Fluorocarbon-23 Measure of Cat Cerebral Blood Flow by Nuclear Magnetic Resonance

James R. Ewing, MS, Craig A. Branch, PhD, Susan C. Fagan, PharmD, J. A. Helpern, PhD, Robert T. Simkins, DO, Shazad M. Butt, and K. M. A. Welch, MD

We employed fluorocarbon-23 (trifluoromethane) as a nuclear magnetic resonance gaseous indicator of cerebral blood flow in seven cats. Pulsed inhalation of this indicator and switching between two coils allowed the acquisition of both an arterial input and a cerebral response function, making possible multicompartmental curve fits to cerebral uptake and clearance data. The brain-blood partition coefficient for trifluoromethane was 0.9 for both gray and white matter. Fast-compartment blood flows were normal and showed appropriate CO2 reactivity. Slow-compartment blood flows did not demonstrate CO2 reactivity, probably because cranial as well as white-matter blood flows were lumped together in the slow compartment. Although cerebral blood flow was stable during administration of 60% trifluoromethane, the compound did prove to be a mild cardiac sensitizer to epinephrine in five cats. (Stroke 1990;21:100-106)

Trifluoromethane (Freon-23, fluorocarbon-23, or FC-23) has shown promise as a nuclear magnetic resonance (NMR) indicator for measuring cerebral blood flow (CBF).1-4 To date, however, multicompartment blood flow studies have not been reported, partly because no estimate of the arterial input function has been available. A two-compartment measurement of CBF is essential in surface coil experiments because the volume from which signal arises is not well defined and includes both gray and white matter in significant proportions. We report a two-compartment NMR determination of CBF in cats, a description of the side effects of FC-23 administration, and a reassessment of the brain-blood partition coefficient, $K_{ab}$, of FC-23 in cat brain.1,2

**Materials and Methods**

For the NMR CBF studies, surgical anesthesia with 20 mg/kg i.m. ketamine and 1.5 mg/kg i.m. xylazine was induced, the cat's cranial muscles were reflected, and a 2-cm-diam. single-turn surface coil was fixed against the skull. One femoral artery and one femoral vein were cannulated, and arterial blood was allowed to flow through Tygon tubing to a 5-mm-i.d. six-turn solenoid coil and then to the vein (Figure 1). The other femoral artery and vein were cannulated so that blood pressure and blood gases could be measured and fluids or drugs could be infused. The cat was then placed in a nonmagnetic animal holder and positioned in a 1.9-T topical magnetic resonance5 magnet (Oxford Instruments, Oxford, England). Once in the magnet, the cat was paralyzed with 0.08 mg/kg pancuronium bromide and ventilated at a rate of 27 respirations/min with a tidal volume of 50 ml, through 9 m of 3.5-mm-i.d. Tygon tubing. Anesthesia was maintained with 2 mg/kg i.v. ketamine and 5 mg/kg i.v. thiopental every 40 minutes, a strategy that in dogs produces a stable CBF at approximately 80% of normal values.6

In a typical NMR CBF experiment, a sequence of normocapnia, hypercapnia, normocapnia was used. All cats were ventilated with a mixture of 30% O2 and 70% N2 during nonstudy normocapnic conditions and with a mixture of 30% O2, 65% N2, and 5% CO2 during nonstudy hypercapnic conditions. After completion of the CBF studies, each cat was killed, and its brain was removed and used to estimate the solubility of FC-23 in brain.6

NMR procedures for both data acquisition and data processing are substantially those reported.7 PIN diode7 switching was used to interleave the arterial and cerebral signals with repetition times for data acquisition of approximately 200 msec for each coil; 64 free induction decays (FIDs) were thus accumulated every 13.1 seconds. The accumulated FIDs were exponentially weighted (line broadening of 20 Hz) and Fourier-transformed, and then the absolute value of the spectral resonance area was determined. This integrated area was proportional to the concentration of FC-23 in the brain or the artery, plus noise.
Reflecting our interest in the total signal, the signal-to-noise ratio (S/N) was calculated as S/N (root mean square of signal integrated over interval) / (root mean square of noise integrated over interval). This calculation of S/N, although related, is not the traditional NMR calculation of S/N.8

