Direct measurement of nitrous oxide kinetics†

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Editor’s key points

• The digit symbol substitution test measures effects of low doses of anaesthetic on cognition.
• The test was shortened to measure the dynamics of onset and offset of anaesthetic effect.
• The effects were matched with modelled cerebral nitrous oxide concentrations.
• The rate of cerebral equilibration is consistent with a blood flow of 120 ml 100 g⁻¹ min⁻¹.

Background. Using conscious subjects, measurement of the effects of low concentrations of anaesthetic agents can allow the dynamics of onset and offset of the agent to be measured and kinetic values estimated. However, the tests have to be rapid and preferably assess cerebral function.

Methods. We used a short version of the digit symbol substitution test (DSST) that allowed frequent measurement of the impairment caused by nitrous oxide. We compared 10 min of onset and offset of breathing 5% and 30% nitrous oxide in 30% oxygen, compared with 30% oxygen only. End-tidal nitrous oxide concentrations were used to predict the concentration in a central compartment, according to a range of T₁/₂ values chosen to be consistent with possible cerebral blood flow values.

Results. We studied 19 volunteers and estimated a mean response. Only 30% nitrous oxide decreased the DSST. When DSST scores were related to the values in the predicted central compartment, the best dose–effect relationship was found when the T₁/₂ was 37 s, consistent with a regional blood flow of about 120 ml 100 g⁻¹ min⁻¹.

Conclusions. The onset of nitrous oxide effect on DSST is rapid, consistent with the perfusion of metabolically active cerebral cortical tissues. The rate of onset is greater than previous measures based on a motor test which involved the function of subcortical structures in the central nervous system.

Keywords: cerebrovascular circulation; nitrous oxide; pharmacokinetics

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Transfer of drugs into and out of the brain is a principal interest of anaesthetic kinetics. To measure anaesthetic effects, a valid means of quantification is required. With an adequate measure of anaesthetic effect, such as a test of cognitive function, the precise time course of the onset and offset of the effects should provide insight into the cerebral kinetics of an anaesthetic agent. Current measures of anaesthetic effect are not sufficient. The standard measure of ‘depth’ of anaesthesia is conventionally defined using a single categorical feature, most frequently, the presence or absence of movement after a standard stimulus,¹ and forms the standard of anaesthetic potency, MAC. More recently, EEG signals have been processed to give a continuously distributed value, quantified using an arbitrary scale to indicate brain activity or ‘depth’ of anaesthesia. Such signals probably measure anaesthetic effects on cerebral cortical activity but do not necessarily measure effects mediated by other parts of the nervous system, such as the spinal cord. Conventional measures of anaesthetic potency such as MAC are mainly mediated by the spinal cord.² ³ The processed EEG signal gives a useful continuous graded measure of anaesthetic effects that can be followed as anaesthetic levels change, which allows drug kinetics to be assessed.⁴ However, because EEG signal processing incurs a delay for signal acquisition and analysis, these measures lag actual brain activity.⁵ Other measures of graded response may avoid this delay, but these do not assess cortical function.⁶ ⁷

Drug actions can be quantified if they cause responses that can be assessed by a graded measure. Using subanaesthetic doses, we described the dose–effect relationships for different subjective and objective features effects of nitrous oxide, sevoflurane, and alcohol,⁸ and used a similar measure to assess the kinetics of nitrous oxide effects with repeated measures of a psychomotor test.⁹ These tests can be done promptly, and should allow better assessment of kinetics than the processed EEG which incorporates delays.⁵ ¹⁰ ¹¹ Our findings were consistent with previous kinetic data for nitrous oxide. However, we were concerned that the test we had used involved a substantial, subcortical, motor component. Since the cerebral cortex is probably the most relevant site of...
anaesthetic action, in relation to nervous system functions such as awareness, we wished to use a test that more closely reflected cognitive function. We developed a measure to follow the kinetics of cognitive function, mediated predominantly by cortical activity and less dependent on motor activity. The digit symbol substitution test (DSST) assesses reasoning ability but usually takes 90 s to complete, which would be too imprecise to follow the onset of nitrous oxide accurately. We shortened the test to 40 s, and determined the best fit between drug effect and predicted central nervous system level during nitrous oxide administration.

**Methods**

Lothian Research Ethics Committee 1 approved the study. We recruited healthy volunteers who were not taking any centrally acting medication, and obtained their written consent. Female subjects participated within the first 2 weeks of their menstrual cycle. The subjects were asked not to consume any alcohol for 12 h before the study, and to get a good night’s sleep. They took a light breakfast or lunch and were allowed their usual caffeine intake until 4 h before testing. Other drinks were allowed up to 2 h before testing.

