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# Electrophysiologic Characteristics of Nitrous-Oxide-Associated Peripheral Neuropathy: A Retrospective Study of 76 Patients

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**Background and Purpose** The electrophysiologic characteristics of peripheral neuropathy secondary to nitrous oxide ($N_2O$) abuse remain unclear. The paper therefore aimed to summarize the electrophysiologic characteristics of $N_2O$-associated peripheral neuropathy and identify the risk factors of severe nerve injury.

**Methods** The electrophysiologic results and clinical data of patients with peripheral neuropathy secondary to $N_2O$ abuse at our hospital between 2018 and 2020 were analyzed retrospectively, and their electrophysiologic changes were summarized.

**Results** Most patients exhibited decreased sensory and motor nerve conduction velocities (75% and 76%), decreased sensory nerve and compound motor action potentials (57% and 59%), and prolonged distal motor latency (59%), while a response was absent in 36%. These findings indicate that $N_2O$ abuse can result in generalized injury to sensory and motor nerves. Electrophysiologic results indicated axonal neuropathy in 37 cases (49%), demyelinating peripheral neuropathy in 4 (5%), and mixed neuropathy in 12 (16%). Peripheral nerve injury was more common in the lower limbs (72%) than in the upper limbs (42%, $p<0.0001$). The upper and lower limbs were primarily affected by sensory nerve demyelination (35%) and motor axonal injury (67%), respectively. Subgroup analysis indicated that longer $N_2O$ exposure and longer disease course were associated with more-severe motor axonal injury in the lower limbs.

**Conclusions** $N_2O$-associated peripheral neuropathy can lead to sensory and motor nerve injury, with axonal injury being the most common. Injuries were more severe in the lower limbs. Prolonged $N_2O$ exposure and disease course increased the severity of motor axonal injury in the lower limbs.

**Keywords** nitrous oxide abuse; peripheral neuropathy; electrophysiologic study; severity; vitamin B12.

## INTRODUCTION

Nitrous oxide ($N_2O$) is an oxidizing agent that is primarily used for anesthesia and in food manufacturing, such as in cream whipping.[1,2] $N_2O$ is also used as a recreational drug to produce stimulant and euphoric effects after inhalation. It acts rapidly, reaching its peak effect in approximately 1 minute, with its effects waning after about 2 minutes. $N_2O$ trafficking and consumption have recently become increasingly common and occur primarily in bars, night clubs, and other entertainment venues; people can also find it in private homes and house parties, and most consumers are teenagers.[3,4] Vitamin B12 metabolism is relevant to the development of $N_2O$-induced neuropathy.[5-7] $N_2O$ users have been reported to exhibit symptoms of peripheral neuropathy (most commonly), myelopathy, and encephalopathy.[5,8,9] Layzer et al.[10] were the first to report cases ($n$=3) of peripheral neuropathy caused

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by $N_2O$. They used nerve conduction testing to suggested that this type of peripheral neuropathy most commonly involved axonal injury. Hsu et al.[11] reported a patient with multiple peripheral demyelination-predominant polyneuropathy, and Morris et al.[12] reported a patient with severe axonal degeneration. These findings indicate that $N_2O$-associated peripheral neuropathy often manifests as acute sensorimotor polyneuropathy. However, only a few studies have summarized the electrophysiologic characteristics of $N_2O$-associated peripheral neuropathy.[13,14] The present study therefore aimed to summarize the electrophysiologic characteristics of $N_2O$-associated peripheral neuropathy and determine the factors that affect severe nerve injury.

## METHODS

### Subjects

This study analyzed patients diagnosed with $N_2O$-associated peripheral neuropathy at Shengjing Hospital of China Medical University between February 2018 and August 2020 who had clinical symptoms of numbness and/or weakness in the distal limbs or electrophysiologic results that indicated peripheral neuropathy. The patients had various $N_2O$ inhalation histories, and none had any personal or family history of neurologic or psychiatric diseases. Patients were excluded if their peripheral neuropathy was possibly caused by diabetes, alcohol poisoning, digestive system disease, long-term vegetarianism, infection and immunity, toxins, chemical substances, or genetic factors. All patients underwent electromyography (EMG). The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Shengjing Hospital of China Medical University (IRB No. 2020PS047K).

### Methods

#### Clinical data

Clinical data of the patients were collected, including clinical symptoms and signs, laboratory test results (hemoglobin, vitamin B12, folate, and homocysteine), and electrophysiologic and imaging results. Vitamin B12 deficiency was defined as a vitamin B12 level lower than the lower normal limit (180 pg/mL), and hyperhomocysteinemia was defined as a homocysteine level higher than the upper normal limit (15 μmol/L).