Using the first protocol in 10 cats, after 8 minutes of data collection to determine baseline noise, N₂ was replaced with FC-23 for 6 minutes, giving inhaled concentrations of 65% by volume. FC-23 uptake and clearance was followed in cerebral tissue for 27 minutes (Figure 2). Other than the indicator and its concentration, these experiments essentially duplicate our earlier ones using chlorofluorocarbon-22 (CFC-22).2 However, with FC-23 as the indicator, we did not obtain a sufficiently high S/N to reliably fit two compartments of blood flow to the uptake and clearance data.

Two- and sometimes three-compartment CBF studies were achieved using the second protocol in seven cats by repeated administrations of FC-23. After 8–10 minutes of data collection to determine baseline noise, N₂ was repeatedly replaced with FC-23 during 2-minute intervals. Six to ten of these pulses were performed for each CBF study, and the total time for accumulation of NMR data was 56.2 minutes.

A Van Slyke apparatus9 was used to estimate \( \lambda_{\text{brain}} \) for FC-23. Brains were taken from seven 3–5-kg ketamine-anesthetized cats, and gray and white matter were dissected out, minced, diluted with varying amounts of distilled water (30–90% by volume for gray and 50–90% for white matter), and homogenized. Using methods described elsewhere,9,10 the samples were injected into the chamber of the Van Slyke apparatus and the solubility of FC-23 in the tissue was calculated by fitting a least-squares regression line of solubility versus percent water, giving an estimated zero intercept for each cat. The mean zero intercept was then calculated by averaging the weighted slopes.11 Blood was treated in a similar manner, except that no dilution was necessary. The values for \( \lambda_{\text{brain}} \) and \( \lambda_{\text{white}} \) were estimated by dividing the extrapolated solubility of FC-23 in the tissue at 39°C by the solubility of FC-23 in blood at 39°C. Variance was estimated using standard methods for ratios.12

The octanol-water partition coefficient13–15 of FC-23 was also estimated by Van Slyke methods. Three solubility determinations were made for octanol, and seven solubility determinations were made for water.

In the second study, cardiac sensitivity to epinephrine was studied via lead-II electrocardiography (ECG) in five ketamine-anesthetized cats. Electroencephalogram (EEG) was monitored via right frontal, parietal, and occipital subdural needle electrodes. The cats were ventilated with a control mixture of 30% O₂, the balance N₂. For 10 minutes before the start of the cardiac sensitivity study and during the test condition, the ventilation mixture was switched to 30% O₂, the balance FC-23. A total of four to six cardiac sensitivity studies were performed by alternating control and 70% FC-23 mixtures, followed by another control and a 40% CFC-22 inhalation cardioc sensitivity study. During each study, ECG, EEG, and systemic arterial blood pressure (BP) were recorded before, during, and after infusion of 0.1 \( \mu g/kg \) (two cats), 1 \( \mu g/kg \) (two cats), or 10 \( \mu g/kg \) (one cat) over 10 seconds.

In the third study, an indicator-dilution experiment was employed to assess whether CBF was altered in ketamine/thiopental-anesthetized cats when FC-23 was administered at 60% by volume. Nine cats were surgically prepared as in the NMR CBF experiment and ventilated with a control mixture of 30% O₂, the balance N₂. For 1 hour before the start of this third study, the ventilation mixture was switched to 30% O₂, 10% N₂O, the balance N₂. Ten minutes before CBF measurement, N₂ was replaced with FC-23. At time \( t = 0 \), N₂O was replaced by N₂ and simultaneous arterial and cerebral venous blood samples were taken for up to 60 minutes. At the end of the run, the blood samples were analyzed for N₂O concentration using a modified trace N₂O monitor (Sensors Inc., Grand Rapids, Michigan), and CBF was calculated using standard (Kety-Schmidt) practices that produce a well-accepted and model-independent estimate.16,17