Each subject attended for a single session, containing three half-hour periods. In each period, a different gas mixture was given. The gases were given in a random order. The gas mixtures were 30% oxygen, balance nitrogen; 5% nitrous oxide, 30% oxygen, balance nitrogen; or 30% nitrous oxide, 30% oxygen, balance nitrogen. The test gas mixtures were made up by one investigator, into 80 litre Douglas bags, in a separate room before the start of the study.

For the tests, subjects sat comfortably at a desk and breathed through a tight fitting silicone rubber facemask connected to a two-way non-rebreathing valve (Hans Rudolph Inc., Kansas City, MO, USA), and a wide-bore breathing system. The input gas was initially air. When required, the Douglas bag was switched into the supply. The subject was instructed to take two large breaths to clear the supply tube and then breathe normally. Exhaled gas was actively scavenged using a T-tube reservoir system. A Datex CD2-O2 Normocap® analyser (Datex, Helsinki, Finland) continuously sampled from the mask and measured concentrations of nitrous oxide and carbon dioxide. The analyser was calibrated before each experiment, using calibration gas mixture from the manufacturer. The signals were acquired (Micro 1401, CED Ltd, Cambridge, UK) and recorded (Spike 2, CED) on a computer. The CO₂ signal was used to mark the end of expiration and nitrous oxide values averaged from the preceding 50 ms. Artifacts caused by coughs or swallowing were excluded.

We tested subject performance with a DSST that we have used before. Each test sheet of paper shows a key which matches nine single digits, 1–9, with simple symbols. Below this reference key is a table of boxes containing random single digits. The subject is given the test sheet face down. At a signal to start, the paper is turned over and the subject starts to write the appropriate symbol in the box under each successive number, as quickly as possible. The first 10 symbol substitutions allowed familiarization with the symbol set: as the subject continued to write symbols, timing was started and the number completed in the next 40 s was recorded. (This time is shorter than the usual time of 90 s.) A fresh sheet, with a different digit-symbol key and a new set of numbers, was used for each test. Comparison of the effects of different gases (0%, 5%, and 30% nitrous oxide) was done using the last two test results of each subject, at the end of the 10 min episode of gas breathing. To relate the effect of 30% nitrous oxide to the predicted central concentration, the means of the scores and the predicted central nitrous oxide values for all the subjects were calculated for each time point, starting with the two tests done just before the test gas started, and ending at the end of the washout period.

The test gases were chosen in a random order. The identity of the test gas was concealed from the subject and the investigator responsible for test administration, and no communication was allowed between the two investigators during the three test procedures. Each half-hour period started with the subject breathing air via the breathing system and mask. After two practice tests at the start of a 10 min run-in period, tests were done just before, after, and at the end of breathing the test gas and again after breathing air, each given for 10 min. The sequence of tests and gas breathing is shown in Figure 1. After each period, the mask was removed for a 5 min break before the next period started. After the three periods, the subjects were asked to state which gas they thought was which.

End-tidal nitrous oxide values at each time $C_{ET}(t)$ and the time of each end-tidal value $t$ were used to predict the concentration in a central compartment, using the following equation:

$$\frac{dC_{central}(t)}{dt} = k(C_{ET}(t) - C_{central}(t))$$

In this equation, $k$ is the rate constant for the transfer of nitrous oxide between the arterial blood (assumed to have the same partial pressure as end-tidal gas) and the central compartment. Values for $C_{central}$ were calculated using a Visual Basic function for Microsoft Excel that we have used previously. This function uses log-linear interpolation and numerical convolution to calculate values of $C_{central}$ based on the measured end-tidal values and a given value of $T_{1/2}$, which is the half-time for equilibration. This is related to the rate constant by the equation

$$T_{1/2} = \frac{\ln 2}{k}$$

To assess how well the predicted central concentration was related to the effect, we plotted the mean of the test results against the mean predicted central concentration. If the relationship was perfect, then the plot should follow a single line during onset and offset. If the test result and
the central concentration were not in phase during onset and offset, then looping or hysteresis would be evident. The area of the loop was estimated and expressed as positive, if the effect was ‘ahead’ of the central concentration, that is, if the central effect (DSST decrease) came on more rapidly than the predicted central concentration increased. In the plot of absolute values that we have used, this would form an anticlockwise loop.

Data were managed using Excel 2003 (Microsoft, Seattle, WA, USA) and statistical analysis was with Prism (version 5.00, GraphPad Software, San Diego, CA, USA).

