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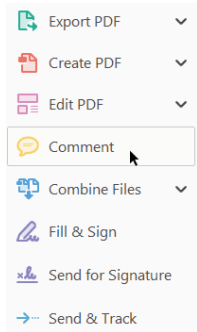
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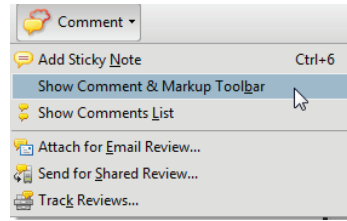


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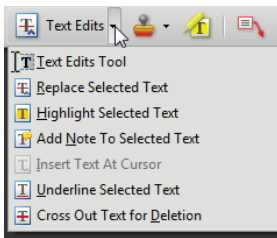


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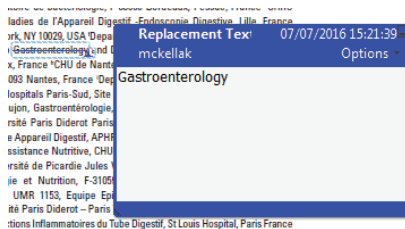
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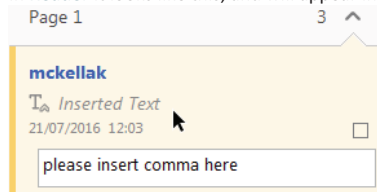


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Invited Review

Past, present, and future of nitrous oxide

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Abstract

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Introduction: For a drug that has been omnipresent for nearly 200 years, nitrous oxide's (N₂O) future seems less certain than its illustrious past. Environmental concerns are coming to the fore and may yet outweigh important clinical benefits.

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Sources of data: After determining the scope of the review, the authors used PubMed with select phrases encompassing the words in the scope. Both preclinical and clinical reports were considered.

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Areas of agreement: The analgesic and anaesthetic advantages of N₂O remain despite a plethora of newer agents

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Areas of controversy: N₂O greenhouse gas effect and its inhibition of key enzymes involved in protein and DNA synthesis have provided further fuel for those intent on eliminating its further clinical use.

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Growing points: The use of N₂O for treatment-resistant depression has gained traction.

Areas timely for developing research: Comparative studies for N₂O role in combatting the prescription opioid analgesic epidemic may well provide further clinical impetus.

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Introduction and history

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In 1772, Joseph Priestley, an English Chemist, discovered nitrous oxide (N₂O) referring to it as phlogisticated

nitrous air in his book 'Experiments and Observations on Different kinds of Air' that was published in 1775. A significant advance was provided by the

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famous Scottish engineer, James Watt, who designed a delivery device which was described in a 1794 book that he co-authored with Thomas Beddoes entitled ‘Considerations on the Medical Use and on the Production of Factitious Airs.’ The term ‘factitious’ refers to the fact that N₂O needs to be synthesized experimentally as it does not exist naturally.

Beddoes established the Pneumatic Institution for Relieving Diseases by Medical Airs in 1798 in Bristol and hired Humphrey Davy, then a 19-year-old chemist from Cornwall, to supervise ongoing experiments that included N₂O. Davy reported on these experiments in his vast tome entitled ‘Researches, Chemical and Philosophical, chiefly concerning Nitrous Oxide, or Dephlogisticated nitrous air, and its Respiration’ published in 1800. Davy commented on N₂O’s possible utility for alleviating pain during surgery as follows ‘As nitrous oxide in its extensive operation appears capable of destroying pain, it may probably be used with advantage during surgical operations in which no great effusion of blood takes place.’ Davy left Bristol to take up a prestigious post at the Royal Institution in London where he lectured extensively on N₂O and went on to discover three elements, sodium, chlorine and iodine as well as the arc lamp which was a precursor to the Davy Lamp that was used in the coal mines.

The first demonstration of the medical use of N₂O was provided by Gardner Quincy Colton for a tooth extraction performed on the dentist Horace Wells, in Hartford Connecticut in 1844. So impressed was Wells that he immediately began to use N₂O on his patients. A subsequent public demonstration in Boston by Wells in the next month was regarded as a failure because the medical student ‘volunteer’ cried out in pain when his tooth was extracted. In the following year, Morton’s public demonstration of ether¹ eclipsed N₂O’s surgical use because of superior potency, ease of transporting the liquid ether and because ether was already being produced in industrial quantities for other applications. After the 1870s N₂O found its place as a staple for dental anaesthesia and in the 1930s it replaced chloroform as the drug of choice for labour analgesia. Over the ensuing years N₂O’s popularity has waxed and waned reaching its nadir in the last two decades when new hospitals that

were built in Europe no longer installed hospital-wide pipes for easy access to N₂O. Despite this mandate a task force convened by the European Society of Anaesthesia opined that there was still a role for the gas in anaesthetic practice.²

Physical properties

While using N₂O as an ‘inert’ gas with which to measure cerebral blood flow, Kety *et al.* needed to resolve the blood:brain solubility coefficient.³ The Bunsen solubility coefficients for N₂O in blood:gas and brain:gas were determined to be 0.412 and 0.437, respectively, in humans.³ Thus, the brain:blood partition coefficient (the ratio of N₂O dissolved per gram of brain to that dissolved in 1 ml of blood) was calculated to be 1.06. N₂O’s low solubility in tissues promotes rapid elimination and hence recovery.^{4,5} N₂O’s favourable pharmacokinetic profile also benefits uptake; the time required for the ratio of end-expired to inspired concentrations to approximate 1.0 during inhalation of 40% N₂O was ~10 min.