#### Electrophysiologic study

The tested sensory nerves were the median, ulnar, superficial peroneal, and sural nerves. The electrophysiologic parameters included the sensory nerve conduction velocity (SNCV) and sensory nerve action potential (SNAP). The tested motor nerves were the median, ulnar, common peroneal, and tibial nerves. The electrophysiologic parameters included distal motor latency, motor nerve conduction velocity (MNCV), and compound motor action potential (CMAP).

Demyelinating neuropathy was considered to have occurred when two or more nerves met the following criteria: SNCV or MNCV <80% of the lower normal limit, or distal motor latency ≥120% of the upper normal limit. Axonal neuropathy was considered to have occurred when two or more nerves met the following criteria: SNAP or CMAP <80% of the lower normal limit, or no response. Mixed neuropathy was considered to have occurred if the criteria for both demyelinating and axonal neuropathy were met.[14-16]

### Statistics

All statistical analyses were performed using SPSS (version 26.0, IBM Corp., Armonk, NY, USA). Continuous variables are expressed as mean±SD or median (interquartile range) values. All categorical variables are expressed as frequency (percentage) values. Measurement data were compared and analyzed using the *t*-test for independent samples or analysis of variance. Count data were analyzed using the chi-square test. Differences for which $p<0.05$ were considered significant.

## RESULTS

### Clinical data, and laboratory and imaging examinations

This study enrolled 76 patients (34 males, 42 females) aged 21.2±4.1 (range 14–33) years. The clinical data and laboratory test and imaging results of the patients are listed in Table 1. The most common clinical symptoms were limb numbness and/or weakness (74 cases, 98%), which were present in all limbs (53 cases, 70%) or only the lower limbs (21 cases, 28%). The most common positive neurologic sign was decreased limb muscle strength (60 cases, 79%). Of the 51 patients who had their hemoglobin tested, 86% (44/51 cases) exhibited anemia, 34% (15/44 cases) of which presented with megaloblastic anemia, with a corpuscular volume of 105.3±4.4 fL (normal value=83–101 fL). Vitamin B12 and folate tests were performed together in 44 patients, and 28 (64%) had vitamin B12 deficiency. The folic acid levels of those 44 patients were within the normal range (3–17 ng/mL). Homocysteine testing was performed on 35 patients, and 25 (71%) hyperhomocysteinemia cases were found. Spinal cord magnetic resonance imaging (MRI) was performed on 36 patients, and 20 cases (56%) were found to have high T2-weighted signals. Head MRI was performed on 13 patients; 6 patients (46%) of abnormal brain were found on MRI, comprising 3 cases

**Table 1.** Patient demographics and laboratory results

| | Value | Range |
|---|---|---|
| Sex | | |
| Males | 34 (45) | |
| Females | 42 (55) | |
| Age, yr | 21.2±4.1 | 14–33 |
| Males | 22.7±3.7 | 15–33 |
| Females | 19.9±4.0 | 14–32 |
| $N_2O$ exposure time, days | 365 [90–365] | 15–1460 |
| Males | 240 [90–730] | 30–1095 |
| Females | 365 [90–365] | 15–1460 |
| Disease course, days | 30 [8–30] | 3–1095 |
| Males | 18 [7–30] | 7–1095 |
| Females | 30 [7–45] | 3–365 |
| Clinical symptoms | | |
| Neurologic symptoms | | |
| Numbness and/or weakness in all limbs | 53 (70) | |
| Numbness and/or weakness in lower limbs | 21 (28) | |
| Walking instability | 32 (42) | |
| Headache | 5 (7) | |
| Urinary retention or dysphoria | 10 (13) | |
| Constipation | 3 (4) | |
| Hypomnesia | 7 (9) | |
| Psychiatric symptom | | |
| Illusion | 4 (5) | |
| Other symptoms | | |
| Chest distress | 3 (4) | |
| Skin pigmentation | 7 (9) | |
| Hearing loss | 1 (1) | |
| Reduced or increased appetite | 12 (16) | |
| Positive neurologic signs | | |
| Decreased limb muscle strength | 60 (79) | |
| Hypesthesia of pain and temperature | 46 (61) | |
| Reduced or absent tendon reflex | 45 (59) | |
| Disturbance of deep sensations | 44 (58) | |
| Ataxia | 22 (29) | |
| Romberg sign | 15 (20) | |
| Signs of upper motor neuron injury | 10 (13) | |
| Laboratory examinations | | |
| Hemoglobin, normal range 110–150 g/dL | 131.2±20.9 | 70–170 |
| Normal | 7/51 (14) | |
| Anemia | 44/51 (86) | |
| Vitamin B12, normal range 180–910 pg/mL | 189.9±124.4 | 50–828 |
| Normal | 16/44 (36) | |
| Decreased | 28/44 (64) | |
| Homocysteine, normal range 0–15 μmol/L | 43.0±39.4 | 3–138 |
| Normal | 10/35 (29) | |
| Increased | 25/35 (71) | |
| Spine MRI | | |
| Normal | 16/36 (44) | |
| Abnormal | 20/36 (56) | |
| Brain MRI | | |
| Normal | 7/13 (54) | |
| Abnormal | 6/13 (46) | |

