upon washing the plexus for about 25 min in normal saline.

Acceleration of ciliary activity

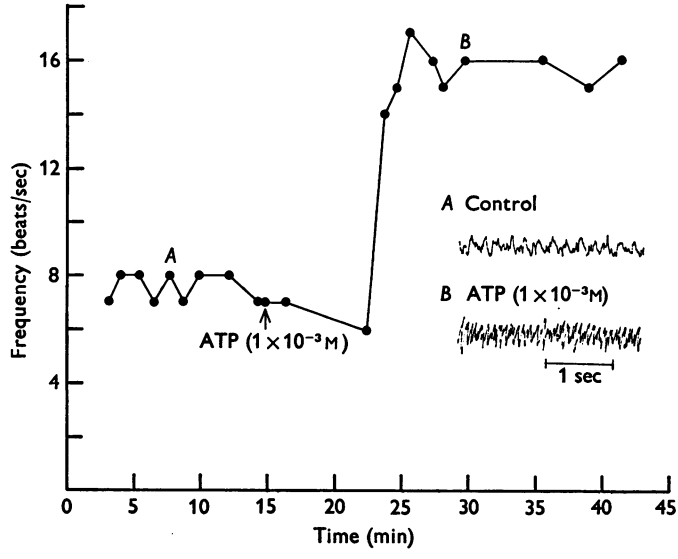
(1) Adenosine triphosphate (ATP): The cilia of the frog choroid plexus are sensitive to the external concentration of ATP. Text-fig. 4 outlines an experiment in which 1 mM-ATP added to the external solution increased



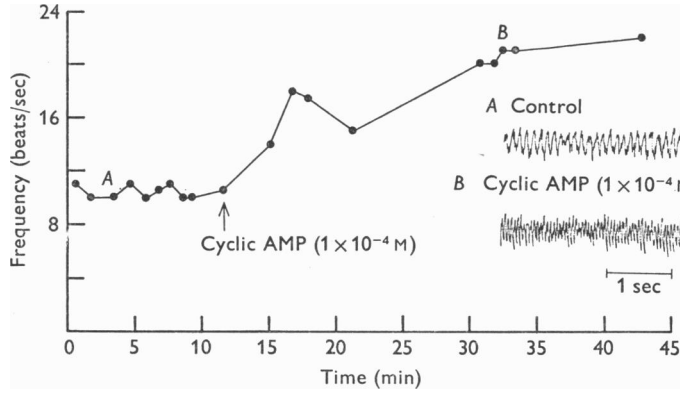
Text-fig. 3. Frequency of beating cilia in a Ringer solution and with the addition of 1 mM-$NiCl_2$. Cilia which showed a stable maintenance of beating frequency were irreversibly inhibited by the addition of $NiCl_2$ which stopped all spontaneous activity. Upon inactivation, the preparation was washed for 1 hr in a $NiCl_2$-free solution which failed to reinstate activity. *A*: pen-recording of frequency monitored by photomultiplier during perfusion with Ringer solution. *B*: recording of partial inhibition of beat frequency obtained 14 min after addition of $NiCl_2$ solution. *C*: complete cessation of ciliary activity after 22 min of $NiCl_2$ application.

the beating frequency from 8 to 16 c/s within 2 min after an initial delay lasting about 5 min. Often the maximum frequency increased by a factor of 2, but the absolute rate never exceeded 30 c/s. In six experiments the frequency increased 68 ± 11 % over the base line level.

(2) Cyclic adenosine 3′,5′-monophosphate (cyclic AMP): Similar results to those produced by ATP were obtained when cyclic AMP was added to the external saline. The frequency increased $61 \pm 11(6)$ % on addition of 1×10^{-4} M cyclic AMP, but, as shown in Text-fig. 5, the time course of the response was quite different.



Text-fig. 4. Frequency of beating cilia in a Ringer solution and with the addition of 1 mM-ATP. Cilia which demonstrated a stable maintenance of beating frequency were excited to an elevated plateau of beating frequency with the perfusion of ATP Ringer solution. *A*: pen-tracing of frequency monitored during perfusion with Ringer solution. *B*: increased beating frequency produced by perfusion with ATP Ringer solution.



