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Comparison of the sedative, cognitive, and analgesic effects of nitrous oxide, sevoflurane, and ethanol

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Background. Anaesthetics which work by different mechanisms may have different patterns of effect. Measurement of these patterns thus may elucidate their mechanisms of action and allow therapeutic choices between the agents.

Methods. We compared the effects of ethanol (∼80 mg per 100 ml), and different end-tidal concentrations of nitrous oxide (15% and 25%) and sevoflurane (0.3% and 0.5%) in volunteers. We measured speed and accuracy in psychomotor tests, reaction time and memory, touch and pain sensitivity to von Frey filaments, and subjective mood for a range of descriptors.

Results. All treatments caused the same degree of overall abnormal feelings, but sevoflurane caused more obtunding (subjective drowsiness, slow reaction times, and loss of memory function) and nitrous oxide was more analgesic. Ethanol caused a marked feeling of drunkenness, but little drowsiness or analgesia.

Conclusions. In the same volunteer subjects, direct comparison of sub-anaesthetic doses of these agents showed a clear and characteristic pattern of effects. These support the possible mechanisms for these disparate agents and may help choose appropriate agents for specific desired anaesthetic outcomes such as sedation or analgesia.

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Anaesthetics are diverse compounds with no obvious similarities in chemical structure, varying from a single atom (xenon) to organic compounds such as halothane and propofol; some are gases, some volatile liquids, and some are solids at room temperature. They interact with a variety of receptor systems, including γ-aminobutyric acid type A (GABA$_A$), glycine, nicotinic acetylcholine (nACh), 5-hydroxytryptamine type 3 (5-HT3), and glutamate,[1] and they affect nervous system function to different degrees to cause different features such as immobilization, sedation, amnesia, and analgesia.

GABA is the principal inhibitory receptor in the nervous system, being found in all regions of brain and spinal cord.[2][3] Most anaesthetics potentiate the effects of GABA on the GABA$_A$ ligand-gated ion channel, and there is a good agreement between GABA potentiation and anaesthetic potency.[4–6] The most important exceptions are ketamine, xenon, and nitrous oxide. These agents appear to have little effect on GABA receptors, but inhibit excitatory *N*-methyl-D-aspartate (NMDA) sensitive glutamate receptors. The nACh, 5-HT3, and glycine receptors are all members of the same cysteine-loop superfamily of ligand-gated ion channels as the GABA$_A$ receptor.[7] Anaesthetics have differing potency on these receptors, but the overall effect is generally inhibitory.[8] Thus, all these mechanisms can reduce overall central nervous system (CNS) activity, and no single receptor mechanism is common to all anaesthetic activity.[9]

A number of clinical endpoints have been identified for anaesthesia, and anaesthetics differ in their capacity to cause them. Immobilization, that is, suppression of a motor response to a noxious stimulus, is the basis for the primary measure of anaesthetic efficacy for inhaled anaesthetics, the minimum alveolar concentration (MAC). The

ability to produce unconsciousness is measured by MAC-awake, the concentration which prevents voluntary responses to an instruction in 50% of subjects. However, this assesses a separate phenomenon—obtunding—and is not an equivalent measure of potency. Thus, nitrous oxide and halothane have higher values of MAC-awake relative to MAC than other inhaled anaesthetics, and are less potent at producing unconsciousness relative to immobilization.[10] In a similar way, nitrous oxide and xenon are more effective analgesics than isoflurane.[11][12]

For both clinical and scientific reasons, it could be useful to establish the relationship between actions at different receptor targets and the different effects of anaesthetics. Clinically, such understanding might allow better choices of anaesthetic compounds (and combinations) to be made in differing circumstances. Scientifically, we might understand the function of different receptors and their role in behaviour. Measuring the effects of sub-anaesthetic doses in volunteers allows these different effects to be distinguished. Volunteers can be given a single agent with no other concomitant medication, which is rarely done in patients. Conscious volunteers can cooperate in the study methods and report their feelings throughout the experiment, which is only possible with patients in the recovery stage. The effects of sub-anaesthetic doses can distinguish important clinical effects, such as subjective sedation, objective measures of arousal, and analgesia and amnesia. Also, sub-anaesthetic doses are directly relevant to recovery, or when anaesthetics are used for sedation rather than full anaesthesia. Thus comparisons of different agents, covering a range of functional measures relevant to anaesthesia, would be helpful, particularly if done in the same subjects.

Ethanol is a useful compound to include in such comparisons. It has much in common with the anaesthetics, enhancing the effects of $GABA_A$ and glycine, and inhibiting NMDA glutamatergic receptors. Because a great deal is known of the effects of ethanol on cognition and psychomotor performance, it is an important reference compound for comparison with the effects of other drugs.