Data are mean±SD, *n* (%), median [interquartile range] values, or *n*/*N* values. The *n* in *n*/*N* is the number of patients who underwent the test or examination; Disease course: duration between initial symptom onset and admission.
$N_2O$, nitrous oxide.

each of brain atrophy and demyelination.

## Electrophysiologic results

Most patients exhibited decreased SNCV and MNCV (75% and 76%, respectively; $p$=0.850) and decreased SNAP and CMAP (57% and 59%, respectively; $p$=0.743). The prolonged distal motor latency and absent response rates were 59% and 36%, respectively. Among the 76 patients, 284 sensory and 276 motor nerves were tested. The EMG results for these nerves are listed in Table 2. The motor nerve potentials of the lower limbs were significantly decreased, by 76% and 63% in the common peroneal and tibial nerves, respectively. Based on the electrophysiologic diagnostic criteria, the most common neuropathy type was axonal (37 cases, 49%), followed by mixed demyelinating and axonal (12 cases, 16%) and demyelinating (4 cases, 5%). Mild electrophysiologic abnormalities were observed in 21 patients (28%) who did not meet the diagnostic criteria for axonal or demyelinating neuropathy. Two patients (3%) had normal electrophysiologic findings.

## Electrophysiologic characteristics

The types and rates of sensory and motor nerve injury were further analyzed based on the distributions in the upper and lower limbs (Table 3). The injury rate was significantly higher in the lower limbs (72%) than in the upper limbs (42%) ($\chi^2$=51.345, $p$<0.0001). The primary type of injury in the upper limbs was demyelination (35%), and that in the lower limbs was axonal injury (54%) ($\chi^2$=20.654, $p$<0.0001). This indicates that both the nerve injury rate and injury type exhibited different distributions between the limbs. In the lower limbs, the injury rate was higher in the motor nerves (76%) than in the sensory nerves (68%) ($\chi^2$=1.700, $p$=0.192). In contrast, that in the upper limbs was higher in the sensory nerves (48%) than in the motor nerves (35%) ($\chi^2$=4.494, $p$=0.034). This indicates that the nerve injury rate in the same limb was associated with the nerve type. We also found that the nerve injury type in the lower limbs was associated with the nerve type, unlike in the upper limbs. The primary injury type in the motor nerves of the lower limbs was axonal inju-

**Table 2.** Electromyography results for various nerves

| Sensory nerves | Median | Ulnar | SPN | Sural | Total |
|---|---|---|---|---|---|
| Total | 76 | 68 | 75 | 65 | 284 |
| SNCV<NV | 18 (24) | 33 (49) | 38 (51) | 23 (35) | 112 (39) |
| m/s | 47.13 [6] | 46.38 [7] | 36.29 [9] | 36.36 [9] | |
| SNAP<NV | 20 (26) | 16 (24) | 23 (31) | 13 (20) | 72 (25) |
| μV/mV | 11.09 [45] | 8.86 [48] | 4.17 [31] | 3.81 [37] | |
| No response | 0 | 0 | 13 (17) | 9 (14) | 22 (8) |
| **Motor nerves** | **Median** | **Ulnar** | **CPN** | **Tibial** | **Total** |
| Total | 76 | 57 | 75 | 68 | 276 |
| MNCV<NV | 18 (24) | 18 (32) | 51 (68) | 46 (68) | 137 (50) |
| m/s | 46.60 [7] | 46.67 [7] | 34.72 [21] | 34.99 [17] | |
| CMAP<NV | 4 (5) | 6 (11) | 32 (43) | 34 (50) | 76 (28) |
| μV/mV | 2.450 [39] | 4.017 [33] | 0.730 [76] | 1.494 [63] | |
| DML>NV | 6 (8) | 9 (16) | 25 (33) | 30 (44) | 70 (25) |
| m/s | 4.620 [5] | 3.523 [7] | 6.216 [24] | 5.829 [21] | |
| No response | 0 | 0 | 17 (23) | 13 (19) | 30 (11) |

Data are *n*, *n* (%), or mean [percentage change] values.
CMAP, compound muscle action potential; CPN, common peroneal nerve; DML, distal motor latency; MNCV, motor nerve conduction velocity; NV, normal value; SNAP, sensory nerve action potential; SNCV, sensory nerve conduction velocity; SPN, superficial peroneal nerve.