Text-fig. 5. Frequency of beating cilia in a Ringer solution and with the addition of 1×10^{-4} M cyclic AMP. Cilia which maintained a stable basal frequency level were excited to an elevated plateau of beating frequency with the perfusion of cyclic AMP Ringer solution. *A*: tracing of frequency monitored during perfusion with Ringer solution. *B*: increased beating frequency produced by perfusion with cyclic AMP Ringer solution.

(3) Theophylline and caffeine: theophylline, a known inhibitor of phosphodiesterase activity, was significantly less effective than either ATP or cyclic AMP in its ability to stimulate ciliary activity. At concentrations of 1 and 5 mM the frequency increased by a maximum of 30 %. Caffeine, on the other hand, produced no significant change in the frequency of the ciliary beat at either 1 or 5 mM.

Miscellaneous compounds

Acetylcholine increased the rate of ciliary beating; 30–50 % above the base line levels some 10–12 min after application. Anticholinesterases (physostigmine (1 mM), neostigmine (1 mM) and di-isopropylfluorophosphate (1–10 mM)) epinephrine (1 mM), propranolol (2 mg/50 ml.) and ouabain (1×10^{-3} M), all failed to affect the base line level of ciliary activity in the frog choroid plexus.

TABLE 1. Effect of cilia on the ventricular unstirred layer of choroid plexus

		Unstirred layer thickness (μm)			
		Control	Experiment		*P* value
A	(1)	300 ± 5 (7)	Ni^{2+}	365 ± 9 (10)	$\ll 0{\cdot}0005$
	(2)	295 ± 4 (5)	Ni^{2+}	370 ± 7 (7)	$< 0{\cdot}0005$
B	(1)	305 ± 11 (10)	DNP	415 ± 12 (7)	$< 0{\cdot}0005$
	(2)	210 ± 11 (12)	DNP	350 ± 13 (8)	$< 0{\cdot}0005$

In these four experiments the thickness of the ventricular unstirred layer was measured 5–12 times in the presence of 1 mM-ATP during the first 2–3 hr, the control period. During the subsequent experimental period the thickness of the unstirred layers was measured 7–10 times in the presence of (*A*) 1 mM-$NiCl_2$ or (*B*) 1 mM-DNP. The thickness of the unstirred layers was obtained from the time course of the build up and decay of streaming potentials. These were obtained by the addition of 100 mM sucrose to the ventricular solution. The magnitude of the streaming potentials was unaffected by the presence or absence of Ni^{2+} or DNP.

The effect of cilia on stirring the c.s.f.

It is readily apparent that the cilia stir the cerebrospinal fluid next to the surface of the choroid plexus. To obtain a quantitative estimate of the extent of this stirring we have measured the thickness of the 'unstirred layer' adjacent to the epithelium when (i) the cilia are beating at their maximum rate, in the presence of 1 mM-ATP, and (ii) when ciliary activity was blocked by 1 mM-$NiCl_2$ or 1 mM-DNP.

The thickness of the unstirred layers was obtained from the time course of the build up and decay of 'streaming potentials' (see p. 65). The results are summarized in Table 1. In the presence of ATP the half-times for the build up and decay of the streaming potentials were about 64 sec and this

corresponds to an unstirred layer of about 300 μm. Addition of Ni^{2+} or DNP, which stopped ciliary activity (Text-fig. 2 and 3), increased the thickness of the unstirred layer to about 400 μm. In four control experiments there was a small, but insignificant ($P > 0{\cdot}2$), decrease in the thickness of the unstirred layer (about 20 μm) over a comparable time period. Thus it may be concluded that the cilia can effectively stir the c.s.f. within 100 μm from the surface of the cell. Historically, it was pointed out by Gray (1928) that the stirring of cilia extends 4–5 times the length of the cilium.