Sevoflurane and nitrous oxide are agents with very different profiles of action. Sevoflurane enhances GABA inhibition via the $GABA_A$ receptor with little effect on NMDA receptors[13] whereas the reverse pattern is found with nitrous oxide.[8] Galinkin and colleagues studied doses up to about 0.3 MAC in volunteers, and found sedation was greater, and psychomotor function and memory more impaired, with sevoflurane than with nitrous oxide. In contrast, nitrous oxide caused substantial analgesia in the cold-pain test, whereas sevoflurane did not.[14] They also studied the effects of nitrous oxide and ethanol, but in a study designed to assess interactions between the two agents.[15] The dose of ethanol used (up to 0.5 g kg^{-1}) had little effect on its own. No other study has directly compared ethanol and nitrous oxide.

We set out to compare the effects of nitrous oxide, sevoflurane, and ethanol in volunteers. We used doses of the two inhaled anaesthetics similar to those used by Galinkin and colleagues[14] but chose a larger dose of ethanol to reliably affect performance. We assessed psychomotor performance, cognitive function, and mood using a broadly based battery that can reliably discriminate the effects of ethanol from benzodiazepines. Effects on pain perception were assessed using mechanical stimulation.

Methods

Volunteers

The study was approved by the Lothian local research ethics committee. We recruited eight volunteers, four female and four male, aged 19–28 yr and weighing 47–87 kg (mean 66). All gave written informed consent, were healthy, were light to moderate social drinkers, had negative pregnancy tests if female, and were not taking any medication that might have interfered with CNS function or drug absorption or elimination.

Design

We used a nested within-subjects design, with four sessions. Treatments were given double-blind in random sequence as follows:

- Session E: a single dose of ethanol, calculated to produce peak plasma ethanol concentrations in the range 80–100 mg per 100 ml.
- Session P: placebo.
- Session N: nitrous oxide, given in two 45 min administrations with a 10 min interval between, at doses of 15% and 25%.
- Session S: sevoflurane, given in two 45 min administrations with a 10 min interval between, at doses of 0.3% and 0.5%.

Performance and pain threshold testing were carried out before treatment and twice during the treatment period (once during each inhalation sub-period). The order of the sessions was determined at random using latin squares, and the order of the two inhaled treatments within the sessions was also randomized.

Assessments

We used the following tests.

Spiral Maze[16]

This pen and paper maze consisted of a white path bounded by a black spiral, with circular obstacles. The pen was placed at the centre of the spiral and the path traced around the spiral as rapidly as possible while avoiding touching the black sides and the obstacles. Time taken was recorded with a stopwatch and the error score obtained by marking one point if the line touched an obstacle or the side of the track, two points if it penetrated.

Zig-zag tracking[17]

This tracking task consisted of a grey zig-zag track with circular obstacles, displayed on a touch screen computer display. The volunteer followed the grey track with a pen as rapidly as possible while trying to avoid the obstacles. The task was scored in the same way as the Spiral Maze.

Four-choice reaction task[18]

Four stimulus locations were arranged in a square pattern on the pen computer screen, and below this were four corresponding buttons. Stimulus locations were highlighted one at a time in pseudorandom order. The volunteer was instructed to tap the appropriate button as quickly as possible. The next stimulus appeared immediately after the button press. No feedback was given if the response was incorrect. The mean response time for correct responses and the number of incorrect responses were recorded.

Logical working memory

(Kyllonen, personal communication): A set of three rules appeared one after the other on the computer screen, each being shown for 3 s, for example, 'The dog comes before the pig'; 'The chair does not come after the table'; and 'The furniture comes after the animals'. A set of eight response choices then appeared, from which the volunteer tapped on the correct one, in this case 'Dog—Pig—Chair—Table'. Twenty-four problems were presented, and the total number of correct responses recorded.

Word list learning

The investigator read out a list of 15 words, and the volunteer then wrote down as many as s/he could remember. After an interval of at least 20 min, during which other tests were performed, the volunteer was again asked to write down as many words as possible. The number of correct and incorrect words for each recall occasion was recorded.[19]

Visual analogue scales[20]

Twenty-four scales, including the 16 described by Bond and Lader[21] were used to assess mood. Each scale consisted of a 10 cm line presented on the pen computer screen, the ends of which were marked with antonyms (e.g. alert—drowsy). Volunteers made a mark on each line to indicate how they felt at that moment.

Pain sensitivity[22]

With the volunteer's eyes closed, the investigator touched the inner aspect of the forearm with von Frey hairs, chosen at random. The volunteer responded on feeling a touch on the forearm, and then on feeling a pricking sensation. This allowed touch and pain thresholds to be assessed. The investigator then pressed the thickest filament of the set three times on the forearm, and the volunteer rated the pain on a 10 cm visual analogue scale.

