

Effect of Nitrous Oxide Concentration on Event-related Potentials during Painful Tooth Stimulation

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Effects of inhaling three levels of nitrous oxide in oxygen on event-related brain potentials (ERPs) and pain report were examined in 10 volunteers undergoing painful electrical stimulation of tooth pulp. Previous work by the authors demonstrated that inhalation of nitrous oxide 33 per cent in oxygen, iv injection of 0.1 mg fentanyl, oral administration of 975 mg aspirin, and electrical acupunctural stimulation all reduced ERP amplitudes obtained at vertex during painful tooth pulp stimulation. The authors report here the demonstration of a dose-response relationship between increasing concentrations of nitrous oxide in oxygen and measures of ERP amplitude and pain report. Subjects inhaled room air, nitrous oxide 25 per cent, 37 per cent, and 50 per cent in oxygen while ERPs were recorded and pain reports were given. The procedure was repeated on three separate days with each subject experiencing all levels of treatment on each day. Analyses of variance revealed that both ERP amplitude and pain report significantly decreased as dosage increased, and a significant linear trend was observed for the positive-going ERP waveform deflection between 160 and 240 ms. Pain report scores decreased significantly ($P < 0.001$) and proportionally as dosage increased, but there was not a significant linear trend. Inhalation of nitrous oxide in oxygen increased peak latency for the negative component at 50 ms and the positive component at 90 ms but not for later components. These outcomes demonstrate that amplitude measures of the vertex ERP obtained with dental dolorimetry correlate consistently with pain and analgesia. Simultaneous assessment of brain electrical activity and subjective report appears to be a useful approach for the assessment of analgesia in humans. (Key words: Anesthetics, gases; nitrous oxide. Brain: evoked potentials. Pain: Experimental; measurement.)

THERE IS NOW AMPLE EVIDENCE that certain characteristics of the vertex event-related potential (ERP) correlate closely with reported pain in laboratory studies in which volunteers undergo painful dental stimulation. When electrical stimulus intensity is raised, more dental pain is experienced and ERP amplitude is increased with no change in peak latency.^{1,2} Moreover, various analgesic treatments reduce ERP amplitude with little or no alteration of peak latency. Chapman and Benedetti³ observed the effects of inhaling nitrous oxide 33 per cent

in oxygen on the dental ERP and noted a decrease in several amplitude measures derived from the ERP waveform. Similarly, reductions of dental ERP amplitude and pain report have been seen with the administration of 0.1 mg fentanyl, iv,⁴ and 975 mg aspirin,⁵ as well as with acupunctural stimulation,⁶ while infiltration of lidocaine 2 per cent in saline around the root of the tested tooth eliminated the entire ERP waveform as well as report of pain.⁷ Although analgesia has been reliably demonstrated to reduce dental ERP amplitude, none of the previous studies have examined dose-response assessment of analgesic effects.

The purpose of this paper is to expand our understanding of nitrous oxide analgesia as inferred from a combination of ERP and subjective pain report measures by evaluating the effects of multiple doses on response to painful dental stimulation. Our goals were to determine: 1) whether decreases in ERP amplitude proportional to the varying concentrations of inhaled nitrous oxide could be demonstrated; and, 2) whether such changes would parallel alterations in subjective pain reports.

Methods

SUBJECTS

Ten paid, healthy, male volunteers ranging in age from 19 to 28 years served as subjects, having signed fully descriptive informed-consent agreements approved by the Human Subjects Committee at the University of Washington.

DOLORIMETRY

For each subject a healthy, unfilled, central incisor was stimulated via a 3.5-mm conductive rubber electrode (cathode) mounted in a plastic shaft hand held by the subject. Quality of the contact between the stimulating cathode and tooth was ensured by continued observance of a digital resistance meter. The anodal electrode was taped to the left zygomatic arch. Stimuli were generated by a Grass S-44 stimulator and controlled by constant current and stimulus isolation units. Stimulus intensity was determined by display of the 5-ms square wave pulses on a calibrated oscilloscope. These pulses ranged in intensity from 10 to 80 μ A depending on the subject, and the mean intensity was $55.53 \pm 22.85 \mu$ A. In each case the stimulus used was one that the subject judged as "strong pain." The stimuli were presented manually

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by the experimenter at a rate of approximately one per second. Minor variations in interstimulus interval helped prevent unwarranted expectancy effects from contaminating recordings. A detailed description of the dental dolorimetry system employed has been provided by Martin and Chapman.⁸

The pain ratings also obtained, scored 1-6, were derived from verbal reports based on a scale of six categories: 1) very faint sensation, 2) very faint pain, 3) faint pain, 4) mild pain, 5) moderate pain, and 6) strong pain.