Preliminary experiments using ependymal tissue from the lateral ventricle placed in a dilute solution of red blood cells, support this conclusion. Cilia beating on the ependymal surface generate circular currents of the red blood cells as far as 200 μm away from the surface.

DISCUSSION

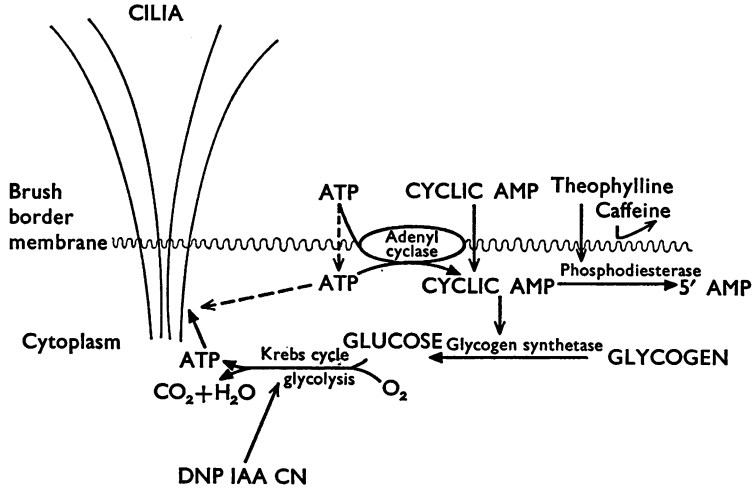
The epithelial cells covering the frog choroid plexus and ependyma are ciliated. About 30–40 cilia, 20 μm in length, emerge from the central region of each cell and project into the cerebrospinal fluid. The presence of cilia on the cells lining the ventricles and canals of the central nervous system has been well documented in recent years through the use of both transmission and scanning electron microscopy in amphibians, reptiles, birds and mammals, including man (Pontenagel, 1962; Carpenter, 1966; Ferraz de Carvalho, 1970; Murakami, 1961; Doolin & Birge, 1969; Brightman & Palay, 1963; Luse, 1956; Weindl & Joynt, 1972; Allen & Low, 1973; Scott, Paull & Krobisch Dudley, 1972; Scott, Kozlowski, Paull, Ramalingam & Krobisch-Dudley, 1973; Dohrmann & Bucy, 1970; Wislocki & Ladman, 1958; Bruni, Montemurro, Clattenburg, Singh, 1972). In general, central nervous system cilia have the same structure as those described by Fawcett & Porter (1954) in the oviduct of the mouse and of the human. It is highly probable that the contractile mechanisms and the control of ciliary motion within the central nervous system are basically those that govern motion in other ciliated cells and tissues (see Stephens, 1971; and Kinosita & Murakami, 1967, for reviews of these subjects).

Control of ciliary activity

Although it is well established that the ability of the cilium to undergo rhythmic beating is an intrinsic property of the organelle, it is recognized that the frequency of beating can be modulated by a variety of agents which interfere with cellular metabolism. A scheme to rationalize our observations is shown in Text-fig. 6.

The central point in the scheme revolves around the fact that the rate of

ciliary beating is related to the intracellular supply of ATP. In a variety of isolated cilia preparations it has been established that the rate of ciliary beating is a direct function of the ATP concentration, e.g. in triton extracted *Necturus* oviduct (Eckert & Murakami, 1972). Consequently, the inhibitory effects of DNP, IAA, and CN^- on intact choroidal cilia are readily explained on the basis that these agents block the production of ATP from glucose via glycolytic metabolism. This view is supported by observations that metabolic inhibitors did not abolish ciliary activity



Text-fig. 6. A model for the control of ciliary beating on the epithelium of the choroid plexus. The epithelium is covered on its ventricular surface by a population of actively beating cilia. The beating of these cilia is arrested when placed in solution of DNP, IAA, and CN^- and accelerated in solutions containing ATP, theophylline and cyclic AMP. The rate of ciliary beating is presumably a function of the intracellular supply of ATP.

reactivated by ATP in triton extracted models (Naitoh & Kaneko, 1973). The presence of numerous glycogen granules in amphibian choroid plexuses (e.g. Carpenter, 1966) indicates that glycogen is probably the source of substrate for ATP production.