Data are presented as mean (SD) unless otherwise stated.

Results

We recruited 42 subjects, but 13 failed to attend. A leak in the gas analyser sampling line required 10 sets of data to be rejected. We acquired valid data from six females and 13 males, mean age 21 yr, range 19–23, weight 72 (12) kg, height 178 (12) cm. The end-tidal nitrous oxide concentration at the end of the administration of 5% nitrous oxide was 4.0 (0.9%) and after 30% nitrous oxide was 27.6 (1.1). Subjects could not identify 5% nitrous oxide consistently, but only one subject was unable to identify 30% nitrous oxide correctly. (This subject showed no change in DSST.)

To compare the effects of the three gases, we compared the sum of the scores at the end of gas administration (i.e. the maximal effect, tests 8 and 9 in the sequence). As expected, DSST was affected by 30% nitrous oxide (repeated-measures analysis of variance $P < 0.07$, Dunnett’s test for 30% vs 0%, $P < 0.05$), with a mean difference of $-2.0$ (95% confidence interval $-3.95$ to $-0.05$). There was no difference between the test results using air and using 5% nitrous oxide.

The relationship between the mean DSST values at each test time and the predicted central concentrations at these times is shown in Figure 2, for $T_{1/2}$ values set from 12.5 to 75 s. These plots show looping: with the least $T_{1/2}$, the predicted brain concentration ‘leads’ the DSST decrease, and when $T_{1/2}$ is 50 s and greater, the DSST decrease ‘leads’ the predicted brain concentration. The relationship between loop area and the $T_{1/2}$ used to predict the central concentration is also shown fitted to a quadratic relationship. The $T_{1/2}$ when the area was zero would be 37 s with 95% confidence values of 32, 41.

Discussion

We modified the DSST to allow frequent measurements. The disadvantage of this approach is that a smaller duration of sampling will reduce the sensitivity and increase the variation in the test result. This would not be suitable if the test was intended to assess accurately the performance of a single subject, which was the initial intention of this particular test design. However, in this study, we had a sufficiently large sample of subjects to allow mean changes to be used as an index of the drug effect. We had to allow sufficient time between tests to change the test materials, and had to limit the tests to the most relevant times of the study to avoid tiring the subjects and sustaining motivation, so testing was not done at those times when the changes in effects would be less pronounced. We also used a model based on mean responses. The ‘noise’ in this abbreviated test is too great to allow fitting individual dose–response relationships. Consequently, we are using a ‘population’ model, sometimes known as the ‘giant rat’ approach, and should only draw very general conclusions from the results. When this approach is applied to binary data such as

![Time plan of the study](https://academic.oup.com/bja/article-abstract/109/5/776/305837/778)

**Fig 1** Time plan of the study. Each test period consisted of a 30 min period of mask breathing, separated into 10 min of air breathing, test gas for 10 min, and then reversion to air. Two practice DSSTs were done for 40 s, separated by 20 s, at the start of air breathing. Two further tests for baseline measurements were done starting 2.5 min before switching to the test gas and then tests were repeated at 1 min intervals for 5 min after the gas change occurred. A similar plan was used for the switch back to air.
response to surgical stimulus, the accuracy of estimates of the gradient of the dose–response may be limited.\textsuperscript{14} However, when used for continuous measures, it is a suitable and parsimonious method of modelling responses.\textsuperscript{15,16}

The test gases were given in a random order. We were unable to find an order effect, when we analysed the results at the end of the test gas administration. Since the effects of 5% nitrous oxide were not detected (as might be predicted from previous studies),\textsuperscript{12} it was not expected that the very low concentrations found after more than 25 min of washout of 30% nitrous oxide would have had a detectable effect. Although the test results at the end of the administration of 30% nitrous oxide showed a slight increase, we could not detect any overall change in the test results from the start to the end of each gas administration period (MANOVA, using factors of subject, test gas, and time).