Equipment

Subjects breathed gas mixtures from a T-tube reservoir system via a tight fitting silicone rubber face mask connected to a large two-way non-rebreathing valve (Hans Rudolph, Inc., Kansas City, MO, USA). Gases were delivered from an anaesthetic machine (Aestiva5, GE Healthcare, Hatfield, Herts, UK) which delivered oxygen, nitrous oxide, and air from compressed supplies, and vaporized sevoflurane from a calibrated vaporizer. Gas in the mask was sampled continuously by a respiratory gas analyser (Datex AS5, Datex Ohmeda Ltd, Hatfield, Herts, UK) to measure concentrations of end-tidal oxygen, carbon dioxide, nitrous oxide, and sevoflurane. The inspired oxygen was always ∼20%. Gas mixtures were controlled by a separate individual so that the investigators testing the volunteers did not know the gas compositions being given.

The spiral maze and word list learning were carried out using pen and paper. Other tests were administered on an Apple MessagePad MP2000 pen computer.[20] von Frey hairs were used to assess pain sensitivity (Somedic Hörby, Sweden).

Blood alcohol concentrations were estimated from breath using a Lion Alcolmeter model S-D2 (Lion Laboratories, Barry, South Glamorgan, UK).

Procedures

Volunteers were instructed to refrain from eating for 4 h before each session, and to eat only light meals before that time. A maximum of one cup of tea or coffee was to be drunk at breakfast time, to be the same on each test day. No further caffeine was permitted until the completion of all test procedures. They were instructed not to consume any alcohol from 24 h before the start of the test session until at least 24 h after, or any tobacco from 2 h before the start of the session until the completion of all test procedures.

Each volunteer first took part in a practice session in which all tests were demonstrated and practised twice, once with the face mask off, the second time with it on.

In each of the four main drug sessions, a baseline test administration was first carried out. The volunteer was then given a drink containing either vodka or water (placebo) mixed with an equal volume of orange drink concentrate. The volume was calculated to give a dose of 0.8 g kg^{-1} body weight for males, up to a maximum of 66 g (200 ml of 37.5% vodka); or 0.7 g kg^{-1} for females up to a maximum of 55 g (167 ml). To mask the taste of vodka, the drink was sprayed with peppermint breath freshener, and the volunteer sucked a Tyrozet® lozenge (containing the local anaesthetic benzocaine) for 1 min before consuming the drink. The drink was consumed within 10 min. Two test administrations were then carried out starting at 20 and 75 min after the start of the drink.

For each test administration (including baseline), the procedure was as follows (Fig. 1): a breathalyser reading was first taken, then the volunteer put on the face mask. After
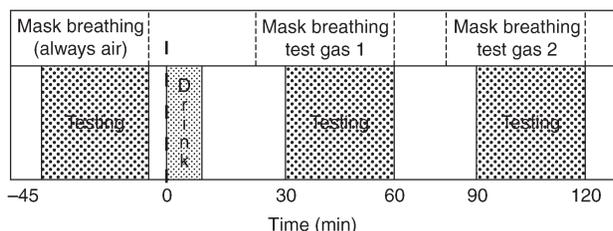
Fig 1 Timeline for study sessions. Test gases 1 and 2 were low dose and high dose (random order) in the nitrous oxide and sevoflurane sessions, air otherwise.

10 min breathing through the mask, visual analogue scales were completed, followed by the test battery, then a second set of analogue scales. Thus, the performance testing started at 30 and 85 min post-drink. The pain assessment was carried out, and after completion of the pain scales the mask was removed. Word list learning was performed only in the first test administration after the drink. The word presentation and first recall were at the beginning of the test battery, and the second recall at the end. All other tests were performed at every administration, and the order of presentation was randomized between volunteers, but was always the same for a given volunteer.

For the placebo and ethanol sessions, and for the baseline period, inhalation was of air. For the sevoflurane and nitrous oxide sessions, one of the inhalation periods was high dose (25% nitrous oxide or 0.5% sevoflurane) the other low dose (15% nitrous oxide or 0.3% sevoflurane). The order of the high and low dose inhalations within the session was randomized.

Statistical analysis

The mean scores for each test measure over the two assessment points were analysed using an analysis of covariance (PROC GLM in SAS statistical software package) with the pre-treatment (baseline) value as a covariate, and treatment and session number in the model. If an overall statistically significant effect of treatment was found ($P<0.05$), then pairwise comparisons were carried out using *t*-tests to compare differences between treatments, and differences between dose levels of the inhaled treatments.

Scores from the visual analogue scales were combined into two factors, functional integrity and mood, as previously described,[20] before being analysed as above. In addition, the scales for sober—drunk, alert—drowsy, and normal—abnormal were analysed individually, because these subjective effects were of specific interest.

Data for the word list recall task, where no baseline assessment was used, were analysed using analysis of variance.