As is the case in other ciliated epithelia extracellular ATP stimulates ciliary activity in the choroid plexus (Vorhaus & Deyrup, 1953; Usuki, 1959; and Murakami, Machemer-Röhnisch & Eckert, 1974). There are at least two explanations for these observations. The first is that extracellular ATP is able to directly supplement the supply of ATP to the cilium. This explanation is improbable because (i) plasma membranes are thought to be impermeable to nucleotides, (ii) the intracellular ATP concentration

is in the range of 0·5–5 mM (Long, 1961), (iii) in the triton extracted oviduct preparation ciliary beating is reactivated by 1×10^{-4} M-ATP, whereas in the intact preparation extracellular ATP concentrations as low as 1×10^{-6}M activates the cilium (Murakami *et al.* 1974), and (iv) extracellular ATP does not maintain ciliary activity in the presence of metabolic inhibitors (Murakami *et al.* 1974). The second explanation is the extracellular ATP stimulates the intracellular production of ATP. Such an increase in ATP production could be mediated by cyclic AMP. Evidence offered to support this interpretation is that (i) high concentrations of cyclic AMP in the extracellular fluid stimulates ciliary activity in the choroid plexus (Text-fig. 5) and glycogenolysis in the liver (Robison, Butcher & Sutherland, 1971), (ii) theophylline stimulates ciliary activity in the plexus (p. 71) and inhibits the break-down of cyclic AMP to 5′ AMP by phosphodiesterase (Robison *et al.* 1971) (Caffeine failed to stimulate ciliary activity in the plexus and this is probably due to inability of the molecule to gain access to the phosphodiesterase), and (iii) in the oviduct extracellular ATP fails to maintain ciliary activity in the presence of metabolic inhibitors (Murakami *et al.* 1974).

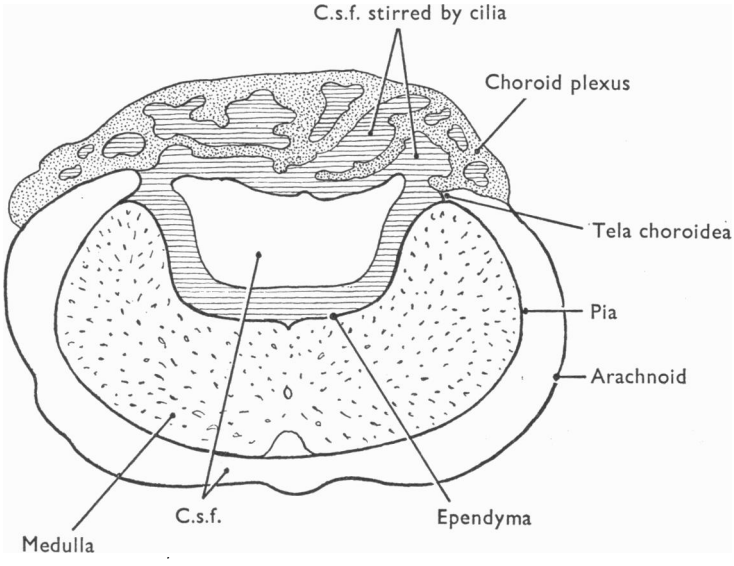
Not shown in the scheme in Text-fig. 6 is our observation that La^{3+}, Ni^{2+}, and to a lesser extent Co^{2+} inhibit ciliary beating in the plexus. This effect is likely to be due to a direct effect on the contractile processes in the cilium as Naitoh & Kaneko (1973) found that Ni^{2+}, Sr^{2+}, Ba^{2+} and Mg^{2+} were able to stop ciliary beating in triton extracted *Paramecium.*

Functions of cilia in the brain

We have shown that the cilia of the frog choroid plexus normally beat at a frequency of about 13 c/s, and that the cilia effectively stir the c.s.f. within 100 μm of the surface of the epithelium. Given that similar results were obtained for the cilia on the ependyma, it may be estimated from the dimensions of the frog brain (see Text-fig. 7) that greater than 75 % of the c.s.f. within the ventricles is mixed as a result of ciliary activity. This conclusion is consistent with earlier observations that cilia generate currents of c.s.f. within the ventricles of amphibians (Vonwiller & Wigodskaya, 1933; Chu, 1942; and Adam, 1953).