**Table 3.** Distribution differences of the rates and types of neuroelectrophysiologic abnormalities

| | Demyelination | Axonal injury | Mixed* | Total | $\chi^2$, *p* |
|---|---|---|---|---|---|
| Sensory nerve (upper limb) | 51 (35) | 36 (25) | 18 (13) | 69 (48) | 12.44, 0.0004 |
| Sensory nerve (lower limb) | 61 (44) | 58 (41) | 23 (16) | 96 (69) | |
| $\chi^2$, *p* | | 1.134, 0.567 | | | |
| Motor nerve (upper limb) | 45 (34) | 10 (8) | 8 (6) | 47 (35) | 45.199, <0.0001 |
| Motor nerve (lower limb) | 77 (54) | 96 (67) | 65 (45) | 108 (76) | |
| $\chi^2$, *p* | | 31.617, <0.0001 | | | |
| Sensory nerve (upper limb) | 51 (35) | 36 (25) | 18 (13) | 69 (48) | 4.494, 0.034 |
| Motor nerve (upper limb) | 45 (34) | 10 (8) | 8 (6) | 47 (35) | |
| $\chi^2$, *p* | | 8.978, 0.011 | | | |
| Sensory nerve (lower limb) | 61 (44) | 58 (41) | 23 (16) | 96 (69) | 1.700, 0.192 |
| Motor nerve (lower limb) | 77 (54) | 96 (67) | 65 (45) | 108 (76) | |
| $\chi^2$, *p* | | 7.503, 0.023 | | | |
| Sensory and motor nerves (upper limb) | 96 (35) | 46 (17) | 26 (9) | 116 (42) | 51.345, <0.0001 |
| Sensory and motor nerves (lower limb) | 138 (49) | 154 (54) | 88 (31) | 204 (72) | |
| $\chi^2$, *p* | | 20.654, <0.0001 | | | |

Data are *n* (%) values.
*Both demyelination and axonal neuropathy.

ry (67%), and that in the sensory nerves was demyelination (44%) ($\chi^2$=7.503, *p*=0.023).

## Analysis of factors influencing nerve injury severity

Since the results described above indicated that motor nerve axonal injury in the lower limbs was the most-prominent injury, the patients were divided into two groups based on the degree of motor nerve axonal injury in this region. The inclusion criterion for the severe group (*n*=24) was CMAP ≤1 mV or an absent response; the remaining patients constituted the mild group (*n*=52). Factors that influenced the degree of motor nerve axonal injury in the lower limbs were further analyzed (Table 4). There were significant differences in disease course and exposure time between the two patient groups (*p*<0.001), suggesting that these factors influence the degree of motor nerve axonal injury in the lower limbs. We therefore further applied logistic regression analysis to the two independent variables of disease course and exposure time with the outcome set as severe motor nerve axonal injury in the lower limbs (no=0, yes=1). This analysis indicated that a lon-

**Table 4.** Analysis of factors affecting the severity of nerve injury according to electrophysiologic results

| | Severe group | | | Mild group | | | |
|---|---|---|---|---|---|---|---|
| | Total | Females | Males | Total | Females | Males | *p* |
| Number | 24 | 13 (54) | 11 (46) | 52 | 29 (56) | 23 (44) | 0.896 |
| Age, years | 21.5±4.5 | 19.2±3.1 | 24.2±4.5 | 21.0±4.1 | 20.1±4.4 | 22.2±3.3 | 0.687 |
| *p* | 0.004 | | | 0.062 | | | |
| Exposure time, days | 365 [180–821] | 365 [180–730] | 365 [180–1095] | 365 [90–365] | 365 [90–365] | 365 [180–730] | <0.0001 |
| *p* | 0.580 | | | 0.432 | | | |
| Disease course, days | 30 [26.3–97.5] | 30 [30–90] | 30 [7–120] | 15 [7–30] | 15 [7–30] | 15 [7–30] | <0.0001 |
| *p* | 0.541 | | | 0.740 | | | |
| Hemoglobin, g/L | 128.9±18.6 | 122.1±17.0 | 137.6±17.9 | 132.3±22.1 | 123.1±19.7 | 146.1±18.5 | 0.429 |
| *p* | 0.077 | | | 0.002 | | | |
| Vitamin B12, pg/mL | 188.0±145.2 | 190.6±186.1 | 185±111.8 | 190.7±112.9 | 185.9±122.2 | 195.7±106.5 | 0.628 |
| *p* | 0.470 | | | 0.629 | | | |
| HCY, μmol/L | 35.8±22.9 | 39.9±23.0 | 24.9±23.1 | 50.8±45.2 | 54.1±48.3 | 48.1±44.4 | 0.721 |
| *p* | 0.497 | | | 0.999 | | | |
| Spinal MRI | 8/13 | 4 | 4 | 12/23 | 7 | 5 | 0.587 |
| Brain MRI | 3/6 | 1 | 2 | 3/7 | 1 | 2 | 0.797 |