Molecular actions of N₂O

N₂O’s effects are largely confined to postsynaptic targets where it blocks both the NMDA subtype⁶ as well as the AMPA-Kainate subtype of the glutamate receptor.⁷ The behavioural properties of N₂O are likely to be produced by antagonism of the NMDA receptor subtype.⁸ To some extent the analgesic mechanisms may also involve inhibition of the T-type calcium channels.⁹

Clinical applications

As an anaesthetic gas, N₂O has many unique properties that have historically been used to great benefit in the operating room. These include a high FA/FI ratio allowing for rapid onset and offset, anxiolytic as well as analgesic and amnestic properties, lack of an odour and lack of irritation to the tracheobronchial tree. These same properties have made it increasingly popular in areas outside of the OR including paediatric procedural sedation, the emergency room,

obstetrics, and potentially psychiatry, for attenuation of treatment-resistant depression.

5 The operating room

Traditionally, N₂O has served a very specific and important role in the administration of general anaesthesia. Before the development of low-solubility volatile anaesthetics, specifically sevoflurane and desflurane, the second gas effect property of N₂O allowed for a more rapid onset and emergence from general anaesthesia.^{10,11} Although the second gas effect is still applicable to sevoflurane and desflurane, its clinical impact is less important.¹² More recently, the ENIGMA randomized clinical trial brought into question whether N₂O was safe to use as part of a general anaesthetic.¹³ The trial found that N₂O increases the risk of post-operative cardiac events (but not mortality); however, the study was not powered to detect such events. The ENIGMA II randomized clinical trial, which was specifically designed to detect increased risk of cardiovascular events or death in patients receiving N₂O, refuted the earlier ENIGMA findings.¹⁴ The authors found no correlation between N₂O and adverse cardiac events in high-risk patients undergoing non-cardiac surgery. They did, however, find a statistically significant increase in postoperative nausea and vomiting (PONV) in those receiving N₂O; the increased risk was not apparent when prophylactic antiemetics were administered prior to the end of the surgery.¹⁵ A meta-analysis revealed that the increase in the risk for PONV with N₂O is time-dependent with a relatively low risk if exposure is <1 h.¹⁶ While the use of N₂O should not be limited because of concern for cardiac complications, the risk of PONV is certainly increased. N₂O may continue to serve a (more limited) role as part of a balanced anaesthetic regimen.

An important product of supplementation of a general anaesthetic with N₂O is that it provides a deeper plane of anaesthesia thereby obviating the risk of awareness as was demonstrated in a thorough meta-analysis.¹⁷ Furthermore, a prospective clinical trial demonstrated that intra-operative awareness can be reduced more effectively with the addition of N₂O than can be achieved with BIS

monitoring.¹⁸ Unlike other volatile anaesthetics, nitrous oxide increases systemic vascular resistance (SVR) as well as plasma norepinephrine levels.¹⁹ Furthermore, when N₂O is used in conjunction with other volatile anaesthetics, it decreases the amount of other volatile agents needed to obtain 1 MAC, thereby attenuating the cardiac depression and decrease in SVR caused by the potent inhalational anaesthetic agents. Additionally, N₂O is neither irritating to the airway nor does it have a pungent odour which proves useful in mask inductions of paediatric and developmentally delayed patients.

While the acute analgesic properties of N₂O have been well-documented, it is now becoming apparent that intra-operative administration of N₂O may result in a decrease in chronic post-surgical pain. In an analysis of the Hong Kong sub-population of patients ($n = 640$) that were enrolled into the original ENIGMA trial, ~10% of patients had new-onset severe pain lasting at least 3 months after surgery; patients randomized to receive N₂O were significantly less likely to experience chronic post-surgical pain (CPSP).²⁰ In a 12-month follow-up to the ENIGMA II trial, more than 12% of patients reported presence of pain at the surgical site¹⁴ although exposure to N₂O did not reduce the risk for CPSP. However, in further planned analysis of the Asian patients from the Hong Kong sub-population of the ENIGMA II trial ($n = 674$) there was a significant reduction in the risk for the reporting of CPSP that was especially evident in those with homozygous variants for the methylenetetrahydrofolate reductase gene.¹⁴

Paediatric use

While use of N₂O in the OR may be decreasing, its use is growing in popularity for sedation for minor procedures particularly in the paediatric population. Because N₂O obviates the need for intravenous access, it is particularly appealing in this population. There have been multiple large studies examining the efficacy and safety of N₂O for a variety of procedures in the paediatric population. In their large, multicenter prospective study, Annequin *et al.* demonstrated

the efficacy and safety of a 50% nitrous oxide/50% oxygen mixture for analgesia and anxiolysis during a variety of paediatric procedures.²¹ Their study included 1019 patients aged 0–18 undergoing procedures including lumbar puncture, bone marrow aspiration, laceration repair, minor procedures (such as surgical dressing or venous cannulation) minor surgeries (such as foreign body extraction and abscess drainage), fracture reductions, dental caries and pulmonary endoscopy.²¹ Median procedural pain evaluations for children were 9 (0–30) on a 1–100 Visual Analogue Scale and 1 when evaluated by either parents or nurses on a 0–10 point numerical scale. Of the 643 children older than 6 years old who were able to self-report, 93% stated that would accept N₂O analgesia again. Only minor side effects were observed in 381 (37%) of patients that included euphoria (20.1%), nausea and vomiting (3.7%), dizziness (1.6%) and deep sedation (2.1%). Other symptoms considered minor side effects were: change in visual or auditory perception, dream, paraesthesia, restlessness, and nightmare or hallucination. All side effects had disappeared within 5 min of discontinuation of the N₂O. In 2006, Onody *et al.* demonstrated a similar safety profile during 35 828 administrations of 50% N₂O in a largely paediatric population.²² Zier *et al.* conducted a prospective observational study of nitrous administration at concentrations up to 70% in 5779 patients on 7802 occasions in patients ages 33 days to 18 years old.²³ The vast majority of patients (90.8%) received a concentration of N₂O that was greater than 50%.

