*British Journal of Anaesthesia* 1996; **76**: 854–859

# Recovery of respiratory ciliary function after depression by inhalation anaesthetic agents: an *in vitro* study using nasal turbinate explants

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## Summary

We have developed a human tissue preparation suitable for measurement of cilia beat frequency derived from nasal turbinates. Cilia beat frequency of turbinate explants from 11 patients did not change significantly over a 10-day observation period while maintained in an incubator, with mean cilia beat frequency of 13.1 (SEM 0.3) Hz to 14.4 (0.2) Hz (ANOVA for repeated measures, $P = 0.168$). We have used this preparation to investigate recovery of ciliary function after depression by inhalation anaesthetic agents. Eight or nine turbinate explants were exposed to three times the minimum alveolar concentration (MAC) of halothane, enflurane or isoflurane for a period of 1 h and thereafter to a period of air washout. After exposure to the inhalation agent there was a significant reduction in cilia beat frequency with all three agents: halothane 14.3 (0.4) Hz to 9.5 (0.3) Hz; enflurane 13.7 (0.6) Hz to 10.5 (0.5) Hz; isoflurane 15.9 (0.6) Hz to 10.6 (0.3) Hz. Cilia beat frequency returned to values after air washout that were not significantly different from baseline after 90 min of washout of halothane and 60 min of washout of enflurane and isoflurane (repeated measures ANOVA, unpaired *t* test; $P = 0.01$ at 60 min and $P = 0.31$ at 90 min washout for halothane; $P = 0.83$ at 60 min washout for enflurane; $P = 0.26$ at 60 min washout for isoflurane). (*Br. J. Anaesth*. 1996; **76**: 854–859)

**Key words**

Anaesthetics volatile, halothane. Anaesthetics volatile, enflurane. Anaesthetics volatile, isoflurane. Lung, trachea.

Mucociliary clearance is an important defence against respiratory tract infections. The depressant effects of inhalation anaesthetic agents on mucus transport *in vivo* are well established in both animals and humans [1, 2], however, the mechanisms involved have not been elucidated. Mucus transport depends on the volume and physical properties of the mucus and on the function of the beating cilia. Cilia beat frequency is an important determinant of mucus transport rate; there is a logarithmic relation between the two such that modest reductions in cilia beat frequency are associated with substantial reductions in mucus transport rate [3, 4].

In a previous study [5] we have demonstrated depression of human respiratory cilia beat frequency with 3 MAC of the three inhalation anaesthetic agents, halothane, enflurane and isoflurane. In this investigation we have studied the duration of cilia beat frequency depression by measuring the recovery characteristics of cilia beat frequency after a period of air washout.

We have developed an alternative source of human respiratory ciliated epithelium suitable for measurement of cilia beat frequency. This is derived from explants of the inferior nasal turbinates removed from patients at elective surgical turbinectomy for a primary diagnosis of tissue hypertrophy. The preparation of these specimens, measurements of cilia beat frequency and the effects of 3 MAC of the inhalation agents followed by a period of air washout are described.

## Methods

### TISSUE PREPARATION

We collected the inferior nasal turbinates removed from patients at elective surgery with a primary diagnosis of tissue hypertrophy without any history of allergy. The turbinates were rinsed thoroughly in medium 199 (M199) (containing 2.2 g litre$^{-1}$ of $NaHCO_3$ and L-glutamate, GibcoBRL Life Technologies) to remove as much blood and mucus as possible. Discs of the surface ciliated epithelium were removed using a 4-mm biopsy punch (Stiefel Laboratories, Wooburn Green, Bucks, UK). The specimens were rinsed in a mixture made from 100 ml of M199 and 2 ml of antibiotic–antimycotic solution (containing penicillin 10 000 u., streptomycin 10 mg, and amphotericin 23 μg ml$^{-1}$, diluted 1 : 100; Sigma Chemical Co.). We placed the specimens in 10 ml of culture medium made from 100 ml of medium 199, 10 ml of fetal calf serum (Gibco Life Technologies), 2 ml of antibiotic–antimycotic solution and 1 ml of mixed additives containing insulin 250 μg ml$^{-1}$, transferrin 250 μg ml$^{-1}$, and hydrocortisone 36 μg ml$^{-1}$. The specimens were then