Data are mean±SD, median [interquartile range], *n* (%), or *n*/*N* values. The *n* in *n*/*N* is the number of patients with abnormal MRI results; Disease course: duration between initial symptom onset and admission.
HCY, homocysteine.

**Table 5.** Logistic regression analysis of factors influencing severity of motor nerve axonal injury in the lower limbs

| Variable | B | SEM | Wald | *p* | Odds ratio | 95% CI |
|---|---|---|---|---|---|---|
| Exposure time | 0.003 | 0.001 | 5.372 | 0.020 | 1.003 | 1.000–1.005 |
| Disease course | 0.041 | 0.015 | 7.887 | 0.005 | 1.042 | 1.012–1.072 |

CI, confidence interval; SEM, standard error of the mean.

ger exposure time and disease course were associated with more-severe motor axonal injury in the lower limbs (exposure time: odds ratio [OR]=1.003, 95% confidence interval [CI]=1.000–1.005, $p$=0.02; disease course: OR=1.042, 95% CI=1.012–1.072, $p$=0.005) (Table 5).

## DISCUSSION

Recreational $N_2O$ use has recently become increasingly popular in both Western and Asian countries, especially among teenagers and young adults.[17-21] The results of the present study reaffirm that $N_2O$ users tend to be young. Myelopathy and (particularly) peripheral neuropathy are the most common $N_2O$-associated neuropathies. All of the patients in the present study were diagnosed with peripheral neuropathy, of which 20 exhibited abnormalities on spinal cord MRI, suggesting concomitant myelopathy. Consistent with previous studies, the common clinical symptoms of $N_2O$ abuse are numbness and/or limb weakness. Neurologic signs often include weak or lost tendon reflexes, decreased sensitivity to pain and temperature, and decreased muscle strength in the limbs, which are consistent with peripheral neuropathy.[22-29]

The exact mechanism underlying $N_2O$-associated peripheral neuropathy has not been elucidated. Previous studies suggested that the pathologic effect of $N_2O$ is related to vitamin B12, which plays a key role in maintaining myelin production and metabolism. $N_2O$ irreversibly induces vitamin B12 oxidation, thereby inhibiting the methionine synthase activity and blocking methylation and DNA synthesis, which affects myelin methylation and leads to the demyelination of central and peripheral nerves.[5-7] $N_2O$ can also exert neurotoxic effects by antagonizing the accumulation of NMDA receptors and homocysteine. Injury due to oxidative stress and changes in cytokine and growth factor levels are also considered to be related to $N_2O$-associated neuropathy.[17,30-33]

Homocysteine and vitamin B12 levels were therefore assessed in the present study. The results indicated that the hyperhomocysteinemia rate (71%) was higher than the vitamin B12 deficiency rate (64%). Consistent with previous studies, the present study also found that the low vitamin B12 deficiency rate may be due to many patients taking vitamin B12 supplements before visiting the clinic.[34] Even if serum vitamin B12 levels are normal, there may be vitamin B12 deficiency in the cells. Buizert et al.[8] reported a case of subacute combined spinal degeneration, in which hematologic tests indicated normal serum vitamin B12 and folic acid levels but signifi-

cantly elevated methylmalonic acid levels; elevated homocysteine or methylmalonic acid levels indicate a lack of functional vitamin B12 at the cellular level.[8,35] Homocysteine is therefore more suitable as a test indicator than vitamin B12 when examining patients suspected of $N_2O$ abuse.

Some differences exist in the electrophysiologic characteristics of the different etiologies of peripheral neuropathy. Peripheral neuropathy due to chemotherapeutic agent use manifests primarily as a pathology in sensory nerve axons,[36-42] whereas peripheral neuropathy due to copper and folic acid deficiency manifests as sensory rather than motor peripheral neuropathy.[43,44] These peripheral neuropathies primarily manifest as sensory neuropathy. However, the present study found that peripheral neuropathy due to $N_2O$ generally affects sensory and motor nerves, and the rate and type of nerve injury are associated with limb distribution and nerve type. The nerve injury rate is higher in the lower limbs, and injury is more severe in the distal than in the proximal limbs. Consistent with the neuroelectrophysiologic findings, we also observed that weakness and numbness were more severe in the distal and lower limbs than in the proximal and upper limbs. This length-dependent characteristic is also common in peripheral neuropathy with other etiologies.[14,36,45,46] The motor nerve potentials of the lower limbs exhibited the most-significant changes, suggesting that motor nerve axonal injury is most severe in the lower limbs.