Adverse events occurred in only 4.3% of patients and included nausea (1.6%) and vomiting (2.2%). Nine patients (0.1%) had events that could be considered potentially life-threatening all of which resolved spontaneously and did not require admission.²³ Overall Zier *et al.* did not find that adverse events were increased with concentrations of N₂O greater than 50%.²³ Adverse events were more likely, however, with administration lengths of >15 min.²³ A recent prospective analysis by the Paediatric Sedation Research Consortium found that serious adverse events occurred in only 0.2% of 1634 nitrous administrations for paediatric procedural sedation which included only three episodes of airway obstruction or

desaturation events.²⁴ They confirmed the findings of Zier *et al.* that higher concentrations of N₂O (>50%) were not associated with increased nausea or serious adverse events. Additionally, over half of these N₂O administrations were performed by non-physicians including advance practice nurses and physician assistants. Taken together, these studies show that N₂O in concentrations up to 70% can be administered safely to paediatric patients by trained professionals.

Many smaller, randomized studies have been undertaken to examine the efficacy, superiority and safety of N₂O for specific procedures including venipuncture, laceration repair, lumbar puncture, botulin injection and various imaging procedures. The overall quality of these studies is limited by their small size, but they make a case for considering N₂O as the primary analgesic for many procedures in the paediatric population.

Venipuncture

Venipuncture for obtaining samples for laboratory analyses can be stressful procedures for paediatric patients. Seventy-percent N₂O has been proven to be more effective than an emulsion mixture of the local anaesthetics lidocaine and prilocaine (EMLA) cream alone.²⁵ Additionally, Furraya *et al.* and Henderson *et al.* showed greater analgesic efficacy of 70% N₂O vs 50% for venous cannulation.^{26,27} When compared to oral midazolam plus EMLA cream, 50% nitrous plus EMLA cream resulted in a decrease in total procedure time, improved rate of successful IV access, and overall better experience for paediatric patients.²⁸ Adding EMLA or intradermal lidocaine to N₂O administration decreases the rate of movement or withdrawal with cannulation but does not decrease self-reported pain scores.²⁹

Laceration repair

In a randomized prospective analysis of 30 children comparing local anaesthetic with 100% oxygen administration to local anaesthetic plus 50% N₂O, pain scores decreased significantly in the children receiving N₂O.³⁰ A larger study randomized 204 children to receive local infiltration alone; local

infiltration and oral midazolam; local infiltration and 50% N₂O; or local infiltration, oral midazolam, and 50% N₂O.³¹ In this study, N₂O significantly reduced pain scores and the addition of oral midazolam to N₂O did not increase analgesic efficacy, but prolonged discharge times and increased the incidence of adverse events such as dizziness and irritability.³¹ These results were confirmed by Bar-Meir *et al.* in their study of 60 children undergoing laceration repair.³² They also found that N₂O was more effective in older patients (>3 years of age). A small randomized study of 32 paediatric patients found that N₂O was as effective as intravenous ketamine but with reduced recovery times.³³

Lumbar puncture

There are few studies that directly address the use of N₂O for analgesia during lumbar puncture (LP). In large, non-randomized studies of N₂O for procedural analgesia in paediatric patients a significant proportion of the patients underwent LP and pain scores were reduced by N₂O overall.^{21,34} To date there has been only one study that specifically addresses the use of N₂O for sedation during LP.³⁵ This prospective observational study included only 39 patients of which seven received 50% N₂O alone and 32 received N₂O in addition to topical anaesthetic. Overall pain scores as evaluated by patients, physicians, nurses, and parents were low. More randomized studies of analgesic modalities are needed to prove the optimal analgesic regimen for lumbar puncture.

Injections

Patients with spastic paraplegia, often as a result of cerebral palsy, undergo frequent botulin injections to relieve spasticity. Because these injections are required frequently and in multiple injection sites, adjunctive pain control is required. In a randomized, double-blind study involving 50 paediatric patients N₂O, up to 70%, was found to be superior to enteral midazolam in lowering pain scores as determined by an objective observer, parents, and nurses. Of note, there were eight adverse events reported with N₂O administration which included nausea, vomiting, headache and brief desaturation

below 92%; however, five of the eight parents who witnessed these events rated the overall encounter better than sedation practices that they had experienced in the past.³⁶ In contrast, two studies evaluating the combination of EMLA cream and 50% N₂O found that adequate analgesia was not universally achieved.^{37,38} The authors concluded that not all patients, particularly those less than five years of age (who are unable to self-administer N₂O), are suitable for nitrous sedation. As was found with venipuncture, the higher the concentration of N₂O the more likely that it will be an effective analgesic for botulin injection.^{26,27}

Fracture reduction

Fracture reduction is another common and very painful procedure often performed in the emergency department on paediatric patients. Hennrikus *et al.* found that self-administered 50% N₂O alone provided inadequate pain relief in 46% of fracture reductions³⁹; however, when N₂O was combined with a haematoma block, the frequency of inadequate analgesia was reduced to just 12% and only 7% of the children would choose a different analgesic technique.⁴⁰ No complications were reported in either study that included a total of 154 patients.⁴⁰ When compared to a Bier block, the analgesic effects were found to have similar efficacy, although 50% N₂O significantly reduced procedure length.⁴¹ A randomized trial comparing ketamine and midazolam to 50% N₂O and a haematoma block found no significant difference in pain scores as both provided acceptable analgesia; however, the time to recovery was significantly different with a mean-time of only 16 min in the N₂O group versus 83 min in the ketamine/midazolam arm.⁴² Adverse events including hypoxia and vomiting were also higher in those receiving parenteral sedation with ketamine and midazolam.⁴²