The theory behind the indicator-dilution measurement of CBF is well established.18–22 Normally, there are two distinct compartments of blood flow, one for gray matter and another for white matter. Accordingly, the time-dependence of the concentration of
FIGURE 2. Uptake and clearance of trifluoromethane ($^{19}$F Intensity) in artery (foreground, solid line) and brain (background, points) of cat after single, 6-minute administration. Using arterial input function, single-compartment clearance curve is fitted through experimental points (background, solid line).

the indicator in cerebral tissue, as measured by the signal in a surface coil, is

$$C(t) = \sum_{i=1}^{N} W_i C_i(t) + S_n$$

$$= \sum_{i=1}^{N} P_i k_i e^{-k_i t} \int_{0}^{t} C_i(\tau + \Delta_i) e^{k_i \tau} d\tau + S_n \quad (1)$$

where $N$=the number of tissue compartments (1, 2, or 3), representing gray matter, white matter, and possibly extracerebral tissue; $C_i(t)$=the processed NMR signal from the head coil, integrated across an interval that includes the signal above the noise (this processed signal is proportional to the concentration of the indicator in the tissue); $W_i$=a proportionality constant that includes the relative contribution (the weight) of the $i$th tissue compartment to $C(t)$ and the efficiency of detection of the indicator in the $i$th tissue compartment; $P_i$=a proportionality constant, $W_i$ times scaling differences between the amplitude of the arterial signal and the tissue signal for a given concentration of the indicator; $C_i(t)$=the tissue concentration of the indicator in the $i$th tissue compartment as a function of time; $k_i$=the clearance constant of the $i$th tissue compartment; $C_a(t)$=the concentration of the indicator in arterial blood; $\Delta_i$=the time from measurement of the signal in the arterial coil to the arrival of that part of the input at the cerebral tissues; and $S_n$=the additive baseline noise ($S_n$ would be the background count if a radioactive indicator were used). Blood flow in the $i$th tissue compartment is calculated as $f_i = k_i \lambda_i$, where $f_i$=the specific blood flow (in milliliters per 100 g per minute) in the $i$th tissue compartment and $\lambda_i$=the brain:blood partition coefficient for the $i$th tissue compartment.

All two-compartment curve fits proceeded via a maximum-likelihood (ML) analysis of indicator-dilution data. ML convergence was considered to occur when the Newton-Raphson procedure employed arrived at an extreme of the object function (the natural logarithm of the likelihood), the number of iterations was $<100$, values for the clearance parameters ($k, s$) lay between 0.0 and 3.0 min$^{-1}$, no two clearance parameters were essentially the same (i.e., values were not within 10% of each other), and the relative weight of a compartment was $>5\%$ of the total.

In attempting three-compartment curve fits, a simplex algorithm was used because of its good stability in (possibly) overdetermined models. Three-compartment curve fits were accepted as convergent when the maximum percent difference between two
TABLE 1. Solubility of Trifluoromethane in Cat Brain, Cat Blood, and Water

<table>
<thead>
<tr>
<th>Solute</th>
<th>n</th>
<th>Solubility x 10^-6 (mol/l/mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gray matter</td>
<td>6</td>
<td>20.11±2.96</td>
</tr>
<tr>
<td>White matter</td>
<td>7</td>
<td>20.12±3.12</td>
</tr>
<tr>
<td>Blood</td>
<td>21</td>
<td>22.32±2.40</td>
</tr>
<tr>
<td>Water</td>
<td>7</td>
<td>18.34±2.01</td>
</tr>
</tbody>
</table>

Data are mean±SD.

vertices of the search space of the object function (the natural logarithm of the likelihood) was <10^-3, the number of iterations was <1000, values for the clearance parameters lay between 0.0 and 3.0 min^-1, no two clearance parameters were essentially the same, and the relative weight of a compartment was >5% of the total.