As mentioned above, peripheral neuropathy associated with $N_2O$ exposure is often attributed to vitamin B12 deficiency. Tani et al.[28] compared the EMG presentations of peripheral neuropathy caused by $N_2O$ exposure and isolated vitamin B12 deficiency, and found that the rate and degree of motor nerve axonal injury were more significant in the $N_2O$ group. The prominent changes in motor axons of patients with $N_2O$ exposure may indicate a distinct pathophysiologic mechanism. The nerve excitability test in patients with $N_2O$ exposure performed by Tani et al.[28] indicated a decreased accommodation toward depolarizing current and increased superexcitability, which may be related to the loss of or conduction block of the largest nerve fibers caused by $N_2O$ toxicity in the axonal paranodal region. The most common injury type in the motor nerves was axonal, and that in the sensory nerves was demyelination. Motor nerves contain more large nerve fibers than the sensory nerves, which may have a closer relationship with this difference. EMG and nerve biopsies may be more helpful to for explaining this problem from the perspective of pathophysiology, which requires further study.

The present study found an association between the degree of nerve injury and the duration of $N_2O$ exposure and disease course. A longer exposure time and disease course were associated with more-significant nerve potential changes, suggesting that motor axonal injury was more severe in the lower limbs. Exposure duration plays an important role in predicting the degree of nerve injury. Previous studies have also found that long-term $N_2O$ consumption is more likely to result in neurologic symptoms. The present study also found significant symptoms and rapid progression even in patients with short exposure durations. This may be related to consumption of high doses of $N_2O$ over a short period, and may also differ among individuals based on their sensitivity to $N_2O$.[17] The degree of nerve injury is also related to the amount of $N_2O$ consumed. Alt et al.[47] suggested that an $N_2O$ dose exceeding 80 g/day increases the risk of permanent nerve injury. The present study did not calculate the amount of $N_2O$ consumed overall or during a single inhalation, and so could not analyze the relationship between $N_2O$ consumption and the degree of nerve injury. However, the exposure duration somewhat reflects consumption. The present study did not find any association between the vitamin B12, homocysteine, or hemoglobin level and the degree of nerve injury. Li et al.[14] compared the EMG findings of patients with normal and abnormal vitamin B12 and/or homocysteine levels, and found no association between nerve injury and vitamin B12 or homocysteine level. In the present study, the electrophysiologic results illustrated the characteristics and extent of injury in $N_2O$-associated peripheral neuropathy and provided evidence for a better understanding of the pathogenesis and pathophysiology of this neuropathy.

The present study had some limitations. First, it had a retrospective design and some patients lacked vitamin B12, homocysteine, and hemoglobin data. Second, some patients lacked a complete medical history regarding exposure time, quantity of $N_2O$ consumed, and whether they inhaled large $N_2O$ doses within a short period of time, all of which may have affected the analysis of factors influencing the degree of injury. A prospective experimental design with a larger sample is therefore necessary to clarify these issues.

In conclusion, in $N_2O$-associated peripheral neuropathy, SNCV, MNCV, SNAP, and MNAP were significantly decreased, and distal motor latency was significantly prolonged. Axonal neuropathy was the most common $N_2O$-associated peripheral neuropathy. Motor nerve axonal injury in the lower limbs was the most-characteristic injury. Motor axonal injury in the lower limbs was more severe in the presence of a longer $N_2O$ exposure duration and disease course.

## Availability of Data and Material

The datasets generated or analyzed during the study are available from the corresponding author on reasonable request.

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## Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

