CHANGES IN AMPLITUDE AND LATENCY OF THE P300 COMPONENT OF THE AUDITORY EVOKED POTENTIAL WITH SEDATIVE AND ANAESTHETIC CONCENTRATIONS OF NITROUS OXIDE


SUMMARY

The P300 component of the auditory evoked response was recorded from six subjects whilst they listened via headphones to a series of clicks which were interrupted unpredictably by a tone burst. They were instructed to press a button as quickly as possible after hearing the tone whilst breathing first air and then a series of increasing concentrations of nitrous oxide. Both the amplitude and the latency of the P300 changed in a dose-dependent manner with nitrous oxide. At nitrous oxide concentrations which prevented recall of any events that occurred whilst breathing the gas, four subjects continued to respond to the tone by pressing the button. In three subjects, the P300 wave was still detectable with a nitrous oxide concentration at which the task was no longer performed. These results show that there is retention of the ability to perform a reaction time task when there is a complete loss of recall of the task. There may be some recognition of an auditory stimulus, as manifest by a P300 wave, albeit reduced greatly in amplitude, in the absence of a motor response to it. The P300, therefore, merits investigation as a tool for studying conscious awareness under anaesthesia.

KEY WORDS


The widespread use of light anaesthesia in conjunction with neuromuscular block produces the potential for patients to be awake but unable to communicate this state to the anaesthetist. However, there is still no reliable method of assessing if an apparently anaesthetized patient is awake or not, and further research is needed to study the interface between the conscious and the unconscious state.

Several methods have been evaluated during surgical anaesthesia and, of these, both the isolated forearm technique and the recording of auditory evoked potentials give some information about various stages of awareness [1]. The isolated forearm technique [2] attempts to detect conscious awareness directly by eliciting specific hand movements on command. Whilst the ability to obey a command under anaesthesia must carry a high risk of conscious awareness, the converse is not true, and it may underestimate the true incidence of awareness [3]. Furthermore, there are technical difficulties with prolonged or repeated occlusion of the limb and with arm movements interfering with the surgical field.

The early cortical components (25–100 ms) of the auditory evoked response show dose-dependent changes in amplitude and latency that are independent of the anaesthetic agent and have been validated as an indicator of awareness against the isolated forearm technique [4]. However, the
method does have disadvantages. First, the evoked potentials are small (0.5 μV) and it takes time to average out the signal from the background EEG. Second, interpretation of the averaged data requires some expertise. Third, these early cortical responses signal only the arrival of a sensory volley at the primary auditory cortex. While this is a pre-requisite for awareness to occur it does not, on its own, imply any form of cognitive processing. An apparently normal early cortical evoked potential may occur in the absence of any conscious awareness of the eliciting stimulus, and when conscious awareness does occur, it does so at an increased latency [5]. Furthermore, using the middle latency responses to predict awareness overestimated its true incidence as detected by the isolated forearm technique [4]. Thus there is no clear relationship between the middle latency responses and conscious awareness. However, later event-related potentials imply a degree of cognitive processing [6] and might, therefore, approximate more closely to a conscious awareness of the stimulus.

Such an event-related potential is the P300. This wave occurs when a regular stimulus is interrupted unpredictably, for example by missing out a stimulus or by replacing it with a different one. The P300 is large (10–20 μV) and has a peak latency of about 300 ms from the unpredictable stimulus. It has been studied with sleep [7], sedative drugs [8], nitrogen narcosis [9] and low doses of nitrous oxide, up to 40% inspired [10–12]. It has also been studied during and after general anaesthesia with isoflurane [13]. This study describes the effect on the P300 in normal volunteers of breathing nitrous oxide to give end-tidal concentrations in the range 20–65%.

SUBJECTS AND METHODS

The study was performed in six anaesthetists who were healthy (ASA I) and of Senior Registrar or more senior status. Approval for the study was obtained from the local Ethics Committee.

Subjects were requested to fast for 5 h before the study and not to drive home afterwards. The subject was made comfortable lying on a bed and a 20-gauge i.v. cannula was placed in the dorsum of the left hand as a precautionary measure. Full resuscitation equipment was available and two anaesthetists (in addition to the subject) were present throughout all experiments.