For an assumed time delay Δ between measurements of the arterial and head curves, the rates and weights of two or three flow compartments and additive noise were iteratively fitted to find ML estimates (MLEs) of them. We varied Δ across a range of values until a maximum in its likelihood and a set of MLEs were found. The set of parameters that were estimated were compartmental weights [P_i] and rates [k_i] where i = 2 or 3, S_m, and Δ. Six parameters were estimated for a two-compartment fit and eight for a three-compartment fit.

All values are reported as mean±sample standard deviation (SD).

Results

The Van Slyke solubilities of FC-23 in gray and white matter, blood, and water are summarized in Table 1 and lead to estimates of 0.90±0.16 for λ_g for gray matter and 0.90±0.17 for λ_w for white matter. 60% of previously published estimates is identical to that in a hydrogen clearance measurement of cortical blood flow in ketamine-anesthetized cats.28 Mean CBF measured by NMR of FC-23 does not differ significantly from that calculated in the Kety-Schmidt technique (t=0.257, df=14, p>0.8); hypercapnic fast-compartment blood flow was 141.3±35.1 ml/100 g/min and that for the slow compartment was 12.6±5.8 ml/100 g/min.

We hypothesized that the second, slow compartment might have contained some admixture of extracerebral epinephrine increased heart rate and BP significantly, and arrhythmias were induced during the control state, CFC-22 inhalation, and FC-23 inhalation.

Control CBF measured by the Kety-Schmidt technique was 41.0±11.6 ml/100 g/min (Paco_2 30.6±3.3 mm Hg), very close to that obtained in N_2O-anesthetized cats using the intra-arterial injection of xenon-133 technique with stochastic analysis.26 In the nine cats, CBF during inhalation of 60% FC-23 was not different from control CBF. The mean difference in CBF was -1.9 ml/100 g/min, and SD was 12.16. With this sample size, using a paired t test, we could not reject the null hypothesis that CBF was the same during the control state and FC-23 inhalation (p>0.6).

The T1 of FC-23 is acceptably short for use as an NMR indicator; using an inversion-recovery procedure27 in a ketamine-anesthetized cat, we estimated the in vivo T1 of the fluorine-19 in FC-23 to be 1.22±0.02 seconds. S/N for FC-23 in the indicator-dilution CBF studies was 17.8±2.3, not as good as the figure of 45 obtained in three CFC-22 runs. To improve S/N, we employed a pulsed-input function.

As shown in the oval window of Figure 3, the NMR spectrum of FC-23 is a doublet. The main section of Figure 3 displays data acquired over 55.9 minutes, from 256 accumulated FIDs, processed as described in "Materials and Methods." Arterial data are in the foreground, and head data are in the background. The magnitude of each point was proportional to the intensity of the fluorine-19 signal and therefore to the concentration of FC-23. The curve in the foreground, obtained from the arterial coil, was therefore proportional to the concentration of FC-23 in the arterial blood and the curve in the background was proportional to the concentration of FC-23 in brain tissue.