Results

The main effects of treatment are summarized in Table 1. For all tests used, at least one measure showed a significant treatment effect. Sevoflurane significantly slowed

Table 1 Summary of treatment effects on performance, pain perception, and subjective state. *Significantly different from placebo; †significantly different from ethanol; ‡significantly different from nitrous oxide (all $P<0.05$). N is used to indicate the number of responses made correctly or incorrectly, as appropriate

Test/measure	Placebo	Ethanol	Nitrous	Sevoflurane	*P*-value
Four-choice reaction task					
N incorrect	5.00	8.16	8.70	24.58	0.12
RT correct (s)	0.536	0.629	0.658	0.907*†‡	<0.01
Logical memory					
N correct	19.1	14.1*	14.1*	9.9*‡	<0.01
Spiral Maze					
Total time (s)	26.7	26.9	28.1	33.5*†‡	<0.05
Error score	14.5	20.2	21.6	33.4*†‡	<0.01
Zig-zag tracking					
Total time (s)	45.5	46.5	46.6	50.3	0.11
Error score	29.8	52.4*	44.8	87.9*†‡	<0.001
Word list recall					
Recall 1 N correct	8.74	7.51	7.75	5.88	0.084
Recall 2 N correct	6.68	3.82*	5.25	2.38*‡	<0.01
von Frey testing					
Touch	6.58	7.10	7.77*	7.69*	<0.05
Pain	10.11	10.78	11.99*†	10.31‡	<0.05
Analogue rating	1.40	0.97	0.91	1.89	0.16
Visual analogue					
Functional integrity	33.9	42.0*	46.6*	58.0*†‡	<0.01
Mood	26.8	26.9	25.9	29.7	0.58
Drunk	8.9	64.5*	29.7*†	31.4*†	<0.01
Drowsy	32.2	39.3	44.5*	63.9*†‡	<0.01
Abnormal	21.6	43.2*	47.4*	48.6*	<0.01

reaction times on the four-choice test, and performance was slower and less accurate on the spiral maze. Both ethanol and sevoflurane increased errors on zig-zag tracking and impaired delayed correct recall in word list learning. All three drug treatments impaired performance on the logical working memory task. The effects of sevoflurane were significantly greater than for any other treatment in the case of four-choice reaction time, spiral maze speed and accuracy, and zig-zag tracking accuracy. For logical working memory and delayed word recall, the effects of sevoflurane were greater than for nitrous oxide.

Visual analogue scales showed significant effects of all treatments on functional integrity, with sevoflurane having the greatest effect. A similar pattern was shown by the measure of drowsiness, although in this case the effects of ethanol were not significant. All treatments showed significant effects on the drunk scale, although here the effect of ethanol was significantly greater than for the other two treatments (Fig. 2). All treatments had comparable effects on feelings of abnormality.

Both nitrous oxide and sevoflurane increased the touch threshold, but only nitrous oxide affected the pain threshold. The effect of nitrous oxide on this measure was significantly greater than for any other treatment (Fig. 3).