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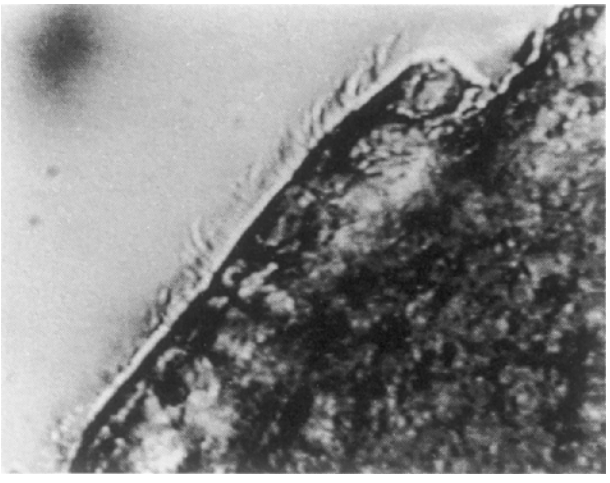

*Figure 1* Photograph of ciliated edge of nasal turbinate explant.

incubated at 37 °C with occasional mixing for 1 h to separate the mucus. We then transferred the specimens into multidishes with 3 ml of culture medium per well and incubated at 37 °C in 5 % carbon dioxide–air. The medium was changed after 24 h and every 3–4 days thereafter.

MEASUREMENT OF CILIA BEAT FREQUENCY

Cilia beat frequency was measured using a modification of the transmitted light technique that we have described previously [6]. The 4-mm disc of tissue was placed in a purpose-built perfusion chamber system and images of the ciliated epithelium were produced using a Nikon microscope by viewing the edge of the disc (fig. 1). The chamber consists of an aluminium block with integral channels at two sides and reversibly sealed coverslips at the top and bottom. The chamber has a depth of 0.85 mm and an internal volume of 1.1 ml. The chamber is perfused using connecting tubing passing from a bottle of Hanks buffered salt solution (HBSS) into the entry port of the chamber and out from its exit port through connecting tubing into a collecting beaker.

HBSS flows from a container immersed in a water bath maintained at 37 °C under the effect of gravity at a flow rate of 0.5 ml $min^{-1}$ measured by a calibrated photoelectric drop counter. Air flows into the container at 1000 ml $min^{-1}$. The perfusion chamber is maintained at 37 ($\pm$0.1) °C using a thermostatically controlled heating element mounted on the underside of the chamber.

Images of the ciliated epithelium were produced using differential interference contrast microscopy (Nikon Diaphot 200, Nikon UK). Using a 60 × objective with a 10 × ocular lens, the image obtained from the microscope was transmitted via a video camera (Panasonic vw-CL 110-AE) to a high resolution video monitor (Sony KX-14CPI). A low pass filtered pinhead photodiode with a cut-off above 25 Hz was attached to the monitor screen and the image of the sample moved so that the cilia interfered with the light reaching the photodiode. The magnification of the optical system and aperture of the photodiode results in 3–5 cilia being captured by the photodiode and as adjacent cilia beat in phase, the frequency of interruption to the light reaching the photodiode by this number of cilia is representative of cilia beat frequency.

The output was transferred to a Gould 20-MHz cathode ray oscilloscope to confirm the detection of ciliary movements. Voltage changes across the photodiode were passed simultaneously via an analogue-to-digital converter to a computer (Dell 386sx) that samples voltage at a frequency of 200 Hz. The voltage signals were collected over a period of 15 s by recording continuously from one edge. These data were then divided into three sequential 5-s intervals for analysis.