The significance of cilia in the hydrodynamics of the c.s.f. in higher animals, including man, has yet to be evaluated. However, it should be noted that ciliary currents adjacent to the ependyma have been observed in dogs (Konno & Shiotani, 1956); rats (Dalen, Schlapfer & Mamoon, 1971); kittens, monkeys, and man (Hild, 1957); rats and man (Worthington & Cathcart, 1966), and that cilia have been implicated in the flow of c.s.f. along the spinal canal in rabbits and rats (Bradbury & Lathem, 1965). In the study by Worthington & Cathcart (1966) on rats, the ciliary

currents were mapped in each of the ventricles, and they concluded that the function of the cilia in the c.n.s. was to keep the c.s.f. in constant motion and to clear cellular debris from the ependymal surface of the ventricular system. It is clear that ciliary currents have to be evaluated along with arterial pulsations, secretion of the c.s.f., and respiratory movements when considering the hydrodynamics of the c.s.f. in the foetus, neonate and adult.



Text-fig. 7. A cross-section of the fourth ventricle in the frog with associated meninges, neural tissue and choroid plexus. Lined areas represent those volumes of c.s.f. actively stirred by c.n.s. cilia. From the dimensions of the frog brain it may be estimated that greater than 75% of the c.s.f. within the ventricles is mixed as a direct result of ciliary activity. The drawing, which is to scale, was obtained from histological sections of the frog brain. The diameter of the brain in this region was about 2 mm.

Cilia may also play an important role in the exchange of solutes and water between the brain and the c.s.f. across the ependyma, and between the blood and the c.s.f. across the choroidal epithelium. This problem is currently under investigation in our laboratory.

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EXPLANATION OF PLATES

PLATE 1

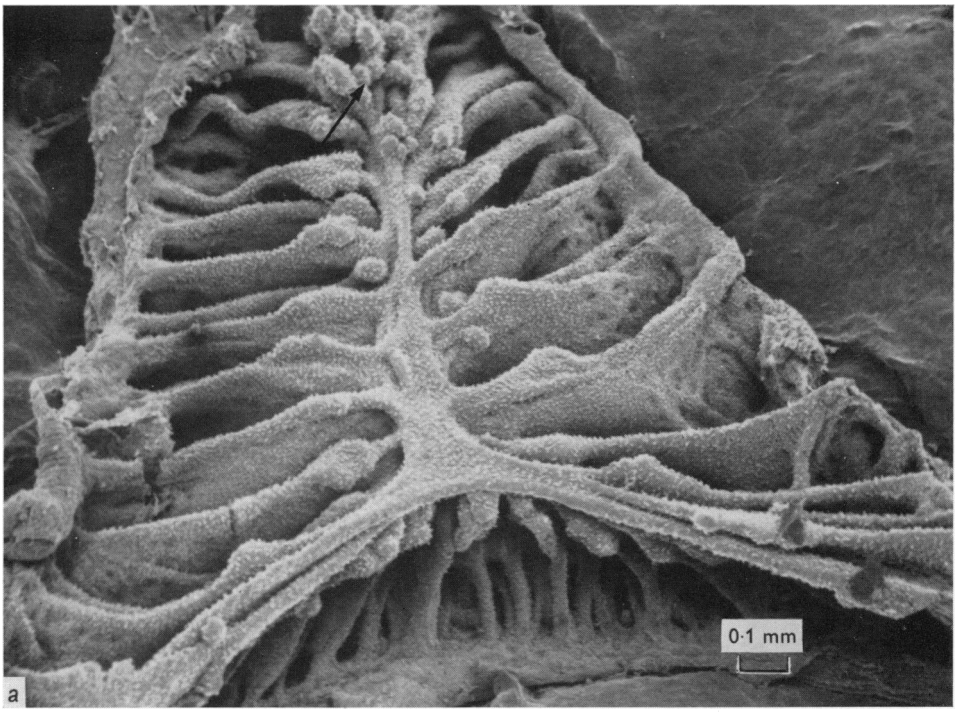
a, low magnification SEM showing the ventricular surface of the choroid plexus from the fourth ventricle in the frog. Note clumps of cilia which give the surface a granulated texture.