The effects of the higher doses of the inhaled anaesthetics were in general larger than for the lower doses (see supplementary table at *British Journal of Anaesthesia* online). This was significant for sevoflurane on logical working memory for the number of correct responses (low dose: 11.4; high dose: 7.7, $P<0.01$), for nitrous oxide on
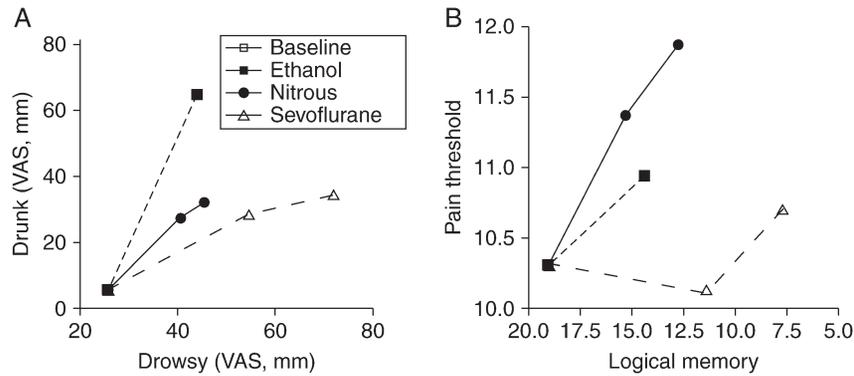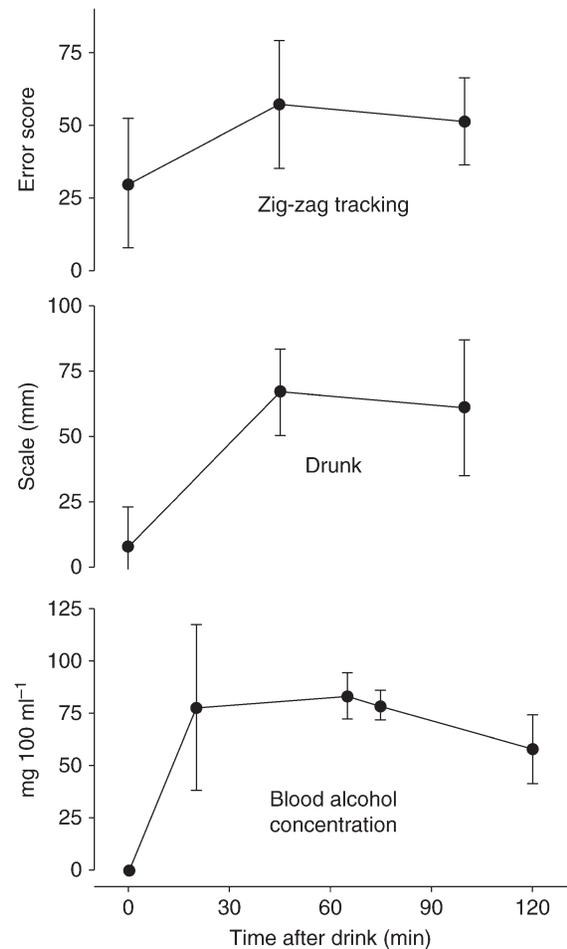
Fig 2 Examples of different effects: the plots compare changes caused by the different agents. (A) Drunkenness compared with drowsiness. Alcohol causes the most drunkenness and the least drowsiness. (B) Pain threshold: nitrous oxide and to a lesser extent alcohol are analgesic; sevoflurane has little analgesic effect and causes the most functional impairment. Pain thresholds refer to the filament number in the von Frey set.
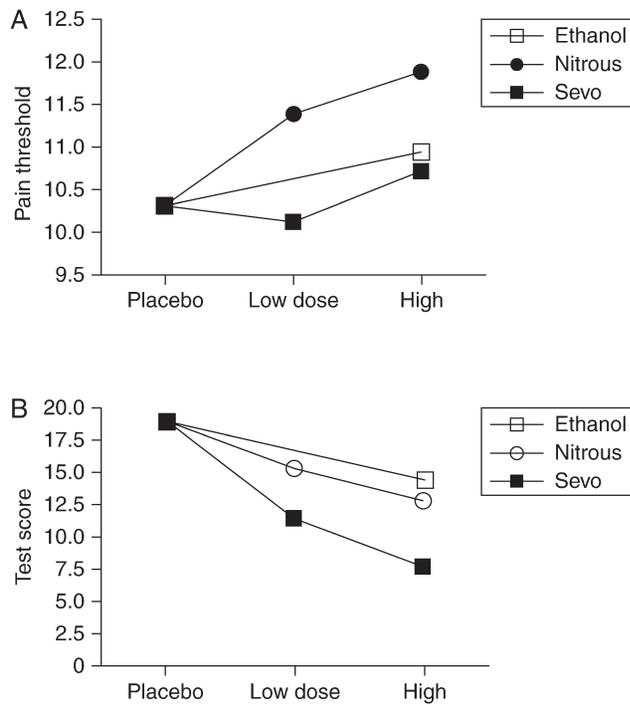
Fig 3 Dose relationship for the different agents for (A) analgesia and (B) logical memory.

Fig 4 Time course of ethanol concentrations and effects (mean, SD).

spiral maze time taken (low dose: 27.4 s; high dose: 28.7 s, $P<0.05$), for nitrous oxide on touch threshold (low dose: 7.00; high dose: 8.25, $P<0.05$), and for sevoflurane with the drowsy rating (low dose: 54.6% of scale length; high dose: 72.1%, $P<0.05$). This is illustrated for logical working memory and drowsiness in Figure 2.

The time course of the action of ethanol on zig-zag tracking, drunkenness, and the blood alcohol concentrations is shown in Figure 4. Peak concentrations of 84 mg per 100 ml (SD 11) were obtained at 65 min post-drink. The changes after the drink were similar for objective and subjective measures, with slightly less effects at the second time-point.

Discussion

We found that sevoflurane has greater effects on both sedation and performance than nitrous oxide. The differences were in psychomotor performance, in working memory, and in formation of new long-term memory. Subjectively, effects were in a group of scales referred to as 'functional

impairment', which include straightforward measures of sedation such as drowsiness, but also measures of awareness of impairment, such as 'clumsy'.[20 21]

The methods we used have some useful features. The most important is that we used within-subject direct comparisons between several drugs and, where possible, used more than one dose of each drug. Comparison of impairment by different drugs using information from different studies is difficult, even when a standardized test battery is used. All drugs are likely to impair all functions to some degree. Comparisons within the same subjects, in a single study, are much more powerful than those between the subjects. A broadly based test battery is also a valuable feature, as differences between drugs are more likely to be seen if a range of different functions are sampled.