Imaging procedures

N₂O has also been studied for a variety of imaging procedures. For voiding cystourethrograms, N₂O may be as effective as enteral midazolam and can eliminate the need for physical restraint during

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catheter placement.^{41–43} However, N₂O may prolong the time to micturition after these procedures.⁴¹ N₂O has also been found to be effective at reducing pain scores, increasing physician satisfaction scores and increasing procedural success during bronchoscopy.^{44,45} One prospective trial found that good or excellent sedation as judged by the endoscopist was achieved by 50% N₂O during gastrointestinal endoscopy.⁴⁶

Emergency department

Much of the experience using N₂O in the emergency room has been in the paediatric population and applications to the adult population have not been studied in great detail. In an Australian study of 85 patients randomized to either local anaesthetic infiltration, local anaesthetic and oxygen, or local anaesthetic and 50% N₂O for abscess drainage, N₂O showed no advantage in either decreasing pain or anxiety.⁴⁷ A more recent prospective non-blinded observation pilot study examined the analgesic effectiveness and staff satisfaction of analgesia provided by a portable N₂O device in adult patients presenting with moderate to severe pain.⁴⁸ The causes of pain were diverse including abdominal pain, dental pain, musculoskeletal pain, chest pain, traumatic pain, headache, cellulitis, burns, abscesses, and wounds.⁴⁸ The authors found that patients had both clinically and statistically significant reduction in mean pain scores 20 min after N₂O administration that were sustained until 60 min post-administration.⁴⁸ Half of the patients received additional pain medications; however, in a *post-hoc* analysis the administration of additional analgesics was not found to result in lower pain scores. The authors intend to conduct a further study to determine whether adequate education of nursing staff can increase the speed at which patients receive adequate analgesia through nurse-driven N₂O administration to increase overall patient satisfaction.⁴⁸ Two limited studies have explored the use of N₂O for reduction of anterior shoulder dislocations.^{49,50} The initial small study found that N₂O provided similar analgesia to IV sedation; however, there was a 20% failure rate in the N₂O

group necessitating supplementation with intravenous sedation.⁴⁹ The second small study compared N₂O with intra-articular lidocaine in 31 patients and found that N₂O was more effective at reducing pain scores.⁵⁰ Yet, a larger randomized trial comparing fentanyl to 50% N₂O reported no difference in analgesic effectiveness for relieving pain from isolated long bone fracture or main joint dislocation.⁵¹

Another small (22 patients) prospective, randomized, double-blind study compared the effectiveness of 50% N₂O vs 100% oxygen for the relief of migraine pain in patients presenting to the emergency department.⁵² Those that received N₂O had a significant reduction in pain scores immediately after treatment; however, 60% of patients required additional analgesia before discharge although this was lower than the 92% in the oxygen only group. Overall, the data for the use of N₂O for procedural sedation and analgesia is less than convincing in adults and more studies are need to prove its clinical utility in this patient population.

Labour analgesia

N₂O has been used for labour analgesia in the United Kingdom since the 1930s and the US since the 1970s. Its rapid onset and offset as well as its analgesic and anxiolytic properties make it seemingly ideal in this setting. However, two extensive reviews have yielded insufficient evidence to demonstrate effectiveness of N₂O in adequately relieving labour pain.^{53,54} Although these reviews identified 22 studies including 12 RCTs, conclusions were limited largely due to heterogeneity in the techniques used and varied widely in the concentration and delivery method of N₂O, adjunctive pain medications (including inhaled potent volatile anaesthetics that are no longer commonly used), and tools and timing of assessment of pain relief.⁵³

Likis *et al.* assessed nine studies that addressed maternal satisfaction with their birth experience and labour pain management as an endpoint of the effectiveness of N₂O.⁵³ Of these only two were adjudged fair⁵⁵ or good⁵⁶ quality and the authors concluded that the non-uniformity of the measurements

of satisfaction made an accurate assessment impossible. Included in this review was Leong *et al.*'s 2000 study of 123 women who were offered epidural analgesia in early labour.⁵⁶ Over half ($n = 68$) declined epidural analgesia and thus received 'usual' care that included scheduled meperidine and self-administered 50% N₂O. Maternal satisfaction was assessed on post-partum day 1 and a greater percentage of those who received epidural analgesia (69%) were satisfied and would repeat the same analgesic option versus 36% in the N₂O/meperidine group. In Waldenstrom *et al.*'s 1999 study, a detailed survey was undertaken in women 2 months after delivery⁵⁵ in which both medical and psychological factors that could have contributed to an overall positive or negative birth experience were explored. N₂O was found to be an independent predictor of a less positive birth experience; however, of the women who delivered with N₂O over 25% of these reported a positive experience.