ERP RECORDING

Samples of brain activity were recorded from vertex (C₂) referred to occiput (O₂) with the zygomatic arch as ground, and electrode resistances were maintained below 5 kohm. Each testing session lasted approximately 1.5 h. Signals were amplified via a model 277J Analog Devices Isolation Unit with an effective bandwidth of 0.2-100 Hz (3 db down) and the signal from Op-Amp was fed directly into a Nicolet 1072 Signal Averager. The input of the Nicolet Digitizer (SD72/4A) was set to a time constant of 4 ms with a sampling rate of 800 Hz. The signal averaging process was monitored on an oscilloscope.

Each measure obtained was derived from a summation average for 192 trials which was produced by summing three averages, each obtained from 64 repeated presentations of identical dental stimuli. To guard against possible artifacts caused by alpha rhythm, neck muscle tension, eyeblinks, or ocular rotation, the consistency of the three 64-trial averages was assessed and atypical recordings were discarded and replaced. As the concentration of nitrous oxide inhaled increased, it became more difficult for the subject to concentrate and perform the task required for the experiment. Special care was taken to monitor simultaneously tooth electrode contact and nasal breathing as well as to minimize muscle movements that might contribute to ERP artifacts when the inspired concentration of nitrous oxide in oxygen was 50 per cent.

The data were quantified in terms of the peak-to-peak amplitudes and peak latencies of the four stable waveform components defined as N50-P90, P90-N160, N160-P240, and P240-N340 (fig. 1). Peak latency and peak-to-peak amplitudes for each 192-trial average obtained were digitized and submitted to analysis.

TESTING PROCEDURE

Each of the 10 volunteers repeatedly underwent testing on three different days, for a total of 30 testing sessions. A baseline ERP recording was performed at the beginning of each session with the subject breathing room air, and another ERP recording was made at each of the

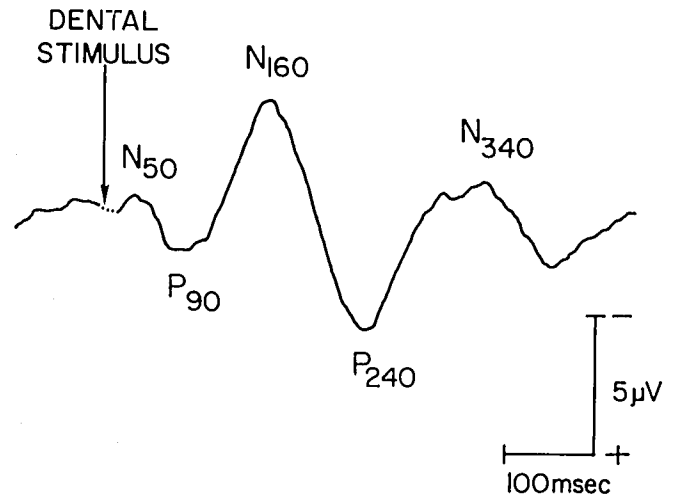


FIG. 1. Typical brain evoked potential recorded from vertex for one subject in response to 192 trials of electrical dental stimulation. The recording represents 600 ms of activity, 100 ms preceding the stimulus, and 500 ms poststimulus. Subscript numbers indicate peak latency from stimulus onset in milliseconds with N specifying negative and P specifying positive going peak deflections.

three N₂O concentrations. Thus, each subject experienced all levels of treatment on each day, and this was repeated three times. Subjects knew that they were receiving differing concentrations of nitrous oxide in oxygen but they were given no information about the exact concentrations or which concentrations were being given at which times.

At the beginning of each session a series of 10 subjective report trials involving repetitive stimulation and slowly increasing stimulus intensities was used for each subject to determine the stimulus levels at which he identified the various points between very faint sensation and strong pain on the rating scale. The mean stimulus intensity at which the subject identified the tooth pulp shock as "strong pain" was selected for testing.