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## REFERENCES

1. Weimann J. Toxicity of nitrous oxide. *Best Pract Res Clin Anaesthesiol* 2003;17:47-61.
2. Becker DE, Rosenberg M. Nitrous oxide and the inhalation anesthetics. *Anesth Prog* 2008;55:124-130.
3. Zheng D, Ba F, Bi G, Guo Y, Gao Y, Li W. The sharp rise of neurological disorders associated with recreational nitrous oxide use in China: a single-center experience and a brief review of Chinese literature. *J Neurol* 2020;267:422-429.
4. van Amsterdam J, Nabben T, van den Brink W. Recreational nitrous oxide use: prevalence and risks. *Regul Toxicol Pharmacol* 2015;73:790-796.
5. Garakani A, Jaffe RJ, Savla D, Welch AK, Protin CA, Bryson EO, et al. Neurologic, psychiatric, and other medical manifestations of nitrous oxide abuse: a systematic review of the case literature. *Am J Addict* 2016;25:358-369.
6. Hathout L, El-Saden S. Nitrous oxide-induced $B_{12}$ deficiency myelopathy: perspectives on the clinical biochemistry of vitamin $B_{12}$. *J Neurol Sci* 2011;301:1-8.
7. Nunn JF. Clinical aspects of the interaction between nitrous oxide and vitamin B12. *Br J Anaesth* 1987;59:3-13.
8. Buizert A, Sharma R, Koppen H. When the laughing stops: subacute combined spinal cord degeneration caused by laughing gas use. *J Addict Med* 2017;11:235-236.
9. Hew A, Lai E, Radford E. Nitrous oxide abuse presenting with acute psychosis and peripheral neuropathy. *Aust N Z J Psychiatry* 2018;52:388.
10. Layzer RB, Fishman RA, Schafer JA. Neuropathy following abuse of nitrous oxide. *Neurology* 1978;28:504-506.
11. Hsu CK, Chen YQ, Lung VZ, His SC, Lo HC, Shyu HY. Myelopathy and polyneuropathy caused by nitrous oxide toxicity: a case report. *Am J Emerg Med* 2012;30:1016.e3-1016.e6.
12. Morris N, Lynch K, Greenberg SA. Severe motor neuropathy or neuronopathy due to nitrous oxide toxicity after correction of vitamin B12 deficiency. *Muscle Nerve* 2015;51:614-616.
13. Fang X, Li W, Gao H, Ma Y, Dong X, Zheng D. Skin hyperpigmentation: a rare presenting symptom of nitrous oxide abuse. *Clin Toxicol (Phila)* 2020;58:476-481.
14. Li HT, Chu CC, Chang KH, Liao MF, Chang HS, Kuo HC, et al. Clinical and electrodiagnostic characteristics of nitrous oxide-induced neuropathy in Taiwan. *Clin Neurophysiol* 2016;127:3288-3293.
15. Van Asseldonk JT, Van den Berg LH, Kalmijn S, Wokke JH, Franssen H. Criteria for demyelination based on the maximum slowing due to axonal degeneration, determined after warming in water at 37 degrees C: diagnostic yield in chronic inflammatory demyelinating polyneuropathy. *Brain* 2005;128(Pt 4):880-891.
16. Albers JW, Kelly JJ Jr. Acquired inflammatory demyelinating polyneuropathies: clinical and electrodiagnostic features. *Muscle Nerve* 1989;12:435-451.
17. Cheng HM, Park JH, Hernstadt D. Subacute combined degeneration of the spinal cord following recreational nitrous oxide use. *BMJ Case Rep* 2013;2013:bcr2012008509.
18. Enticknap JB. A case of fatal accidental N2O poisoning. *Med Sci Law* 1961;1:404-409.
19. Garland EL, Howard MO, Perron BE. Nitrous oxide inhalation among adolescents: prevalence, correlates, and co-occurrence with volatile solvent inhalation. *J Psychoactive Drugs* 2009;41:337-347.
20. Broadfield D. Drug misuse: findings from the 2016/17 crime survey for England and Wales [Internet]. London: Dandy Booksellers; 2017 [cited 2018 Jul 28]. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/642738/drug-misuse-2017-hosb1117.pdf.
21. Randhawa G, Bodenham A. The increasing recreational use of nitrous oxide: history revisited. *Br J Anaesth* 2016;116:321-324.
22. Antezana A, Antezana Siles A, Hidalgo G. Toxic myelopathy secondary to recreational nitrous oxide abuse. *J Neurol Sci* 2015;357:e216.
23. Blanco G, Peters HA. Myeloneuropathy and macrocytosis associated with nitrous oxide abuse. *Arch Neurol* 1983;40:416-418.
24. Greenblum DN, Serby M, Goodgold AL. Sequelae of nitrous oxide abuse. *Psychosomatics* 1982;23:307-311.
25. Gutmann L, Farrell B, Crosby TW, Johnsen D. Nitrous oxide-induced myelopathy-neuropathy: potential for chronic misuse by dentists. *J Am Dent Assoc* 1979;98:58-59.
26. Oussalah A, Julien M, Levy J, Hajjar O, Franczak C, Stephan C, et al. Global burden related to nitrous oxide exposure in medical and recreational settings: a systematic review and individual patient data meta-analysis. *J Clin Med* 2019;8:551.
27. Pema PJ, Horak HA, Wyatt RH. Myelopathy caused by nitrous oxide toxicity. *AJNR Am J Neuroradiol* 1998;19:894-896.
28. Tani J, Weng HY, Chen HJ, Chang TS, Sung JY, Lin CS. Elucidating unique axonal dysfunction between nitrous oxide abuse and vitamin B12 deficiency. *Front Neurol* 2019;10:704.
29. Sahenk Z, Mendell JR, Couri D, Nachtman J. Polyneuropathy from inhalation of N2O cartridges through a whipped-cream dispenser. *Neurology* 1978;28:485-487.
30. Duque MA, Kresak JL, Falchook A, Harris NS. Nitrous oxide abuse and vitamin B12 action in a 20-year-old woman: a case report. *Lab Med* 2015;46:312-315.
31. Keddie S, Adams A, Kelso ARC, Turner B, Schmierer K, Gnanapavan S, et al. No laughing matter: subacute degeneration of the spinal cord due to nitrous oxide inhalation. *J Neurol* 2018;265:1089-1095.
32. Maze M, Fujinaga M. Recent advances in understanding the actions and toxicity of nitrous oxide. *Anaesthesia* 2000;55:311-314.
33. Maler JM, Seifert W, Hüther G, Wiltfang J, Rüther E, Kornhuber J, et al. Homocysteine induces cell death of rat astrocytes in vitro. *Neurosci Lett* 2003;347:85-88.
34. Pugliese RS, Slagle EJ, Oettinger GR, Neuburger KJ, Ambrose TM. Subacute combined degeneration of the spinal cord in a patient abusing nitrous oxide and self-medicating with cyanocobalamin. *Am J Health Syst Pharm* 2015;72:952-957.
35. Chiang TT, Hung CT, Wang WM, Lee JT, Yang FC. Recreational nitrous oxide abuse-induced vitamin B12 deficiency in a patient presenting with hyperpigmentation of the skin. *Case Rep Dermatol* 2013;5:186-191.
36. Fatima K, Majid A. Motor nerve conduction studies in patients with