Importantly, they also found that perception of involvement or control in the birthing process was the strongest psychologic predictor of a positive birth experience followed by midwife support. Pain and anxiety played a role but to a lesser degree than support and individual control. Although the analgesic effectiveness of N₂O is variable and inferior to that of epidural analgesia, there is a subset of patients who are both satisfied with their overall birth experience and would choose N₂O analgesia again. These studies also provide evidence that effective analgesia alone may not be the most important predictor of a positive birth experience, conclusions that were also reported in Hodnett's systematic review which found that personal expectations, the amount of support from caregivers, the quality of the caregiver-patient relationship, and involvement in decision-making were the most important determinants of overall satisfaction with the birth experience.⁵⁷

To further assess the relationship between effective analgesia and overall satisfaction, Richardson *et al.* conducted a large retrospective analysis of prospectively collected data to assess pain relief and overall satisfaction with labour analgesia in 6507

post-partum women using a standardized survey administered on post-partum Day 1 to compare analgesic effectiveness and overall analgesic satisfaction among women who delivered vaginally using nitrous oxide, neuraxial analgesia or both (neuraxial after N₂O).⁵⁸ Overall reports of analgesic effectiveness and satisfaction with epidural analgesia alone and after a trial of N₂O were consistently high. Amongst the 753 (11.2%) patients who ultimately delivered with N₂O alone there was far greater variability of reported analgesic effectiveness. Fifty-two percent rated analgesic effectiveness as high, 27% as moderate and 21% as low effectiveness. Despite the small effect size, overall satisfaction with nitrous oxide analgesia was high with 93% reporting they were highly satisfied with their anaesthetic care and only 1% reported a low satisfaction level. Furthermore, of those who reported poor to moderate analgesic effectiveness by any modality, the parturients who received N₂O alone were more likely to report high overall satisfaction than those who received neuraxial analgesia. In conclusion, although the overall analgesic effectiveness of N₂O is quite variable, analgesia may not be the most important factor in overall maternal satisfaction and there is likely a small subset of parturients who benefit significantly from N₂O administration during labour. Factors predicting the responsive subset have yet to be elucidated.

Depression

N₂O has also recently shown promise in alleviating the symptoms of treatment-resistant depression (TRD). TRD is a severe form of depression that has failed two or more adequate treatment trials. As early as 1990, NMDA receptor antagonists showed efficacy in mouse models as potential antidepressants.⁵⁹ In parallel, a large body of work has defined the role of NMDA receptors in the pathophysiology of major depressive disorder and bipolar disorder.⁶⁰ The first RCT of ketamine, another NMDA receptor (NMDAR) antagonist,⁶¹ for the treatment of depression was undertaken in 2000. Although the study included only seven patients, antidepressant effects were immediate (within 60 min) and dramatic (lasting

for up to three days).⁶¹ Limited evidence (due to low quality of the studies) of its efficacy has been demonstrated in a recent Cochrane Review⁶² and meta-analysis.⁶³

5 Although the molecular effect of N₂O differs somewhat from ketamine, the evidence of the efficacy of ketamine in treating depression and the increasing evidence for the role of the NMDAR in depression prompted a small proof of concept trial by Nagele and colleagues.⁶⁴ This blinded, placebo-controlled crossover trial included 20 patients with TRD. Patients underwent two treatment sessions 1 week apart with either 50% N₂O/50% oxygen or placebo: 50% nitrogen/50% oxygen. Patients showed significant decrease in their depressive symptoms at 2 and 24 h after N₂O treatment. Interestingly, a majority of the patients treated with N₂O continued to report a decrease in their symptoms which confounded the crossover study design. Overall, 70% of patients reported improvement after N₂O treatment and only 35% reported improvement after placebo. Additionally, 20% of those that received N₂O showed treatment response and 15% showed remission (defined as complete resolution of depressive symptoms). N₂O overall was also well-tolerated in these depressed patients. This limited phase II clinical trial showed dramatic effects on patients with TRD; however, subsequent studies are required to determine optimal dosing to treat depression and avoid side effects and study this treatment on a broader population of patients with TRD.

Side effects

Mutagenicity/occupational hazard

N₂O is utilized in a variety of clinical settings and its possible effect on precipitating genetic abnormalities has been a widespread concern. N₂O decreases methionine synthase function, which plays critical roles in folate metabolism and DNA methylation. It may, therefore, lead to damage to existing DNA and/or inhibit proper DNA synthesis.⁶⁵⁻⁶⁷

45 In a small study involving 91 patients undergoing colorectal surgery, it was found that patients randomized to receive 70% N₂O in oxygen had an

increase in quantifiable DNA damage in leucocytes using a comet tail assay.⁶⁸ This, in turn, led the study's authors to suggest that N₂O would be associated with decreased wound healing after surgery. 50 Further, it was suggested by Hogan that N₂O, in clinically relevant doses, was a potent human genotoxin.⁶⁹ In addition, another study involving 52 patients demonstrated that the use of N₂O may have led to a delay in repair of genotoxic damage 55 when combined with sevoflurane.⁷⁰ Several other studies have shown a trivial amount of DNA damage after exposure to N₂O and other anaesthetic gases, but did not evaluate whether these resulted in clinically meaningful effects.^{71,72} 60

Though no single study has been conclusive regarding N₂O's potential genotoxic, mutagenic, or carcinogenic potential, it is considered an occupational hazard. Prior to routine use of waste gas scavenging systems, concentrations of N₂O in operating rooms and dental suites were between 1000 and 2000 ppm.⁷³⁻⁷⁵ A small study evaluating operating room nurses exposed to anaesthetic waste gases that included N₂O without active waste gas scavenging systems showed that they had increased 70 DNA damage.⁷² A retrospective study revealed that dental assistants exposed to unscavenged occupational N₂O had reduced fertility rates compared to non-exposed assistants.⁷⁶ In addition, there was an increased risk of spontaneous abortion in subjects 75 with similar environments with unscavenged exposure.⁷⁷ Interestingly, and as expected, these effects did not occur in a properly scavenged environment, which suggests these potential reproductive health risks are attenuated, or eliminated, with modern 80 scavenging systems.