A Quantiflex anesthetic machine was used to deliver a constant flow at 10 l/min of a mixture of oxygen and nitrous oxide via a Dupaco nasal mask, of standard design for dental clinical inhalation analgesia, using a non-rebreathing system. The mask was equipped with a one-way valve which prevented room air breathing during inspiration, and all subjects were trained to avoid oral breathing during the experiment. An anesthesia bag in the breathing system allowed monitoring of nasal breathing by its partial deflation during peak inspiration. This bag was monitored constantly by visual inspection during the procedure and the subject was reminded not to breath orally when any failure in partial bag deflation was noted. The mixture of nitrous oxide and oxygen was administered at three different settings of the flowmeters: 1) 7.5 l/min of oxygen and 2.5 l/min of nitrous oxide (25 per cent); 2) 6.3 l/min of oxygen and 3.7 l/min of

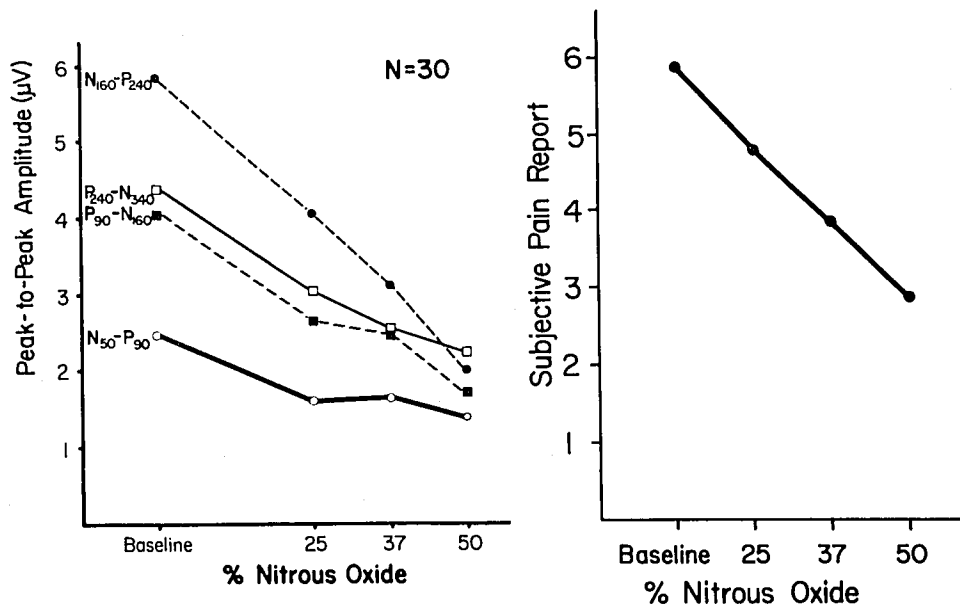


FIG. 2. A. Mean peak-to-peak amplitude scores for room air (baseline), 25 per cent, 37 per cent, and 50 per cent nitrous oxide in oxygen (N = 10). B. Mean subjective pain report for each condition (N = 10).

nitrous oxide (37 per cent); and 3) 5 l/min of oxygen and 5 l/min of nitrous oxide (50 per cent). Each mixture was inhaled for 10 min before the ERPs were recorded.

Results

ERP ANALYSIS

Figure 1 illustrates a typical ERP baseline waveform from a single subject. Figures 2A and 2B illustrate changes in mean amplitude scores across room air and the three dosage levels of nitrous oxide in oxygen as well as category judgment scores. Analysis of variance for a Repeated Measures Two-Factor Design⁹ was carried out for each of the components to evaluate: 1) Session, 2) Dosage, and 3) Session × Dosage interaction effects. In some cases Sheffé tests¹⁰ were conducted to evaluate differences between means at the varying dosages of nitrous

oxide in oxygen. Finally, a trend analysis for linearity⁹ was performed on the data collapsed across the three sessions to assess whether the treatment dosage effect could be characterized as linear across dosage level. In some cases nonparametric analyses of variance¹¹ were used to evaluate whether changes in ERP measures rank ordered in accordance with nitrous oxide dosage. Table 1 lists the specific tests and their significance levels.

There was no significant difference among the means due to Session, indicating that measurements were consistent across the three testing days, but a significant Dosage effect was evident, demonstrating that nitrous oxide inhalation at various dosages modulated ERP amplitudes. There was no Session × Dosage interaction which showed that the drug affected the ERP consistently in all three sessions. All levels of nitrous oxide inhalation affected components P90-N160, N160-P240, and P240-N340, but N50-P90 was affected only at the