We found changes in DSST during the onset and offset of the central effects of nitrous oxide consistent with an equilibration half-time of 30–40 s. The predominant factor controlling the rate of equilibration between arterial blood and central tissue is tissue perfusion. The equilibration rate constant ($k$) can be related to the relative perfusion of the tissue and the tissue/blood partition coefficient with the following equation:

\[
k = \frac{\text{blood flow}}{\text{tissue volume} \times \text{tissue/blood partition coefficient}}
\]

and this is related to $T_{1/2}$:

\[
T_{1/2} = \frac{\log_2(2)}{k}
\]
A $T_{1/2}$ of 35 s is equivalent to a rate constant of $\sim 1.2 \text{ min}^{-1}$. Since the blood/tissue partition coefficient for nitrous oxide in nervous tissue is $\sim 1$, and the specific gravity of brain tissue is close to unity, we must conclude that the perfusion of the site of action that is assessed by the DSST is of the order of 120 ml 100 g$^{-1}$ min$^{-1}$. This is greater than most current measures of cerebral blood flow, and certainly inconsistent with simulation-based values and previous measures obtained with other anaesthetic agents used during clinical levels of anaesthesia. However, our estimate requires qualification, in several respects. The most important is that there is probably little delay in the response of the site, in contrast to the delay imposed by acquisition and processing of an EEG-based signal. We also took advantage of the ‘off-line’ analysis used in this study to temporally align the signals. Thus, the midpoint of the time of the DSST measurement was related to the mean predicted brain concentration values for exactly the same time period. The second is that in conscious subjects, the importance of various other factors such as changes in blood flow, diffusion, neural input, or ‘neural inertia’ are less, whereas these may act to delay drug action during anaesthesia.

Other researchers, using a greater range of doses of anaesthetic agent, could model the entire dose–response relationship (pharmacodynamic features) of drug and effect, usually with a sigmoid model which requires data covering a range of effects from minimal to maximal. The fundamental assumption of this approach is that drug effect is directly related to drug concentration, in a time-invariant manner. An assumed mathematical function such as a standard sigmoid relationship then allows a fit to be made for each subject, although in some studies, fits are not necessarily obtained for all subjects. For several reasons, we had to use a group mean approach. Clearly, we had to limit the dose range we used. There is considerable range of individual responses to nitrous oxide. One subject in this study was unable to correctly identify 30% nitrous oxide, and in other studies, subjects became unresponsive when given 40%. The lower part of the dose–response relationship for nitrous oxide and reduction in the DSST score is not linear. However, a part of the dose–response relationship for nitrous oxide then allows a fit to be made for each subject, allowing a range of effects from minimal to maximal. The fundamental assumption of this approach is that drug effect is directly related to drug concentration, in a time-invariant manner. An assumed mathematical function such as a standard sigmoid relationship then allows a fit to be made for each subject, although in some studies, fits are not necessarily obtained for all subjects. For several reasons, we had to use a group mean approach. Clearly, we had to limit the dose range we used. There is considerable range of individual responses to nitrous oxide. One subject in this study was unable to correctly identify 30% nitrous oxide, and in other studies, subjects became unresponsive when given 40%. The lower part of the dose–response relationship for nitrous oxide and reduction in the DSST score is not linear. However, a loop display such as we used, to assess goodness of the fit of predicted central concentration to the drug effect, is not disadvantaged by a curvilinear relationship: a best fit is still indicated by a minimal area between the onset and offset parts of the plot. The use of group mean values may obscure some details in these relationships, because of the varied responsiveness to nitrous oxide. Although the single-compartment model we used to predict central concentrations from end-tidal values is simplistic, it has been used by others. However, even if the assumptions involved are not valid, such as equivalence of arterial with end-tidal values, or only a small delay in transit from the lung to the brain, factors such as these would reduce the calculated rate constant, and cannot explain our finding of a very large value. In fact, conscious subjects are less likely to have features, such as impaired gas exchange, or slow circulation, which invalidate the assumptions of this model.

In a similar study of conscious volunteers using a motor test, we found a greater half-time, of the order of 2 min, equivalent to a rate constant of about 0.34 min$^{-1}$. This could be because a substantial component of that test consists of limb movement, mediated by different structures that could have less blood flow. Studies in men report blood flow values in the cervical spinal cord that are much less than cerebral values. However, discrimination between grey and white matter is important and recent animal studies using nuclear magnetic resonance found that when measurements were confined to grey matter, spinal cord and cerebral blood flows were similar.

Studies of the dynamics of other continuous agent–response relationships are of interest. These range from spinal reflexes to the brainstem, but generally bispectral index-based responses seem to be more prompt, perhaps because the cortex is better perfused. The most likely argument for the large value for the rate constant we have found is that we have studied a response that is dependent in large part on a local highly perfused part of the brain. Specific tasks can increase regional cerebral perfusion by 50%, and it may be that the act of performing the test increases the rate of onset and offset of the anaesthetic in the specific cortical region responsible. In our previous study, we only found significant effects in a proportion of subjects. The test we have developed in the present study has been able to quantitatively assess the rate of onset and offset of a specific anaesthetic effect in a complete group of subjects.

**Declaration of interest**

None declared.

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