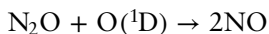
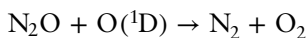
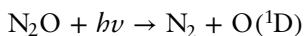
The acknowledgement that N₂O may contribute to potential problems has led to the advent of occupational exposure limits (OELs). In the United States, the National Institute for Occupational Safety and Health, a division of the Centers for Disease Control and Prevention, recommends an OEL of 25 ppm, which is lower than most other recommendations.⁷⁸ In the United Kingdom, the Control of Substances Hazardous to Health Regulations set forth by the Health Services Advisory Committee, limits the OEL of N₂O to 100 ppm.⁷⁹ 90

Despite concerns about design flaws, introduction of bias, or sample size in most studies, the weight of evidence of N₂O's association with potential health risks cannot be ignored, although proper waste gas scavenging systems and limiting occupational exposure to less than stated OELs likely eliminates this risk.

10 Greenhouse gas emissions

According to the United States Environmental Protection Agency, N₂O accounts for ~6% of all human-derived U.S. greenhouse gas emissions. This amount is of growing concern, as N₂O is now recognized as one of the most environmentally damaging gases in the atmosphere.

N₂O produced at the surface is relatively inert until it reaches the middle stratosphere and undergoes photolysis and other chemical reactions that use free oxygen.



The reformed NO contributes to damage to the ozone layer.^{80,81}

In addition to its destructive nature on the ozone layer, N₂O traps heat 300 times more effectively than carbon dioxide, even though it makes up a much smaller percentage of all atmospheric gases. N₂O may last up to 150 years in the atmosphere and has a global warming potential of 298, potentially 12 times higher than methane.⁸²

Globally, 40% of N₂O emissions are attributed to human activities, with the largest source originating from the agricultural sector. Agricultural sources include the use of fertilizers and manures, soil leaching and runoff, and solid waste of domesticated animals, mainly cattle. Fossil fuel combustion for sources of mobile and stationary energy is another significant source of human nitrous oxide emissions. Finally, industrial sources of N₂O emissions include production of adipic acid and nitric acid, ingredients in manufacturing plastics and fertilizers, respectively.

Together, these sources make up 98% of man-made nitrous oxide emissions.^{82,83}

Apart from being released into the atmosphere by human activities, N₂O is also emitted by natural processes. In fact, the majority of N₂O emitted into the atmosphere is derived from these processes. Soils under natural vegetation allow certain microbes to decompose vegetative matter that accounts for more than 60% of these natural processes. In addition, N₂O-producing microbes living in ocean water account for another 35% of natural N₂O emissions.^{82,83}

Unfortunately, N₂O's ozone-depletion potential is comparable to several of the hydrochlorofluorocarbons, which are set to be phased out of use by 2030. The Montreal Protocol on Substances That Deplete the Ozone Layer is an international treaty involving 197 countries, and focuses on ozone-depleting substances that contain either chlorine or bromine. It does not include N₂O.⁸⁴⁻⁸⁶ This would leave N₂O as one of the most important threats to the ozone layer.

Addiction potential

Although N₂O is clinically used as a safe anaesthetic, it is a commonly abused drug in the United States and the United Kingdom, especially amongst adolescents.^{87,88} In fact, N₂O is the second most commonly used recreational substance in the UK after cannabis, according to a recent international survey.⁸⁹ Desired effects include euphoria, anxiety, hallucinations, and confusion. Common practice amongst N₂O abusers include inhaling gas from balloons filled by tanks supplied to industrial or clinical use and inhaling released gas directly from whipped cream dispensers.

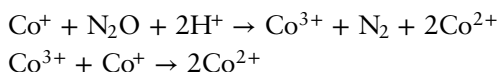
Although the exact mechanism for its addictive potential has not been fully elucidated, it likely stems from nitrous oxide's supraspinal effects to induce analgesia via downstream opioidergic neurons through the release of enkephalins.⁸⁰ In addition to activation of noradrenergic neurons via enkephalins, nitrous oxide may also activate mesolimbic dopaminergic neurons, causing a reinforcement pathway that may lead to further abuse.^{90,91}

It is well documented that longer-term side effects from N₂O abuse include impaired memory and cognition, peripheral numbness, weakness and eventually,

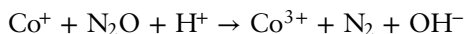
peripheral neuropathy and megaloblastic anaemia.^{80,92} The latter effects stem from N₂O's ability to inactivate vitamin B12, leading to a functional vitamin B12 deficiency with long-term use. In addition, fatal accidents have been reported with recreational use, attributed to hypoxia and asphyxia.^{87,88}

Homocysteine

It is well-known that the use of N₂O is associated with elevated levels of homocysteine. This effect occurs because N₂O indirectly inhibits methionine synthase, a key enzyme in the metabolism of homocysteine. Nitrous oxide oxidizes cobalt I (Co⁺) to Co³⁺, which then leads to the formation of Co²⁺:



(or)



The oxidized cobalt cation prevents cobalamin from acting as a coenzyme for methionine synthase, which in turn, leads to increased homocysteine levels.⁹³ Adequate methionine synthase function is required for proper synthesis of DNA, RNA, myelin and catecholamines. Because methionine synthase plays a crucial role in cellular function, aberrations of its function can result in genetic and protein dysfunction. It has been demonstrated that certain populations may be more susceptible to methionine synthase dysfunction, leading to elevated homocysteine levels. Patients with pre-existing vitamin B12 deficiency, pernicious anaemia, chronic alcoholism or malnourishment may be particularly affected. In addition, a certain subset of the population exhibits polymorphisms of the methylenetetrahydrofolate reductase (MTHFR) gene, leading to reduced enzyme activity. This enzyme reduces 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a key product that is required to convert homocysteine to methionine. Patients with homozygous mutations in the MTHFR gene present with elevated homocysteine levels and homocystinuria.⁹⁴

Cardiovascular system and N₂O

We will first reflect on the physiologic changes that N₂O produces in the cardiovascular system and thereafter consider whether cardiovascular morbidity is more likely to occur when N₂O is added to an anaesthetic regimen in patients, both with and without underlying cardiovascular disease.