The data were transferred to an RM Centra V466 computer and analysed by software in Mathematica 2.2 to provide a power spectrum using fast Fourier transforms. Mean frequencies less than 2 Hz were filtered out to eliminate artefacts of low frequency caused by sudden movements of the light pen or microscope stage. The peak of the power spectrum was taken to represent cilia beat frequency. The whole apparatus is mounted on a concrete block to reduce extraneous vibrations that interfere with photometric techniques of measurement. Acceptable ciliated edges for measurement were deemed to be those devoid of mucus, and at least 60 μm long.

STATISTICAL ANALYSIS

The mean of peak cilia beat frequencies from the power spectrum was computed for each sample at each time point. The data were analysed by analysis of variance for repeated measures. If this was significant the data were analysed by unpaired *t* tests with Bonferroni correction, comparing baseline cilia beat frequency with that measured at subsequent times. Significance was taken as $P < 0.05$.

### *Turbinate cilia survival characteristics*

In order to investigate the survival characteristics of cilia from the turbinate preparations over time, we measured cilia beat frequency of samples obtained from 11 patients. These patients had a mean age of 28.3 (range 21–56) yr, all were non-smokers, receiving no regular medications, who were anaesthetized with propofol for induction of anaesthesia, morphine or fentanyl for analgesia, and isoflurane for maintenance of anaesthesia. All patients received cocaine 200 mg and adrenaline 1 mg applied topically to the nasal cavity before surgical excision.

We measured cilia survival characteristics over a period of 10 days from discs obtained from the 11 patients by measuring cilia beat frequency 1, 3, 6, 8 and 10 days after surgical excision. On each of these days we transferred six discs from any six of the patients into the perfusion chamber and measured cilia beat frequency 1–2 h after removal from the incubator. After measurement, these discs were discarded and on subsequent days this process was repeated with another set of discs from any six of the 11 patients stored in the incubator. Between six and 10 readings of cilia beat frequency were obtained from each disc.

*Effect of 3 MAC of the inhalation agents and a period of air washout*

Samples of human respiratory ciliated epithelium were obtained in the same manner as above from the inferior nasal turbinates of nine patients undergoing turbinectomy surgery for a primary diagnosis of turbinate hypertrophy. The patients were aged 30.1 (range 18–43) yr, seven were male, and all were healthy, non-smokers.

Discs of 4 mm from the patient's turbinates of variable storage period and up to 6 days old were mounted in the perfusion chamber. The chamber was perfused from a delivery bottle of HBSS under the effect of gravity at 0.5 ml $min^{-1}$. Air at 1000 ml $min^{-1}$ was passed through a Tec 3 vaporizer before passing into the delivery bottle. The vaporizer was set at a concentration of 3 MAC for unpremedicated young adults at 37 °C; this represented 2.25 % for halothane, 5 % for enflurane and 3.6 % for isoflurane. We have shown previously that this apparatus delivers the volatile anaesthetic agents into the perfusate and equilibrates within 15 min [5]. Samples from eight patients were exposed to halothane and isoflurane and samples from nine patients to enflurane. After exposure to 3 MAC of one of the three inhalation agents for a period of 1 h, we recorded 6–10 readings of ciliary beat frequency. We then exposed the samples to air alone by perfusing them from a separate delivery bottle of HBSS and measured cilia beat frequency after exposure to air at 30-min intervals for 1.5 h.

## Results

Cilia beat frequency of the turbinate samples did not change significantly over the 10-day observation period while maintained in the incubator, with mean cilia beat frequency ranging from a minimum of 13.1 (SEM 0.3) Hz to a maximum of 14.4 (0.2) Hz (MANOVA for repeated measures, $P = 0.168$) (table 1).

Turbinate explants from eight patients were exposed to 3 MAC of halothane and subsequently to air. Mean cilia beat frequency after exposure to halothane for 1 h was 9.5 (SEM 0.32) Hz, after 30 min of air washout 8.3 (0.50) Hz, after 1 h of air washout 12.6 (0.5) Hz and after 90 min of washout 13.7 (0.4) Hz. These compared with a pretreatment value of 14.3 (0.4) Hz. After 1 h of air washout, cilia beat frequency remained significantly depressed, but after 90 min of air washout it had returned to a value that was not significantly different from the pretreatment value (repeated measures ANOVA, unpaired, $t$ test with Bonferroni correction, $P = 0.01$ at 1 h and $P = 0.31$ at 1.5 h of air washout compared with pretreatment control) (fig. 2).