We found substantial differences between the effects of the different agents. In the four-choice test, for example, reaction times were slowed by 23% by nitrous oxide, but 69% by sevoflurane. Delayed word recall was reduced by 21% by nitrous oxide, but by 64% by sevoflurane. Nitrous oxide increased sedation scores by 13% of the scale length, whereas sevoflurane increased it by 24%. The differences are also consistent—for every test measure where sevoflurane caused impairment compared with placebo, impairment was greater with sevoflurane than with nitrous oxide, except for subjective drunkenness.

The doses of nitrous oxide and sevoflurane used were chosen to be equipotent with respect to anaesthetic potency in young adults, the higher dose in each case being 0.24 MAC.[23 24] The dose of ethanol cannot be compared in the same way, as there is no clearly defined anaesthetic dose of ethanol, so we chose a dose of ethanol that we know causes similar objective impairment as nitrous oxide. Our results showed no objective measure that had significant differences between the effects of nitrous oxide and ethanol. Impairment with ethanol was clearly less than with sevoflurane. The subjective changes showed a different pattern, with feelings of drunkenness being significantly greater with ethanol than with either nitrous oxide or sevoflurane (Fig. 2).

The impairment with nitrous oxide was similar to that noted in other similar studies. Nitrous oxide has a clear dose–effect relationship[25 26] and effects are not detectable at <5%.

Pain testing showed analgesic effects only with nitrous oxide. The clear separation between the analgesic and the sedative effects of nitrous oxide and sevoflurane (Fig. 3) supports previous reports[14] and shows that the differences we found between nitrous oxide and sevoflurane represent real differences in the profiles of action of the two agents, and are not caused by a lack of dose equivalence. Similarly, the greater drunkenness seen with ethanol than the other drugs along with comparable or greater objective effects of the inhaled anaesthetics demonstrates another dissociation between subjective effects and impairment that cannot be accounted for by dosage effects.

Sevoflurane enhances $GABA_A$ receptor-mediated inhibitory neurotransmission, and the fact that other drugs which cause sedation and performance impairment such as the benzodiazepines and barbiturates act on this receptor underscores its importance in anaesthetic action. There are a number of subunits in $GABA_A$ receptors, which confer different functions. For example, $\alpha 1$ subunit containing receptors appear to mediate sedative but not anxiolytic actions of benzodiazepines.[27] The anaesthetic action of etomidate may be mediated by receptors containing β subunits,[28] whereas α subunits appear to be important for volatile anaesthetics.[29] The extent to which actions on different subunit compositions are associated with distinct profiles of action is still not clear. Other potent sedatives have little or no effect on GABA receptors, for example, anticholinergics and antihistamines. GABA probably has a limited role in mediating immobilization, which is the basis of potency estimation by means of MAC.[30 31]

There is little doubt that GABAergic mechanisms are responsible at least in part for the sedative and amnesic actions of sevoflurane.[32 33] The pattern of effects we observed is similar to that of drugs such as temazepam, with slowing of responses, reduction in long-term but not short-term memory, and subjective drowsiness. The doses of sevoflurane we used are sufficient for significant enhancement of GABA transmission.[32] These central sedative effects are important for both positive and negative reasons. The amnesic effect, in particular, is valuable clinically, and is very marked with sevoflurane: this subanaesthetic dose reduced the number of words correctly recalled by about two-thirds, whereas nitrous oxide had little effect. Sedation may be important during recovery, although few clinical problems are likely with short-acting inhalation agents in this respect.

The mechanism of nitrous oxide analgesia is not clear. A mechanism operating through the opioid system has been proposed. However, not all studies could show reversal of nitrous oxide analgesia using naloxone.[34] Noradrenergic transmission may also be involved, but this could be either the primary mode of action or an intermediate stage in the pain pathway.[35 36] An action through direct inhibition of NMDA receptors is also possible. The role of excitatory amino acid transmission in nociception is well established[37] and NMDA antagonists such as ketamine are effective analgesics. Mice with knockout for the NMDA receptor $\epsilon 1$ subunit have reduced sensitivity to nitrous oxide, whereas the effects of sevoflurane are not affected.[38] However, the effects of NMDA antagonists seem to be more on secondary pain and temporal summation of pain than on acute pain perception.[39]

This is the first study to directly compare ethanol and nitrous oxide, in which the dose of ethanol was sufficient to impair objective performance. Ethanol in general had more marked effects on performance, with the pattern of impairments being broadly similar. The analgesic effect is different, since ethanol did not have important analgesic

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effects. There is also distinct dissociation of subjective effects. Ethanol caused much more drunkenness, while volunteers became more drowsy with nitrous oxide. It is not surprising that ethanol made our volunteers feel more drunk than the other treatments, but it raises an interesting question: what action of ethanol is responsible for this effect? Clearly, potentiation of GABA is not a valid explanation. Both sevoflurane, in this study, and temazepam, in a previous study,[40] caused drowsiness rather than drunkenness. Nitrous oxide may be more of an intoxicant than benzodiazepines, but it is less effective than ethanol. Ethanol affects a number of other receptor systems, in particular 5-HT3,[41] and it would be interesting to study the effects of other compounds with this activity.