In a small study involving 20 coronary artery bypass graft surgical patients under neurolept anaesthesia, the cardiovascular parameters and circulating catecholamines were measured just prior to cardiopulmonary bypass in a cohort that was administered either 66% N₂O or air.⁹⁵ Both mean arterial pressure and cardiac output decreased during N₂O exposure compared to the group that received air.⁹⁵ Baroreflex sensitivity (cardiovascular responses to phenylephrine and nicardipine) was compared in groups ($n = 13$) of ASA I–II patients that were administered equi-anaesthetic concentrations of either N₂O, xenon or isoflurane.⁹⁶ N₂O had similar sensitivity to isoflurane while xenon blunted the responsiveness. One hundred patients were administered epinephrine and lidocaine while undergoing transphenoidal hypophysectomy during isoflurane anaesthesia $\pm 60\%$ N₂O⁹⁷; isorhythmic atrioventricular dissociation occurred significantly more frequently in the patients exposed to N₂O with no difference in ventricular ectopy. Cardiac output was assessed in 80 patients over the age of 60 that were anaesthetised with either isoflurane or halothane $\pm 50\%$ N₂O; while the expected changes were observed in the presence of the volatile anaesthetic alone, it was notable that the systemic vascular resistance *increased* when N₂O was added to halothane-anaesthetised patients resulting in a decrease in cardiac index.⁹⁸ Thirty ASA I–II patients received target-controlled infusion propofol $\pm 70\%$ N₂O⁹⁹; the addition of N₂O induced cardiovascular changes that were not clinically significant.⁹⁹

Because homocysteine induces endothelial dysfunction and has atherogenic properties the effect of N₂O on plasma homocysteine levels were monitored in 394 patients randomized to receive a general anaesthetic \pm N₂O; patients receiving N₂O had higher homocysteine levels which became particularly elevated following

prolonged anaesthetics.¹⁰⁰ Fifty-nine surgical patients with cardiovascular disease were randomized to receive a N₂O-free or N₂O-containing general anaesthetic; patients that had received N₂O had higher homocysteine levels and exhibited endothelial dysfunction evidenced by alterations in flow-mediated dilation of the brachial artery.¹⁰¹

Myocardial ischaemia/infarction

In a small study involving 10 patients with ischaemic heart disease, the addition of 70% N₂O to 1% isoflurane exacerbated the myocardial ischaemia in three of the six patients that isoflurane alone produced.¹⁰² In a larger study involving 70 patients undergoing carotid artery surgery patients randomized to receive a N₂O-free anaesthetic were not less likely to develop myocardial ischaemia (diagnosed by EKG or by TEE) than in those patients that received an anaesthetic regimen that contained up to 60% N₂O.¹⁰³ However, in a randomized study involving 90 patients undergoing carotid endarterectomy, those patients receiving a 50% N₂O added to a total intravenous anaesthetic had significantly more postoperative myocardial ischaemia than the N₂O-free anaesthetic group.¹⁰⁴ In a widely quoted study involving 47 abdominal aortic aneurysm surgery, patients randomized to receive a N₂O-free isoflurane/fentanyl anaesthetic regimen had less myocardial ischaemia and required less nitroglycerin for blood pressure control than patients in whom N₂O was used to supplement the general anaesthetic.¹⁰⁵

Because of the plausibility of N₂O's ability to produce myocardial ischaemia through its effect on methionine synthase and endothelial function, Myles' group launched the ENIGMA trial in which 2050 non-cardiac surgical patients were randomized to receive either a N₂O-free or N₂O-containing general anaesthetic and followed-up for a median of 3.5 years to determine the impact on survival and the occurrence of myocardial infarction or stroke. While risk of death (primary endpoint) and stroke were not influenced by exposure to N₂O, there was a statistically significant ($P = 0.04$) increase in the adjusted odds ratio (1.59) for myocardial infarction (95% CI: 1.01–2.51). Because of this adverse outcome a follow-

up study, ENIGMA II, was launched involving 7112 non-cardiac surgical patients at risk for coronary artery disease with a composite primary endpoint of death and major non-fatal cardiovascular events (myocardial infarction, cardiac arrest, pulmonary embolism and stroke) at 1 year after surgery.¹⁰⁶ In the 82% of patients in whom follow-up data were available, exposure to N₂O did not increase the risk of the primary outcome, disability (Katz index of independence in activities of daily living of <8) death, myocardial infarction or stroke.¹⁰⁷ Unlike the original ENIGMA trial the oxygen concentration was maintained the same in each intervention.

Contra-indications

While N₂O overall has a high safety profile, there are several instances in which it is contraindicated.

Closed spaces

The blood: gas partition coefficient of nitrous oxide is 0.46 which is 30 times greater than that of nitrogen (0.014). Because of this, N₂O will enter gas-filled spaces more than thirty times faster than nitrogen (contained in room air) can exit the space. As such, both the volume and pressure within the closed space will increase. In 1955, Hunter described cardiovascular compromise in patients with pneumothorax, pneumoperitoneum and pneumopericardium who were anaesthetised with N₂O.¹⁰⁸ Subsequently, Eger *et al.* found that in dogs anaesthetised with 70–80% N₂O, the intestinal gas volume increased by 80–100% at 2 h and to 200% at 4 h.