*Table 1* Cilia beat frequency (CBF) of turbinate explants over 10 days (mean (SEM))

| Day | CBF (Hz) | Ciliated edges measured |
|---|---|---|
| 1 | 14.0 (0.33) | 109 |
| 3 | 14.5 (0.24) | 99 |
| 5 | 13.8 (0.26) | 123 |
| 8 | 13.1 (0.24) | 112 |
| 10 | 13.2 (0.28) | 97 |

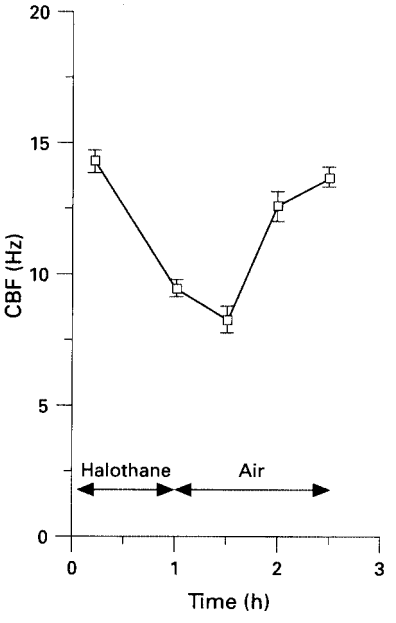


*Figure 2* Cilia beat frequency (CBF) after 3 MAC of halothane for 1 h and air washout for 1.5 h (mean, SEM).

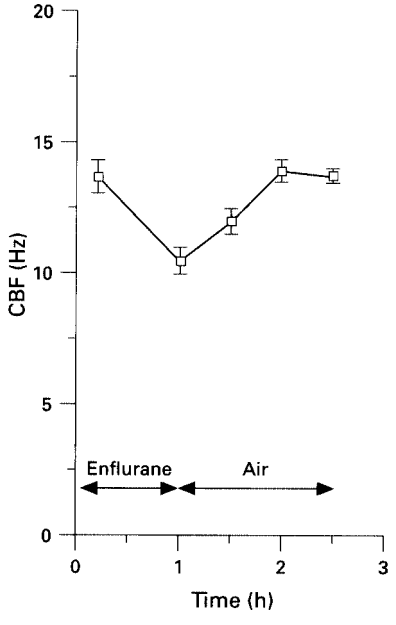


*Figure 3* Cilia beat frequency (CBF) after 3 MAC of enflurane for 1 h and air washout for 1.5 h (mean, SEM).

Mean cilia beat frequency after exposure to enflurane for 1 h was 10.5 (0.51) Hz, after 30 min of air washout 12.0 (0.4) Hz and after 1 h of air washout 13.9 (0.4) Hz compared with a treatment value of 13.7 (0.6) Hz. Mean cilia beat frequency had returned to values that were not significantly different from pretreatment values after an air washout period of 1 h (repeated measures ANOVA, unpaired $t$ test with Bonferroni correction, $P = 0.83$, at 1 h of air washout compared with pretreatment controls) (fig. 3).