Using the arterial curve as an input function, the uptake and clearance head curve data was iteratively fitted by ML procedures, estimating the parameters of a two-compartment CBF model, which are summarized in Table 2. When 0.90 is used for both λ_g and λ_w, these clearance rates lead to a normocapnic fast-compartment (gray matter) blood flow of 70.2±15.3 ml/100 g/min, a slow-compartment blood flow of 11.7±4.5 ml/100 g/min, and a mean CBF of 39.0±18.8 ml/100 g/min. The fast-compartment blood flow is consistent with what is expected in this model; our values for the fast-compartment blood flow were nearly identical to that in a hydrogen clearance measurement of cortical blood flow in ketamine-anesthetized cats.28
clearance components. Accordingly, we attempted to fit three compartments of flow to our data. The results of those curve fits that we accepted as convergent are summarized in Table 3. Three hypercapnic studies and two normocapnic studies were available. In only one pair (runs 380 and 381) was a normocapnia-hypercapnia sequence available. The fast-compartment blood flow estimates were comparable to their corresponding two-compartment estimates. In addition, an intermediate clearance compartment (assuming \( \lambda_{\text{brain}} \) to be 0.9) had blood flow values appropriate to white matter.29-30 Further, in this one study in which we have a normocapnia-hypercapnia sequence, this intermediate compartment did show \( CO_2 \) reactivity. Finally, there also appears to be a sizable compartment with a very slow clearance that could represent blood flow in the skull, based on decreased clearance during hypercapnia.31 This could explain the lack of \( CO_2 \) reactivity in the second compartment of the two-compartment fits.

Discussion

Using FC-23 as an indicator and an NMR surface coil for detection, two and sometimes three compartments of CBF were observed when the indicator was administered in 2-minute pulses to ketamine/thiopental-anesthetized cats. To our knowledge, this is the first publication of the input function of FC-23 in an experiment using NMR to measure CBF. As we have previously demonstrated,7 atraumatic measurements of input are possible using an anesthetic gas monitor.

FC-23 had mild narcotic side effects in the ketamine/thiopental-anesthetized cats but no effect on CBF when administered at a concentration of 60% by volume in \( O_2 \). In ketamine-anesthetized cats, FC-23 was a mild cardiac sensitizer to epinephrine. Since cardiac sensitization to epinephrine in the presence of Freons varies across species,32-35 the effects of FC-23 in humans must await further studies in primates.

If the value of 0.90 for both \( \lambda_{\text{gray}} \) and \( \lambda_{\text{white}} \) is accepted, fast-compartment blood flows are appropriate to this model, during both normocapnia and hypercapnia. An octanol:water partition coefficient for FC-23 of 4.1 means that this physiologically inert compound of very simple structure is freely diffusible in brain.13-15 No sign of diffusion limitation could be observed in our fast-compartment data; hypercapnic
(5% CO₂) blood flows were, appropriately, almost exactly twice those during normocapnia, in agreement with numerous studies using other methods of measuring regional CBF (see References 4 and 26). On the other hand, slow-compartment blood flows were slower than expected and did not show appropriate CO₂ reactivity, probably because extracerebral (cranial) blood flow was also being measured in the skull and because of the nonlinearity of surface coil sensitivity. Blood flow in the skull, lumped into the second compartment, was responsible for both the low second-compartment blood flows and their lack of CO₂ reactivity.

Our findings, although self-consistent, disagree with those of previous studies of CBF using FC-23 as an indicator. Much of the disagreement hinges on the value of λ₉ for FC-23. Our Van Slyke estimates were 60% of other estimates using NMR. As a check on our methods, we measured the solubilities of various gases in water and blood and obtained good agreement with previously published estimates. Our estimate of λ for CFC-22 was also consistent with a previously published value. Recently, another laboratory has used NMR and measured λ for FC-23 to be approximately 0.9 for both gray and white matter. A potential for application of an NMR FC-23 indicator-dilution CBF study in humans is evident. It may eventually be feasible to image human CBF with such a technique's considerable radiation doses. Such a development awaits the demonstration that usefully high concentrations of a potential indicator can be administered to humans without harmful side effects.