chronic renal failure. *Journal of Rawalpindi Medical College* 2010;14:11-14.
37. Jasti DB, Mallipeddi S, Apparao A, Vengamma B, Sivakumar V, Kolli S. A clinical and electrophysiological study of peripheral neuropathies in predialysis chronic kidney disease patients and relation of severity of peripheral neuropathy with degree of renal failure. *J Neurosci Rural Pract* 2017;8:516-524.
38. Julian T, Glascow N, Syeed R, Zis P. Alcohol-related peripheral neuropathy: a systematic review and meta-analysis. *J Neurol* 2019;266:2907-2919.
39. Pal PK. Clinical and electrophysiological studies in vincristine induced neuropathy. *Electromyogr Clin Neurophysiol* 1999;39:323-330.
40. Sandler SG, Tobin W, Henderson ES. Vincristine-induced neuropathy. A clinical study of fifty leukemic patients. *Neurology* 1969;19:367-374.
41. Zajączkowska R, Kocot-Kępska M, Leppert W, Wrzosek A, Mika J, Wordliczek J. Mechanisms of chemotherapy-induced peripheral neuropathy. *Int J Mol Sci* 2019;20:1451.
42. Sultan LI. Evaluation of the clinical and neurophysiologic parameters of peripheral nerve functions in uremic Egyptian patients. *Egypt J Neurol Psychiatr Neurosurg* 2007;44:473-487.
43. Taylor SW, Laughlin RS, Kumar N, Goodman B, Klein CJ, Dyck PJ, et al. Clinical, physiological and pathological characterisation of the sensory predominant peripheral neuropathy in copper deficiency. *J Neurol Neurosurg Psychiatry* 2017;88:839-845.
44. Koike H, Takahashi M, Ohyama K, Hashimoto R, Kawagashira Y, Iijima M, et al. Clinicopathologic features of folate-deficiency neuropathy. *Neurology* 2015;84:1026-1033.
45. Park SB, Lin CS, Krishnan AV, Goldstein D, Friedlander ML, Kiernan MC. Long-term neuropathy after oxaliplatin treatment: challenging the dictum of reversibility. *Oncologist* 2011;16:708-716.
46. Osmani K, Vignes S, Aissi M, Wade F, Milani P, Lévy BI, et al. Taxane-induced peripheral neuropathy has good long-term prognosis: a 1- to 13-year evaluation. *J Neurol* 2012;259:1936-1943.
47. Alt RS, Morrissey RP, Gang MA, Hoffman RS, Schaumburg HH. Severe myeloneuropathy from acute high-dose nitrous oxide (N2O) abuse. *J Emerg Med* 2011;41:378-380.