Mean cilia beat frequency after exposure to isoflurane for 1 h was 10.6 (0.30) Hz and after 1 h of

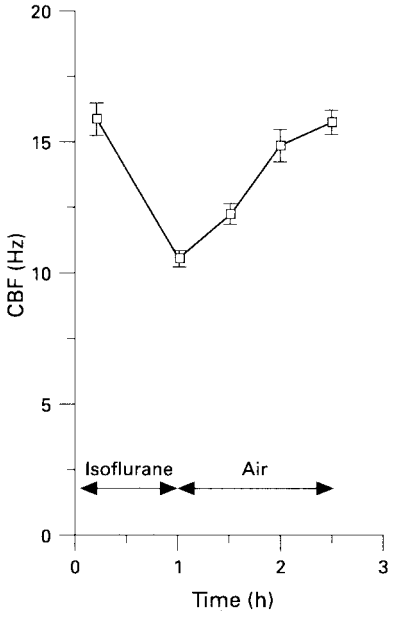


*Figure 4* Cilia beat frequency (CBF) after 3 MAC of isoflurane for 1 h and air washout for 1.5 h (mean, SEM).

air washout 14.9 (0.6) Hz compared with a pretreatment value of 15.9 (0.6) Hz. Mean cilia beat frequency had returned to values that were not significantly different from pretreatment values after an air washout period of 1 h (repeated measures ANOVA, unpaired *t* test with Bonferroni correction, $P = 0.26$ at 1 h of air washout compared with pretreatment controls) (fig. 4).

## Discussion

Cilia beat frequency of the nasal turbinate explants was unchanged during storage in a controlled environment for up to 10 days. Using this preparation there was reversible depression of cilia beat frequency *in vitro* after exposure to 3 MAC of the three inhalation agents halothane, enflurane or isoflurane; however the reduction was more prolonged after exposure to halothane compared with the two other agents.

Almost all human *in vitro* studies of respiratory ciliary function have used cytological specimens from brushings of the respiratory tract [7]. Nasal brushings are favoured because this is less invasive and cilia beat frequency is similar to bronchial ciliated specimens [8] implying nasal cilia are representative of cilia further down the respiratory tract. However, such exfoliated tissue samples may present problems as the cells are disrupted from their basement membrane, they are prone to sample movement which interferes with measurement of cilia beat frequency [9] and we have found that the samples are suitable for analysis only on the day of removal, which causes logistical restraints.

We intended originally to culture the ciliated cells from excised nasal turbinates using techniques described by Devalia and colleagues [10]; however, we noticed that the cilia of the turbinate explants remained functional for more than 1 week, despite no evidence of growth, and therefore explored the use of these preparations. We continued to use the growth factors for tissue storage as described in the earlier study [10], although this may not in fact be necessary.

Historically, *in vitro* samples of respiratory ciliated epithelium were provided by biopsy of the inferior nasal turbinates and used to diagnose such conditions as cystic fibrosis; however, such techniques became unpopular because they were performed under local anaesthesia with its attendant ciliotoxic effects and there was a risk of haemorrhage associated with this procedure. We considered that biopsies obtained from the inferior nasal turbinates had advantages over nasal brushings. Such preparations may provide a better model of *in vivo* ciliary behaviour as much of the adjacent tissue is retained and the use of larger samples reduces the likelihood of sample movement which interferes with measurement of cilia beat frequency. Furthermore, we had found that cilia of turbinate explants remained beating for up to 10 days after excision and could provide a tissue source in the laboratory. In addition, the specimens derived from the turbinates were found to have superior edges from which to measure cilia beat frequency. Using explants from excised turbinates overcomes the problems of haemorrhage from turbinate biopsies referred to earlier and the ability to store the tissue for use more than 24 h after excision overcomes the short-lived effects of topical vasoconstrictors and local anaesthetics on ciliary function [11].

Clearly such samples are derived from patients with abnormal turbinates requiring surgical excision and the possibility that their cilia may be abnormal was considered. We therefore only used tissue from turbinates removed from patients with a diagnosis of tissue hypertrophy causing nasal obstruction without any history of allergy. Other workers have demonstrated that the mucus transport rate of such turbinates is normal, implying normal mucociliary function [11].