### Table 2. Parameters for Two-Compartment Model of Cerebral Blood Flow in Seven Cats: Nuclear Magnetic Resonance Using Pulsed Input of Trifluoromethane

<table>
<thead>
<tr>
<th>Run</th>
<th>Condition</th>
<th>k₁ (min⁻¹)</th>
<th>k₂ (min⁻¹)</th>
<th>Pᵢ/ₚ (Pᵢ+P₂)</th>
<th>Δᵣ (sec)</th>
<th>S₀ (% peak)</th>
<th>Paco₂ (mm Hg)</th>
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<tbody>
<tr>
<td>354</td>
<td>N</td>
<td>0.88</td>
<td>0.23</td>
<td>0.51</td>
<td>4.0</td>
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<td>35.4</td>
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<tr>
<td>356</td>
<td>H</td>
<td>1.42</td>
<td>0.14</td>
<td>0.71</td>
<td>0.0</td>
<td>4</td>
<td>61.5</td>
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<tr>
<td>360</td>
<td>N</td>
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<td>0.07</td>
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<td>0</td>
<td>2</td>
<td>37.4</td>
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<tr>
<td>362</td>
<td>H</td>
<td>2.21</td>
<td>0.18</td>
<td>0.59</td>
<td>0</td>
<td>3</td>
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<tr>
<td>370</td>
<td>N</td>
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<td>0.12</td>
<td>0.53</td>
<td>1.5</td>
<td>1</td>
<td>31.5</td>
</tr>
<tr>
<td>371</td>
<td>H</td>
<td>1.85</td>
<td>0.21</td>
<td>0.54</td>
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<tr>
<td>377</td>
<td>N</td>
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<td>9.0</td>
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<td>0.10</td>
<td>0.54</td>
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<td>4</td>
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<tr>
<td>381</td>
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<td>0.97</td>
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<td>6.0</td>
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<td>390</td>
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<td>5</td>
<td>67.0</td>
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<tr>
<td>Mean±SD</td>
<td>N</td>
<td>0.78±0.17</td>
<td>0.13±0.05</td>
<td>0.45±0.12</td>
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<td>—</td>
<td>34.2±2.2</td>
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<tr>
<td>Mean±SD</td>
<td>H</td>
<td>1.57±0.39</td>
<td>0.14±0.064</td>
<td>0.57±0.18</td>
<td>—</td>
<td>—</td>
<td>60.2±6.9</td>
</tr>
</tbody>
</table>

Data are maximum-likelihood estimates. N, normocapnia; H, hypercapnia.

### Table 3. Parameters for Three-Compartment Model of Cerebral Blood Flow in Cats: Nuclear Magnetic Resonance Using Pulsed Input of Trifluoromethane

<table>
<thead>
<tr>
<th>Run</th>
<th>Condition</th>
<th>k₁ (min⁻¹)</th>
<th>k₂ (min⁻¹)</th>
<th>k₃ (min⁻¹)</th>
<th>Pᵢ/ₚ (Pᵢ+P₂+P₃)</th>
<th>Pᵢ/ₚ (Pᵢ+P₂+P₃)</th>
<th>Pᵢ/ₚ (Pᵢ+P₂+P₃)</th>
<th>Paco₂ (mm Hg)</th>
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<tbody>
<tr>
<td>371</td>
<td>H</td>
<td>2.195</td>
<td>0.447</td>
<td>0.3730</td>
<td>0.406</td>
<td>0.0656</td>
<td>0.147</td>
<td>54.0</td>
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<td>0.3777</td>
<td>0.270</td>
<td>0.0869</td>
<td>0.452</td>
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<tr>
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<td>0.222</td>
<td>0.289</td>
<td>0.0722</td>
<td>0.155</td>
<td>32.1</td>
</tr>
<tr>
<td>381</td>
<td>H</td>
<td>1.229</td>
<td>0.406</td>
<td>0.3923</td>
<td>0.274</td>
<td>0.0229</td>
<td>0.320</td>
<td>47.6</td>
</tr>
<tr>
<td>396</td>
<td>H</td>
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<td>0.478</td>
<td>0.2161</td>
<td>0.449</td>
<td>0.0697</td>
<td>0.073</td>
<td>67.0</td>
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Data are simplex estimates. H, hypercapnia; N, normocapnia.
References


Key Words: cerebral blood flow • nuclear magnetic resonance • cats
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