TABLE 1. Specific Tests and Their Significance

	N ₅₀ -P ₉₀	P ₉₀ -N ₁₆₀	N ₁₆₀ -P ₂₄₀	P ₂₄₀ -N ₃₄₀
Analysis of variance				
Session	F(2, 18) = 0.19 NS	F(2, 18) = 0.06 NS	F(2, 18) = 0.37 NS	F(2, 18) = 1.93 NS
Dosage	F(3, 27) = 4.42 P < 0.01	F(3, 27) = 19.08 P < 0.001	F(3, 27) = 48.79 P < 0.001	F(3, 27) = 20.37 P < 0.001
Session × Dosage	F(6, 54) = 1.38 NS	F(6, 54) = 0.69 NS	F(6, 54) = 1.78 NS	F(6, 54) = 0.57 NS
Linearity	F(1, 9) = 0.853 NS	F(1, 9) = 2.676 NS	F(1, 9) = 6.808 NS	F(1, 9) = 3.209 NS
Friedman nonparametric test for predicted rank order across dosages	Q = 8.28 NS	Q = 17.76 P < 0.0015	Q = 27.72 P < 0.0001	Q = 17.4 P < 0.0019

50 per cent level. A significant linear trend was observed for N160-P240, the major ERP deflection, which indicates that this component was modulated by the inhalation analgesic in a linear dose-response fashion. No other component showed significant linearity, but all except N50-P90 showed significance in rank ordering of amplitude scores according to dosage level.

The linear trend observed for the major deflection could be judged as of borderline significance considering the probability of Type 1 error with the large number of tests done, and thus, it should probably not be interpreted too strictly. While nitrous oxide in oxygen clearly reduced ERP amplitudes in such a way that amplitude diminished as dosage level increased on any of the days, the rather stringent prediction of linearity of response across dosage levels could be demonstrated only by pooling repeated measurements of subjects over three days.

SUBJECTIVE REPORT

Analysis of variance was performed on subjective report scores. There was no significant Session effect, $F(2,18) = 0.02$, but the Dosage effect was significant, $F(3,54) = 12.98$, $P < 0.001$. Mean subjective report scores decreased as dosage increased over averaged sessions ($P < 0.001$), but there was not a significant parametric linear trend.

PEAK LATENCY

Figure 3 shows mean peak latency values averaged across the three sessions for room air and the three dosage levels of nitrous oxide inhalation. It is clear from visual examination that no dose-related trends in mean peak

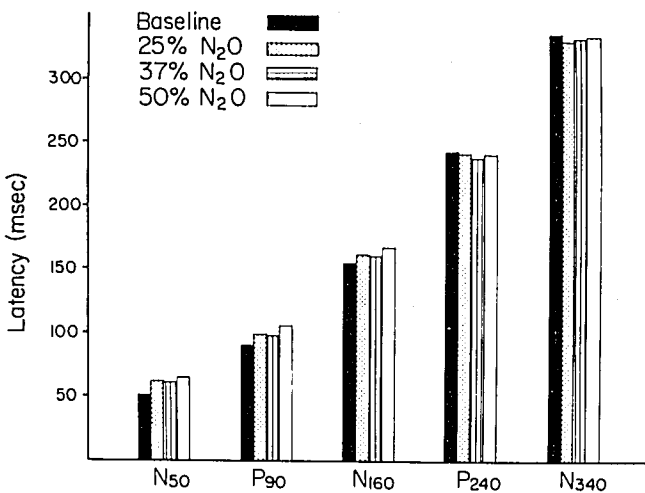


FIG. 3. Peak latency values averaged across the three sessions for baseline, 25 per cent, 37 per cent, and 50 per cent nitrous oxide in oxygen. Significant changes from baseline after inhalation of nitrous oxide occurred at N50 and P90, but not at other peaks.

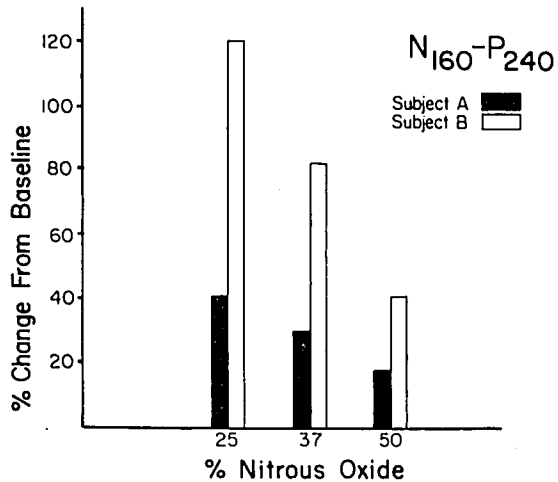
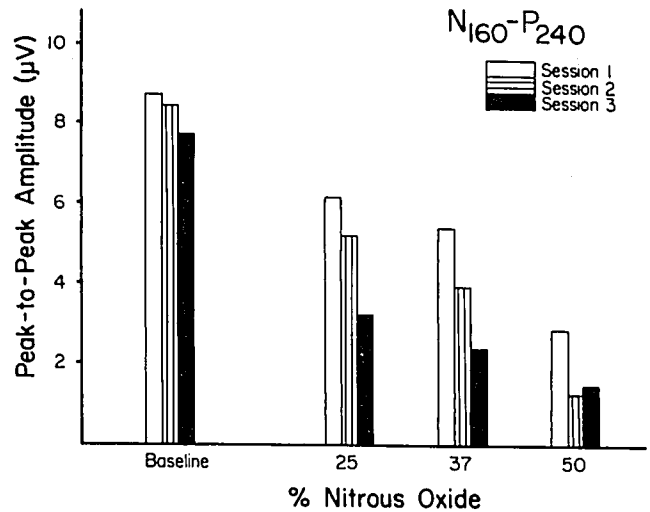