P300 recordings

Clicks (1 ms duration) were presented to the subject via headphones at a rate of 1 s⁻¹. To elicit a P300 wave, one click was replaced periodically, in a pseudo-randomized fashion, by a tone burst (frequency 1.5 kHz, 100 ms duration) at an average frequency of 1 tone to 9 clicks. The subject was asked to concentrate on the clicks and to press a hand-held button as soon as the tone occurred. The EEG was recorded, using silver-silver chloride adhesive electrodes, from the vertex, referenced to linked ear lobes and with an earth electrode on the forehead. All inter electrode impedances were 2–3 kΩ. The EEG signal was amplified using an optically isolated amplifier and passed to a digital signal averager (NEC portable computer controlling a Cambridge Electronic Designs interface unit via CED SIGAV software). Sampling was triggered by the click preceding the tone and 3000 points were sampled over the next 3 s. The record thus showed the response not only to the tone, but also to the preceding and following clicks. Sixteen of these 3-s sweeps were collected and stored, and an average record formed from them using the SIGAV software. Sweeps contaminated by blinks or other large artefacts were rejected automatically using the amplitude-triggered sweep rejection option of the SIGAV program.

Reaction time recording

A second channel of the averaging system was used to record the time that the subject pressed the button in response to the tone. The shortest reaction time for each group of 16 samples was measured.

Nitrous oxide administration and monitoring

Throughout the experiment, the subject’s oxygen saturation was monitored with a pulse oximeter (Ohmeda Biox 3700) via a finger probe. During nitrous oxide administration the subject’s inspired and end-tidal concentrations of oxygen, carbon dioxide and nitrous oxide were monitored using a mini mass spectrometer (Ohmeda 6000). Mixtures of nitrous oxide and oxygen were delivered from a standard anaesthetic machine (Boyle International 2) via a Bain system to a mouthpiece. The subject wore a nose clip and supported the mouthpiece himself. The gas mixture was breathed until the end-tidal nitrous oxide concentration was stable and a P300 record-
ing was performed. A recording was accomplished in all subjects with the end-tidal concentration of nitrous oxide changing by no more than 2-3%. The total gas flow was adjusted as necessary to keep the end-tidal carbon dioxide concentration in the range 4.5–5.5 kPa.

Experience and recall

At the end of each nitrous oxide study the subject was questioned about their mental experience and recall of events which had occurred during breathing the gas; for example hearing the tones, pressing the button and external disturbances such as alarms. Detailed records were kept of what each subject described.

Experimental programme

At the start of the experiment, two sets of recordings of the P300 and reaction times were obtained whilst the subject breathed air. The subject then breathed the first of the nitrous oxide/oxygen mixtures until the end-tidal concentration of nitrous oxide was stable when they were warned that a recording was about to start and reminded to press the button on hearing the tone. When the recordings of P300 and reaction times were complete the subject was allowed to recover whilst breathing room air. At the earliest possible opportunity they were questioned about recall as outlined above. When the end-tidal nitrous oxide concentration was ≤ 2%, the next concentration was administered. This sequence was repeated, increasing the inspired concentration by 10% in each study until the subject no longer co-operated. Failure to keep still enough for EEG recording to be possible or failure to retain the mouthpiece were the usual reasons for terminating the experiment. A final recording was performed with the subject breathing air 15–20 min after nitrous oxide was discontinued. This programme represents a compromise between several conflicting requirements which are discussed later.

Statistics

Regression coefficients and intercepts were calculated using least squares linear regression analysis. All regression analysis was performed on the pooled data for all subjects (except for table II).

RESULTS

Behavioural changes with nitrous oxide

Of the seven subjects who originally volunteered for the study, one became very dysphoric at an end-tidal nitrous oxide concentration of 20% and was unable to co-operate with EEG recordings, so the experiment was abandoned. The results of the remaining six subjects are presented. Of these, subject No. 4 (symbol O in figures) also became dysphoric and unable to keep still enough to provide satisfactory EEG recordings at an end-tidal nitrous oxide concentration of 42%, although we managed to obtain a complete record of psychomotor measures. While breathing increased concentrations, the remaining five
subjects had experiences that were vivid and were described in terms of dissociation; for example, solving difficult problems with bizarre insights into the meaning of life. Despite these sensations, during which they were oblivious to the purpose of the study, they were able to co-operate with and complete the experiment, although there was one other subject in whom we could perform psychomotor testing at an expired concentration of 62%, but EEG recordings were swamped by movement artefacts.