Intrapleural gas volumes doubled within 10 min and tripled in 30–45 min. In contrast, intrapleural and intestinal gas volumes remained constant or decreased with halothane and oxygen administration.¹⁰⁹ Therefore, pneumothorax, bowel obstruction and pneumopericardium are considered contraindications to N₂O. Extrapolating from this, emphysematous blebs may also be a contraindication to N₂O as air/gas can be trapped in these spaces during the respiratory cycle.¹⁰⁹ Similarly, inhaled nitrous oxide can increase the pressure in the middle ear.¹¹⁰

There has been speculation that this is particularly problematic when the eustachian tube is blocked preventing exit of this gas; however, this has not proven to be clinically significant.¹¹¹ In contrast, N₂O expansion into intravitreal air bubbles of sulphur hexafluoride (SF6) or perfluoropropane (C3F8) used in vitreoretinal procedures can cause blindness.¹¹² Anaesthesia textbooks recommend that N₂O should be avoided for 7–10 days after a sulphur hexafluoride bubble and at least a month after a perfluoropropane bubble; however, Fu *et al.* recommend at least a month for SF6 and potentially altogether avoidance of N₂O in patients who have had a C3F8 vitreous bubble placed.¹¹²

This same property also means that N₂O can increase the volume of a venous air embolism. As such, N₂O is considered relatively contraindicated during surgeries where there is a high risk of venous air embolism (VAE). These surgeries include all surgeries in the sitting position, particularly posterior fossa surgeries, laparoscopic surgery and caesarean section. In his 1966 study, Munson found that, in rabbits, N₂O anaesthesia decreases the lethal dose of intravenous air by 30% compared to halothane alone.¹¹³ Later, he showed, in dogs, that N₂O significantly increases pulmonary pressures and dead space and significantly decreases cardiac output in the presence of an air embolus.¹¹⁴ Losasso *et al.* subsequently found in their prospective, randomized trial that N₂O did not increase the incidence of VAE or haemodynamically significant VAE when N₂O was discontinued as soon as the VAE was detected by precordial Doppler.¹¹⁵ Therefore, N₂O can be used during surgeries with a relatively high risk of venous air as long as there is a high level of vigilance for VAE.

N₂O may also increase pulmonary vascular resistance (PVR) and mean pulmonary arterial pressures (mPAP) and is, therefore, contraindicated in patients with pulmonary hypertension. Schulte-Sasse *et al.* found that PVR was significantly increased in patients with pre-existing pulmonary hypertension secondary to mitral valve stenosis.¹¹⁶ A subsequent study in similar patients found that with a higher dose fentanyl induction, N₂O did not increase PVR or mPAP and the authors postulated that the high dose opioid attenuates the catecholamine release caused by N₂O.¹¹⁷

Although the effects on PVR may be attenuated by high dose opioids, it should most likely be avoided in patients with pre-existing pulmonary hypertension.

Additionally, N₂O is relatively contraindicated in patients with a predisposition to postoperative nausea and vomiting. Nausea is a well-known side-effect of N₂O and this was again confirmed in the ENIGMA II trial.¹⁴ These effects may increase with the length of procedure as proposed by Peyton and Wu in their review and meta-analysis.¹⁶ Specifically they found that the risk ratio of PONV increases 20% per hour of nitrous oxide exposure beginning at 45 min after initiation.¹⁶

Conclusion

For a drug that was first used clinically nearly two centuries ago, N₂O has proven to be remarkably durable despite the introduction of several waves of ‘newer’ drugs from ether to xenon. Features such as low cost, rapid pharmacokinetics, ease of use and monitoring and relative safety, have secured for it a place in the armamentarium of anaesthetists the world over.

Why does enthusiasm for the use of N₂O persist?¹¹⁸ Its consistent analgesic effect reduces chronic postoperative pain thereby reducing the need for opioid analgesics in the midst of the crisis of the prescription opioid analgesic epidemic.²⁰ Using the number needed to treat, intra-operative awareness can be more effectively prevented with N₂O than with BIS monitoring!^{17–18} More recently, questions have arisen whether certain vulnerable populations may be at risk from a drug that has inhibits folate-dependent enzymes including methionine synthase and methylenetetrahydrofolate reductase affecting ‘one-carbon’ metabolism that can influence biochemical processes from amino acid to nucleotide synthesis. Myelinopathy, remains a devastating complication albeit exceedingly rare under clinical, non-abuse, conditions. In fact it can be argued that blockade of the NMDA subtype of the NMDA receptor is more likely to result in neuroprotection than in neurotoxicity.¹¹⁹ In the arena of anaesthetic-induced developmental neurotoxicity (AIDN) the preclinical studies suggest an exacerbating effect of N₂O although it does not have the same potential for AIDN as do the potent volatile agents.^{120,121}

Among safety concerns are the volume-expanding potential of N₂O that may cause problems in bowel surgical procedures and in the setting of pneumocephalus or when air embolisation occurs. The potential for cardiovascular-provoking complications from elevated levels of homocysteine arising from the failure to methylate this amino acid in the presence of N₂O remains a concern.¹²² Yet neither the POISE nor the ENIGMA II demonstrated a relationship between N₂O exposure and acute cardiovascular complications.^{107,123}

N₂O continues to be an effective and safe analgesic and supplement for general anaesthesia. However from a societal aspect, environmental concerns may ultimately trump the individual patient's benefit.

Conflict of interest statement

The authors have no potential conflicts of interest.

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