The subjective effects of drugs may give important information about their mode of action. Often, subjective measures such as visual analogue scales are more sensitive indicators of drug effects than objective measures of performance. Drugs have quite distinctive effects on subjective state even when their effects on performance cannot differentiate between them. A systematic approach to investigation of these effects may reveal important properties of individual drugs. Another important feature is the continuous measurement of drug effect, in contrast to the binary features of a measure such as MAC or verbal response. Quantifying the profiles of drug actions on performance and subjective effects in this way is important in elucidating mode of action, measuring actions, and predicting effects in clinical use.

Supplementary material

A supplementary table is available at *British Journal of Anaesthesia* online.

Funding

References

1 Franks NP. Molecular targets underlying general anaesthesia. *Br J Pharmacol* 2006; **147**: S72–81

2 Magoul R, Onteniente B, Geffard M, Calas A. Anatomical distribution and ultrastructural organization of the Gabaergic system in the rat spinal-cord—an immunocytochemical study using anti-Gaba antibodies. *Neuroscience* 1987; **20**: 1001–9

3 Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G. GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. *Neuroscience* 2000; **101**: 815–50

4 Krasowski MD, Jenkins A, Flood P, Kung AY, Hopfinger AJ, Harrison NL. General anesthetic potencies of a series of propofol analogs correlate with potency for potentiation of gamma-aminobutyric acid (GABA) current at the GABA(A) receptor but not with lipid solubility. *J Pharm Exp Ther* 2001; **297**: 338–51

5 Olsen RW. The molecular mechanism of action of general anesthetics: structural aspects of interactions with GABA(A) receptors. *Toxicol Lett* 1998; **101**: 193–201

6 Zimmerman SA, Jones MV, Harrison NL. Potentiation of gamma-aminobutyric acid(A) receptor Cl- current correlates with in-vivo anesthetic potency. *J Pharm Exp Ther* 1994; **270**: 987–91

7 Lester HA, Dibas MI, Dahan DS, Leite JF, Dougherty DA. Cys-loop receptors: new twists and turns. *Trends Neurosci* 2004; **27**: 329–36

8 Yamakura T, Harris RA. Effects of gaseous anesthetics nitrous oxide and xenon on ligand-gated ion channels: comparison with isoflurane and ethanol. *Anesthesiology* 2000; **93**: 1095–101

9 Sonner JM, Antognini JF, Dutton RC, *et al.* Inhaled anesthetics and immobility: mechanisms, mysteries, and minimum alveolar anesthetic concentration. *Anesth Analg* 2003; **97**: 718–40

10 Eger EI. Age, minimum alveolar anesthetic concentration, and minimum alveolar anesthetic concentration-awake. *Anesth Analg* 2001; **93**: 947–53

11 Petersen-Felix S, Arendt-Nielsen L, Bak P, *et al.* Analgesic effect in humans of subanaesthetic isoflurane concentrations evaluated by experimentally-induced pain. *Br J Anaesth* 1995; **75**: 55–60

12 Petersen-Felix S, Luginbuhl M, Schnider TW, Curatolo M, Arendt-Nielsen L, Zbinden AM. Comparison of the analgesic potency of xenon and nitrous oxide in humans evaluated by experimental pain. *Br J Anaesth* 1998; **81**: 742–7

13 Stucke AG, Stuth EAE, Tonkovic-Capin V, *et al.* Effects of sevoflurane on excitatory neurotransmission to medullary expiratory neurons and on phrenic nerve activity in a decerebrate dog model. *Anesthesiology* 2001; **95**: 485–91

14 Galinkin JL, Janiszewski D, Young CJ, *et al.* Subjective, psychomotor, cognitive, and analgesic effects of subanesthetic concentrations of sevoflurane and nitrous oxide. *Anesthesiology* 1997; **87**: 1082–8

15 Zacny JP, Camarillo VM, Sadeghi P, Black M. Effects of ethanol and nitrous oxide, alone and in combination, on mood, psychomotor performance and pain reports in healthy volunteers. *Drug Alcohol Depend* 1998; **52**: 115–23

16 Gibson HB. *Manual to the Gibson Spiral Maze*, 2nd Edn. Sevenoaks, Kent: Hodder and Stoughton Educational, 1978

17 Tiplady B. Zig-zag tracking: a test of psychomotor speed and accuracy designed for repeated administration. *J Psychopharmacol* 2004; **18**: A48

18 Tiplady B, Drummond GB, Cameron E, *et al.* Ethanol, errors, and the speed-accuracy trade-off. *Pharmacol Biochem Behav* 2001; **69**: 635–41