FIG. 4. A (upper). This is an example of one subject's response to nitrous oxide across sessions. Note differing amounts of analgesia were produced. The decrease in brain evoked potential amplitude across sessions for baseline was not typical of the whole group of subjects. B (lower). Peak-to-peak amplitude presented as per cent of baseline amplitude during one session for two subjects. The responses for the two subjects were highly dissimilar demonstrating great individual differences in response to nitrous oxide.

latency collapsed across both sessions and dosage levels revealed that peak latency occurred, but *t* tests of baseline *vs.* mean peak latency was significantly increased at N50 ($t = 2.64$, $P < 0.05$) and P90 ($t = 2.80$, $P < 0.025$) by nitrous oxide inhalation but not at N160, P240, or N340.

Discussion

Our results demonstrate that amplitude of the vertex ERP is altered by inhalation of nitrous oxide in oxygen in a manner roughly similar to that observed with a variety of other analgesic agents³⁻⁷ since amplitude is reduced and the peak latencies of the later components of the ERP waveform remain stable. Uniquely, however, earlier components show an increase in latency in as-

sociation with nitrous oxide inhalation. Our outcomes have demonstrated a dose-response function wherein ERP amplitude decreased with increases in drug concentration, and this was linear, at least in this study, for the major deflection at N160-P240. Since the outcome was of borderline significance, however, we are not confident that strict linearity can be replicated. A parallel phenomenon is present but not strictly linear for the other waveform components, save for the earliest. Subjective pain reports show parallel decreases from baseline with increasing nitrous oxide in oxygen concentration. It appears, therefore, that decreases from baseline in waveform peak amplitude appear to be reliable correlates of nitrous oxide analgesia in human subjects. This demonstration further supports use of the dental stimulation/ERP model for laboratory evaluation of analgesic effects.

It must be noted that the close relationship between ERP amplitude and subjective pain report can be altered in certain circumstances so that amplitude may change while pain judgment remains fixed. Chapman *et al.*¹² showed that decreasing the rate of stimulus repetition led to increases in waveform amplitude but there were no changes in subjective pain reports. This indicates that the waveform amplitude, taken alone, cannot be considered as a measure of pain sensation and that the use of this variable as a indicator of analgesic states in human or animal subjects could lead to erroneous conclusions if parallel indicators of experienced pain are not quantified concurrently. Thus, while our methodology can be used reliably, it requires caution and procedural rigor.

The issue of individual differences in response to nitrous oxide warrants some comment since we have drawn conclusions only from group means. On the average, the effect of nitrous oxide is very predictable, but at the level of the individual it can affect pain sensations erratically. Some of our subjects developed significantly dissimilar degrees of analgesia on the three different days for each of the concentrations delivered as figure 4A illustrates. Moreover, nitrous oxide in oxygen at similar concentrations led to rather different analgesic potencies in two different subjects as figure 4B shows. In one subject, an increase in N160-P240 amplitude occurred after inhaling the nitrous oxide in oxygen 25 per cent with a concomitant increase in subjective pain report. This subject later developed clearly defined analgesia at higher concentra-

tions of nitrous oxide. In contrast, most showed strong and increasing analgesia with increasing concentrations of nitrous oxide in oxygen. Such individual differences are important considerations in generalizing the outcomes of studies such as this one to clinical or other research settings. It is well-known that the patient inhaling nitrous oxide in oxygen may behave inconsistently or even paradoxically, and recent evidence suggests that psychological factors, such as expectations maintained by the subject, may enter into the picture and produce unusual outcomes.[¶] Further work on determinants of individual differences in response to nitrous oxide will help to bridge the gap between laboratory evaluation and clinical practice.

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