In common with other workers [10, 12], we
Control

25% N\textsubscript{2}O

42% N\textsubscript{2}O

51% N\textsubscript{2}O

Click

Time (s)

Tone

FIG. 4. Series of averaged EEG responses with increasing concentrations of nitrous oxide in one subject. Each trace is the average response to 16 stimuli. On the right are the responses to the tones and on the left, for comparison, are the averaged responses to the preceding click.

TABLE I. Calculated values of end-tidal nitrous oxide at which the P300 amplitude would be zero for each of the six subjects, as estimated by the intercepts on the nitrous oxide axis of the individual linear regression lines

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>End-tidal N\textsubscript{2}O (%)</td>
<td>66.4</td>
<td>69.3</td>
<td>56.6</td>
<td>36.4</td>
<td>69.5</td>
<td>62.0</td>
</tr>
</tbody>
</table>

found that the subjects’ minimum reaction times increased with increasing nitrous oxide (fig. 1). Figure 2 shows how the subjects’ accuracy at pressing the button in response to the tone altered with increasing nitrous oxide. At the lower concentrations, the subjects continued to respond to all the tones, albeit with a longer reaction time, but there was a fairly narrow range of concentrations over which the response decreased sharply.

Recall for the recording period was disrupted by lesser concentrations of nitrous oxide than affected the ability to respond. Four of the six subjects continued to respond to some of the tones by pressing the button with concentrations at which they could not recall any events that occurred whilst breathing the gas (points within the shaded area of figure 2).

Changes in the P300 with nitrous oxide

Figure 3 shows a typical averaged EEG response to a click and a tone and illustrates the

Fig. 5. Effect of nitrous oxide on P300 amplitude in each of the six subjects, normalized as a percentage of that subject’s mean amplitude when breathing air at the beginning of the study. r = −0.96; P < 0.0001; X-intercept = 62%. Key to subjects as in figure 1.
temporal relationship between the motor response to the reaction task and the P300. The motor response was approximately 100 ms earlier than the peak P300 latency. The figure also illustrates the fact that, with our experimental paradigm, the P300 was seen on the falling phase of the P200 wave, which implies that simple measurements of peak amplitude are not appropriate. The method of measurement chosen was to draw a straight line to fit the decrease in P200 and to measure the area of the P300 above that line, as represented by the shaded area. A typical series of P300 records obtained from one subject breathing air and then increasing concentrations of nitrous oxide is shown in figure 4; as the nitrous oxide concentration increased, P300 became smaller and later.

Figure 5 shows how the amplitude of the P300 changed with increasing end-tidal concentrations of nitrous oxide. Because the absolute amplitude measurements in any one subject are influenced by such factors as electrode contact and position, it was necessary to normalize the measurements in order to compare them between subjects. Therefore, all amplitudes are given as a percentage of the mean P300 amplitude with the subject breathing air at the beginning of the study, for that subject.

All the subjects showed a step-wise decrease in P300 amplitude with increasing end-tidal concentration of nitrous oxide. The regression line calculated from the pooled data intercepts the x-axis at 62.3 % nitrous oxide. This is at the top end of the range in which the subjects failed to push the button, and well above the range in which recall was lost (fig. 2). It can be seen from the graphs that individuals differed in their sensitivities to nitrous oxide; the calculated nitrous oxide intercepts for each of the six subjects are shown in table I.

All subjects showed an increase in the peak latency of the P300 with increasing end-tidal concentrations of nitrous oxide (fig. 6). The relationship between the P300 amplitude and the performance of each of the subjects was similar to that between end-tidal nitrous oxide concentration and performance (fig. 7). At reductions in P300 amplitude less than 50 % there was very little change in performance, but thereafter it decreased sharply.

**Recovery from nitrous oxide**

A final set of observations was obtained from five of the six subjects 15-20 min after they had finished breathing nitrous oxide (table II). Recovery was quite variable, but three of the five subjects had persistent changes in amplitude or latency of the P300 at this time, although the minimum reaction times are more or less normal. Note that the subject who was most sensitive to nitrous oxide (fig. 5) had the most attenuated P300 at this stage.

**DISCUSSION**

The results of this study have demonstrated that the P300 response of the auditory evoked potential to a randomly presented auditory event showed
dose-dependent changes in amplitude and latency over the range of end-tidal concentrations of nitrous oxide used in routine clinical practice. The calculated regression line for all the data showed that the amplitude of the P300 tended to zero at 62% end-tidal nitrous oxide. Four of the six subjects pressed a button in response to some or all of the tones at a stage when they had no recall of the proceedings and three of the subjects had a detectable P300 at a stage when they made no response at all to the tones. Thus the P300 seems to represent some residual processing of information in the absence of a motor response.