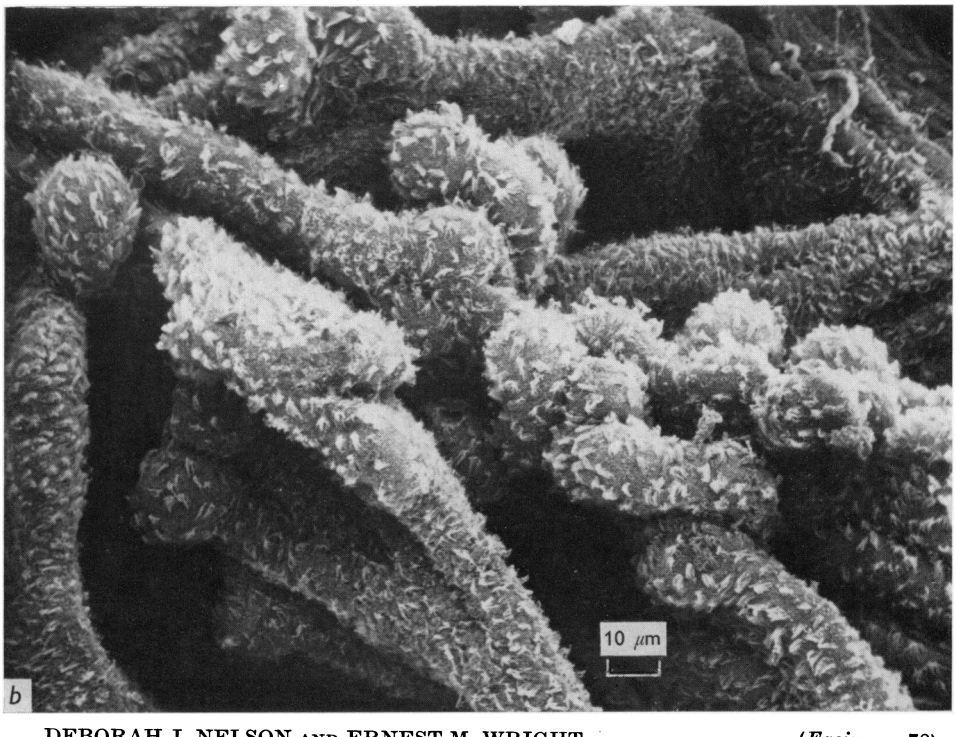
b, SEM micrograph of the surface of the fourth ventricle choroid plexus. The view is an enlargement of the area indicated by the arrow in Pl. 1. Note the clumps of cilia.