19 Lezak MD. *Neuropsychological Assessment*. New York: Oxford University Press, 1995

20 Cameron E, Sinclair W, Tiplady B. Validity and sensitivity of a pen computer battery of performance tests. *J Psychopharmacol* 2001; **15**: 105–10

21 Bond A, Lader M. The use of analogue scales in rating subjective feelings. *Br J Med Psychol* 1974; **47**: 211–8

22 Jorum E, Arendt-Nielsen L. Sensory testing and clinical neurophysiology. In: Breivik H, Campbell W, Eccleston C, eds. *Clinical Pain Management: Practical Applications and Procedures*. London: Arnold, 2007; 27–38

23 Hornbein TF, Eger EI, Winter PM, Smith G, Wetstone D, Smith KH. The minimum alveolar concentration of nitrous oxide in man. *Anesth Analg* 1982; **61**: 553–6

24 Scheller MS, Saidman LJ, Partridge BL. MAC of sevoflurane in humans and the New Zealand white rabbit. *Can J Anaesth* 1988; **35**: 153–6

25 Armstrong PJ, Morton C, Sinclair W, Tiplady B. Effects of nitrous-oxide on psychological performance—a dose–response study

using inhalation of concentrations up to 15-percent. *Psychopharmacology* 1995; **117**: 486–90

26 Fagan D, Paul DL, Tiplady B, Scott DB. A dose–response study of the effects of inhaled nitrous-oxide on psychological performance and mood. *Psychopharmacology* 1994; **116**: 333–8

27 Reynolds DS, Rosahl TW, Cirone J, *et al.* Sedation and anesthesia mediated by distinct GABA(A) receptor isoforms. *J Neurosci* 2003; **23**: 8608–17

28 Zeller A, Arras M, Lazaris A, Jurd R, Rudolph U. Distinct molecular targets for the central respiratory and cardiac actions of the general anesthetics etomidate and propofol. *FASEB J* 2005; **19**: 1677–9

29 Nishikawa K, Harrison NL. The actions of sevoflurane and desflurane on the gamma-aminobutyric acid receptor type A—effects of TM2 mutations in the alpha and beta subunits. *Anesthesiology* 2003; **99**: 678–84

30 Zhang Y, Sonner JM, Eger EI, *et al.* Gamma-aminobutyric acid(A) receptors do not mediate the immobility produced by isoflurane. *Anesth Analg* 2004; **99**: 85–90

31 Katayama S, Irifune M, Kikuchi N, *et al.* Increased gamma-aminobutyric acid levels in mouse brain induce loss of righting reflex, but not immobility, in response to noxious stimulation. *Anesth Analg* 2007; **104**: 1422–9

32 Hirota K, Roth SH. Sevoflurane modulates both GABA(A) and GABA(B) receptors in area CA1 of rat hippocampus. *Br J Anaesth* 1997; **78**: 60–5

33 Wakasugi M, Hirota K, Roth SH, Ito YI. The effects of general anesthetics on excitatory and inhibitory synaptic transmission in area CA1 of the rat hippocampus *in vitro*. *Anesth Analg* 1999; **88**: 676–80

34 Zacny JP, Conran A, Pardo H, *et al.* Effects of naloxone on nitrous oxide actions in healthy volunteers. *Pain* 1999; **83**: 411–8

35 Kingery WS, Agashe GS, Guo TZ, *et al.* Isoflurane and nociception—spinal alpha(2A) adrenoceptors mediate antinociception while supraspinal alpha(1) adrenoceptors mediate pronociception. *Anesthesiology* 2002; **96**: 367–74

36 Dawson C, Ma DQ, Chow A, Maze M. Dexmedetomidine enhances analgesic action of nitrous oxide—mechanisms of action. *Anesthesiology* 2004; **100**: 894–904

37 Dickenson AH, Dray A. Selective antagonism of capsaicin by capsazepine: evidence for a spinal receptor site in capsaicin-induced antinociception. *J Appl Physiol* 1991; **76**: 1848–55

38 Sato Y, Kobayashi E, Murayama T, Mishina M, Seo N. Effect of N-methyl-D-aspartate receptor epsilon(1) subunit gene disruption of the action of general anesthetic drugs in mice. *Anesthesiology* 2005; **102**: 557–61

39 Ilkjaer S, Dirks J, Brennum J, Wernberg M, Dahl JB. Effect of systemic N-methyl-D-aspartate receptor antagonist (dextromethorphan) on primary and secondary hyperalgesia in humans. *Br J Anaesth* 1997; **79**: 600–5

40 Tiplady B, Hiroz J, Holmes L, Drummond G. Errors in performance testing: a comparison of ethanol and temazepam. *J Psychopharmacol* 2003; **17**: 41–9

41 Lovinger DR. 5-HT3 receptors and the neural actions of alcohols: an increasingly exciting topic. *Neurochem Int* 1999; **35**: 125–30