Two aspects of the methodology need to be discussed. First, the nitrous oxide concentrations were always presented to the subjects in ascending order. Whilst this is not ideal statistically, it proved impracticable to randomize the order. Table II illustrates the fact that the recovery of the P300 15 min after nitrous oxide was stopped varied between subjects. The intervals necessary between trials in order to ensure zero carry-over from large to small concentrations would have made the experiment impossible to complete at one time. An alternative would have been to study different concentrations on different days, but that would have introduced new sources of variability such as electrode placings. The design chosen was a compromise, based on the assumption that any carry-over should have least effect from smaller to greater concentrations.

Second, there is no standard method of measuring the amplitude of the P300. In our experiment it appeared on the falling phase of the P200 when air was breathed and gradually separated from this wave with increasing concentrations of nitrous oxide. This makes simple measurements of amplitude inappropriate because there is no suitable fixed point to which such measurements may be referred. The method used was to measure an area which approximated to the area of the response, and which underestimated the P300 the closer it was to the P200; that is, in this experiment it underestimated at smaller concentrations of nitrous oxide. Thus any errors from this source lessened, rather than enhanced, the dose-dependent changes in amplitude observed. It is then necessary to consider what these changes represent.

The P300 is maximal if the stimulus is infrequent and if its occurrence has some meaning to the subject, for example if he has been told beforehand that he has a task to perform when it occurs. In this situation the latency of the P300 is always longer than the associated reaction time.

Table II. Recovery of P300 amplitude and latency and of minimum reaction time with the subjects breathing air 15–20 min after last breathing nitrous oxide. (Data for subject No. 2 were lost.)

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>P300 amplitude (% control)</th>
<th>P300 latency (change from control) (ms)</th>
<th>Reaction time (change from control) (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>84</td>
<td>+48</td>
<td>+16</td>
</tr>
<tr>
<td>3</td>
<td>107</td>
<td>-12</td>
<td>-22</td>
</tr>
<tr>
<td>4</td>
<td>37</td>
<td>+42</td>
<td>+13</td>
</tr>
<tr>
<td>5</td>
<td>91</td>
<td>-1</td>
<td>+2</td>
</tr>
<tr>
<td>6</td>
<td>68</td>
<td>-3</td>
<td>+21</td>
</tr>
</tbody>
</table>
P300 AND NITROUS OXIDE

[14], and so it is believed that the P300 reflects some part of the stimulus evaluation process, rather than the process of producing a motor response. Its latency may be too short for it fully to represent conscious awareness of the stimulus [5], but it represents a very much later stage in the pathway, just before consciousness, than do the early cortical components (about 50 ms) of the evoked potential. The fact that its amplitude tends to zero at 62% end-tidal nitrous oxide suggests that it is not useful as a measure of depth of anaesthesia, as it is not present throughout the range of anaesthetic concentrations usually used to perform surgery. However, we speculate that it might be used as a marker for conscious awareness.

It is important to realize that it is not simply a marker for conscious awareness because in all five subjects in whom satisfactory recordings could be obtained, it persisted at nitrous oxide concentrations with which recall of external events was lost. Some subjects were able to achieve a high degree of accuracy at pressing the button in response to the tones when they had no recall at all. This shows that lack of recall of events is a poor indicator of conscious awareness.

There is also an interesting relationship between the P300 amplitude and ability to respond to the tones. With the smaller concentrations of nitrous oxide, the reaction time lengthened gradually although the button was pushed in response to every tone. Then, at concentrations at which the P300 was already reduced by more than 50%, the response began to fail altogether. Although this failure was apparently abrupt, this is not really the case. There is, in effect, a continuum in which the reaction time gradually lengthens and then increases steeply to infinity. In parallel with this, the P300 gets progressively smaller and, in three of six subjects, it finally disappeared slightly after the motor response. No subject had a motor response to a tone in the absence of a P300. If we accept that the presence of a P300 represents some recognition of the tone, albeit attenuated and not recalled, then we have detected a state, in some subjects, in which the stimulus was recognized, but not acted upon. This implies that a change in the P300 may be used as a sensitive indicator of conscious awareness without recall, although we cannot prove this without more detailed psychometric testing.

We conclude that the P300 warrants further investigation as a tool for exploring the relationship between conscious awareness and anaesthesia.

REFERENCES