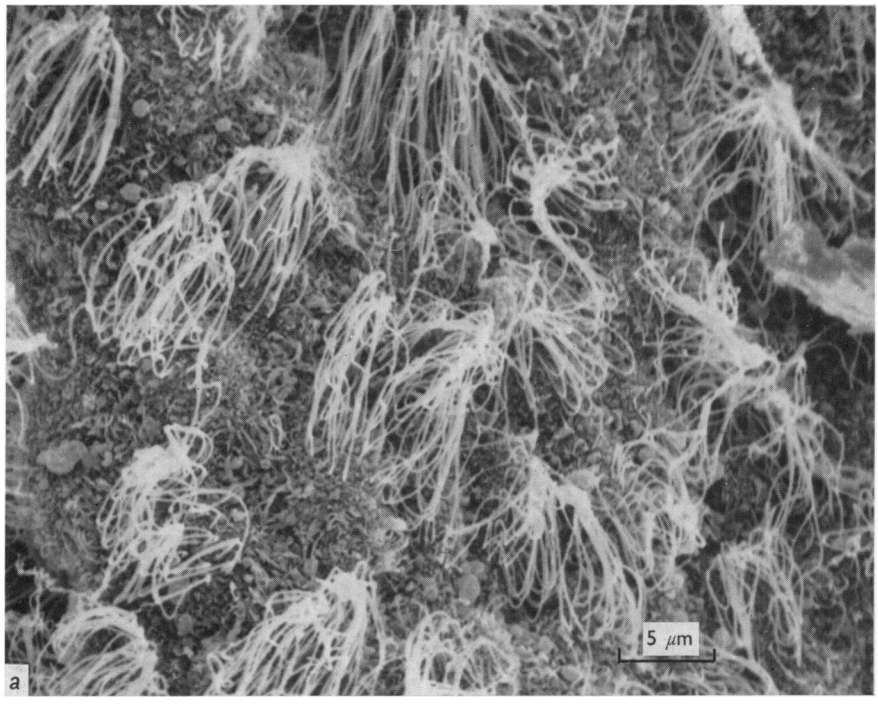
PLATE 2

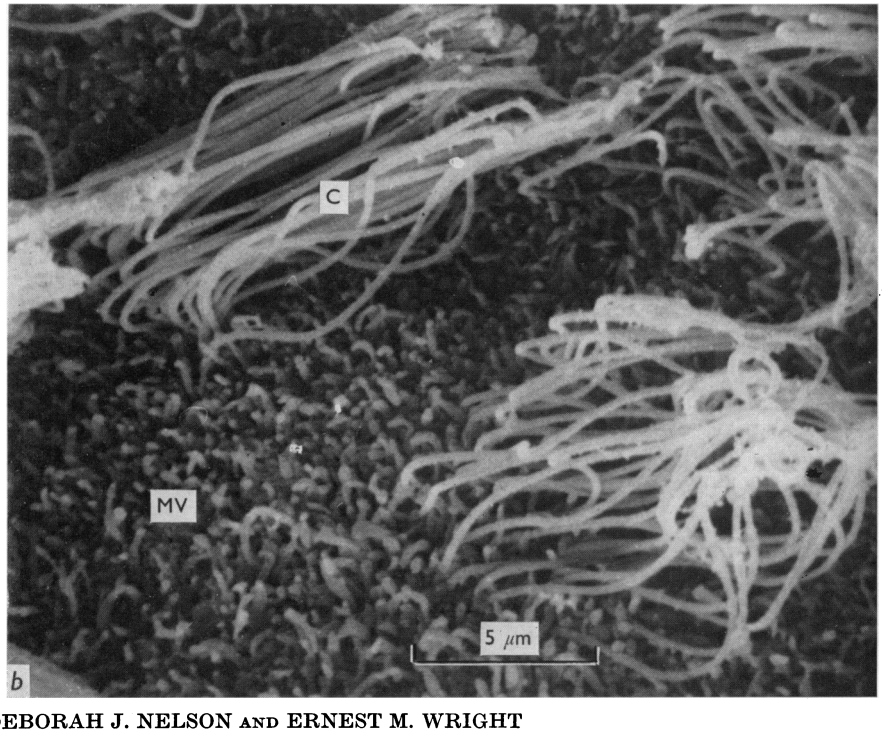
a, scanning micrograph of a portion of the fourth ventricular choroid plexus. At this magnification distinct tufts of cilia are seen. Note that the boundaries between cells appear as furrows in the membrane surface.

b, high magnification SEM of two cilia clumps that demonstrate long gracile cilia (C) which measure approximately 20 μm in length. Note also the surrounding bed of microvilli (MV).


0·1 mm
a


10 μm
b





DEBORAH J. NELSON AND ERNEST M. WRIGHT