Cilia beat frequency of explants was within the range reported by previous workers using different ciliated preparations and different measurement techniques of 11–18 Hz [9]. The range of ciliary beat frequencies we computed from the nasal brushings of 11.2–12 Hz [6] was lower than that measured from the turbinate explants of 11.6–15.0 Hz. This difference represents different patterns of interference with the light beam, the rate of which is deemed to represent ciliary beat frequency. The diameter of the light pen images 3–5 cilia in any plane and cilia throughout the depth of the field. Because adjacent cilia are co-ordinated and beat in synchrony, interference in the light path by multiple cilia imaged with each position of the light pen provides a measure of cilia beat frequency. The depth and co-ordination of adjacent cilia may well be different in cytological specimens compared with explants. With turbinate explants there is a greater depth of ciliated tissue interfering with the light path but the greater ciliary continuity may provide better co-ordination. These factors may explain the different cilia beat frequency computed. Nevertheless, for each specimen, whether nasal brushings or turbinate explants, cilia beat frequency was within the range found by

other workers, was reproducible and predictably responsive to pharmacological agents.

There are few previous studies of the effects of inhalation anaesthetic agents on cilia beat frequency with which to compare this investigation; however, other studies found similar reductions in cilia beat frequency. Using rabbit trachea, Lee and Park found reductions in cilia beat frequency with 3 MAC of halothane of 22 %. This compares with our reductions at these concentrations of 25 %. In the case of enflurane, these workers found reductions in cilia beat frequency at 3 MAC of 26 % which compares with our finding of 26 % [12]. Depression of cilia beat frequency in our turbinate preparations of 33 %, 25 % and 33 % with halothane, enflurane and isoflurane, respectively, differs from that found at 1 h with nasal brushings in our previous study [5], where we found reductions of 28 %, 10 % and 2 % with these three agents. The difference is because of the use of a different tissue preparation and interestingly the results with the turbinate preparations are closer to those found by Lee and Park using rabbit trachea than our measurements from nasal brushings. The turbinate samples may be a more physiological tissue than exfoliated cells to investigate ciliary function.

Cilia beat frequency is an important determinant of mucus transport rate. Forty years ago, Hill investigated the movement of carborundum particles across frog oesophagus and rat trachea [13]. She described a hyperbolic relationship between mucus transport rate and particle velocity such that there were disproportionately larger decreases in mucus transport rates at lower particle velocities. It was hypothesized that this relationship arose because the transfer of power from the beating cilia to the mucus was most effective within a narrow range of cilia beat frequencies.

Puchelle and Zahm studied the relationship between the transport rate of sputum from bronchitic patients across mucus-depleted frog palate and the rheological properties of sputum [14]. Not surprisingly, the rheological properties of increased viscosity, increased elasticity and reduced spinnability were associated with reduced transport rates; however the most important determinant of mucus transport rate found by step-by-step multiple regression was ciliary beat frequency.

Duchateau and colleagues investigated the relationship between nasal cilia beat frequency measured photometrically from biopsies *in vitro* with nasal mucociliary clearance of dye and saccharin *in vivo* in 31 healthy volunteers [3]. A good correlation was found between the logarithm of mucus clearance rate and cilia beat frequency.

Hee and Guillerm similarly described a non-linear relationship between cilia beat frequency and mucus transport rates in sheep such that modest reductions in cilia beat frequency were associated with large reductions in mucus transport rates [4]. These studies suggest that cilia beat frequency has an important role in determining the transport rate of mucus.

The return to baseline values of cilia beat frequency after exposure to 3 MAC of halothane, enflurane and isoflurane for 1 h took 1 h for enflurane and isoflurane and 1.5 h for halothane. Interestingly, cilia beat frequency measured 30 min after ceasing exposure to the inhalation agent demonstrated some recovery with enflurane and isoflurane but a further reduction with halothane.

Differences in recovery time and effects of early washout with the three agents may be related to their different recovery characteristics from general anaesthesia which is dependent on their physical properties. Halothane has a greater lipid–water solubility coefficient than the two other agents and may take longer to diffuse out from the fat soluble tissues of the preparation. The mechanism of continuing reduction in cilia beat frequency after cessation of halothane is unknown but is an interesting finding that merits further investigation. The only comparable work is by Gyi, O'Callaghan and Langton investigating the effects of halothane alone on cilia beat frequency. Their data failed to show a reversal of effect after air washout for 50 min [15]. This concurs with our findings at 1 h.

There are no data available comparing the duration of reduction in mucus transport or clinical data comparing the incidence of infective respiratory problems with different inhalation agents.

The time for reversal of depression in ciliary function found in this study may appear to be clinically insignificant, especially in the light of the longer duration of depression of mucus transport rates demonstrated with inhalation anaesthetics *in vivo*. Forbes and Gamsu measured tantalum bronchographic clearance in dogs anaesthetized with halothane and found that 1.2 MAC of halothane administered for 2 h delayed the clearance of tantalum for more than 4 h after termination of anaesthesia [1]. This may represent additional effects of the anaesthetics on the mucus itself. Alternatively, even relatively short periods of depression of ciliary beat frequency which produce mucus stasis could alter the physical properties of mucus and therefore impair mucus transport for a longer period.

## References


1. Forbes AR, Gamsu G. Mucociliary clearance in the canine lung during and after general anesthesia. *Anesthesiology* 1979; **50**: 26–29.
2. Lichtiger M, Landa JF, Hirsch JA. Velocity of tracheal mucus in anesthetized women undergoing gynecologic surgery. *Anesthesiology* 1975; **42**: 753–755.
3. Duchateau GS, Graamans K, Zuidema J, Merkins FW. Correlation between nasal ciliary beat frequency and mucus transport rate in volunteers. *Laryngoscope* 1985; **95**: 854–859.
4. Hee J, Guillerm R. Discussion on smoke and mucociliary transport. *European Journal of Respiratory Diseases* 1985; **66** (Suppl. 139): 86–88.
5. Raphael JH, Selwyn DA, Mottram SD, Langton JA, O'Callaghan C. Effects of 3 MAC of halothane, enflurane and isoflurane on human nasal cilia beat frequency *in vitro*. *British Journal of Anaesthesia* 1996; **76**: 116–121.
6. Selwyn DA, Gyi A, Raphael JH, Key A, Langton JA. A perfusion system for the *in vitro* measurement of cilia beat frequency. *British Journal of Anaesthesia* 1996; **76**: 111–115.
7. Rutland J, Cole PJ. Non-invasive sampling of nasal cilia for measurement of beat frequency and study of ultrastructure. *Lancet* 1980; **2**: 564–565.

8. Low PP, Luk CK, Dulfano MJ, Finch PJP. Ciliary beat frequency of human respiratory tract by different sampling techniques. *American Review of Respiratory Disease* 1984; **130**: 497–498.
9. Yager J, Chen T-M, Dulfano MJ. Measurement of frequency of ciliary beats of human respiratory epithelium. *Chest* 1978; **73**: 627–633.
10. Devalia JL, Sapsford RJ, Rusznak C, Toumbis MJ, Davies RJ. The effects of salmeterol and salbutamol on ciliary beat frequency of cultured human bronchial epithelial cells, in vitro. *Pulmonary Pharmacology* 1992; **5**: 257–263.
11. Mason JDT, Aspden TJ, Adler J, Jones NS, Illum L, Davis SS. Measurement of nasal mucociliary transport rates on the isolated human inferior turbinate. *Clinical Otolaryngology* 1995; **20**: 530–535.
12. Lee KS, Park SS. Effect of halothane, enflurane and nitrous oxide on tracheal ciliary activity *in vitro*. *Anesthesia and Analgesia* 1980; **59**: 426–430.
13. Hill JR. The influence of drugs on ciliary activity. *Journal of Physiology* (*London*) 1957; **139**: 157–166.
14. Puchelle E, Zahm JM. Influence of rheological properties of human bronchial secretions on the ciliary beat frequency. *Biorheology* 1984; **21**: 265–272.
15. Gyi A, O'Callaghan C, Langton JA. Effect of halothane on cilia beat frequency of ciliated human respiratory epithelium *in vitro*. *British Journal of Anaesthesia* 1994; **73**: 507–510.