



Elucidating Unique Axonal Dysfunction Between Nitrous Oxide Abuse and Vitamin B12 Deficiency

Jowy Tani^{1,2,3}, Hsing-Yu Weng^{1,4}, Hung-Ju Chen¹, Tsui-San Chang⁴, Jia-Ying Sung^{1,3,4*} and Cindy Shin-Yi Lin^{5,6}

¹ Department of Neurology, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan, ² Ph.D. Program for Neural Regenerative Medicine, College of Medical Science and Technology, Taipei Medical University and National Health Research Institutes, Taipei, Taiwan, ³ Taipei Neuroscience Institute, Taipei Medical University, Taipei, Taiwan, ⁴ Department of Neurology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan, ⁵ Graduate Institute of Neural Regenerative Medicine, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan, ⁶ Faculty of Medicine and Health, Brain and Mind Centre, Central Clinical School, The University of Sydney, Sydney, NSW, Australia

OPEN ACCESS

Edited by:

Massimiliano Filosto,
Civil Hospital of Brescia, Italy

Reviewed by:

Fiore Manganelli,
University of Naples Federico II, Italy
James Howells,
University of Sydney, Australia

*Correspondence:

Jia-Ying Sung
sung.jiaying@tmu.edu.tw

Specialty section:

This article was submitted to
Neuromuscular Diseases,
a section of the journal
Frontiers in Neurology

Received: 01 March 2019

Accepted: 14 June 2019

Published: 09 July 2019

Citation:

Tani J, Weng H-Y, Chen H-J,
Chang T-S, Sung J-Y and Lin CS-Y
(2019) Elucidating Unique Axonal
Dysfunction Between Nitrous Oxide
Abuse and Vitamin B12 Deficiency.
Front. Neurol. 10:704.
doi: 10.3389/fneur.2019.00704

Introduction: Abuse of nitrous oxide (N₂O) has an unusually high lifetime prevalence in developed countries and represents a serious concern worldwide. Myeloneuropathy following the inhalant abuse is commonly attributed to the disturbance of vitamin B12 metabolism, with severe motor deficits are often noted. The present study aims to elucidate its underlying pathophysiology.

Methods: Eighteen patients with N₂O abuse or vitamin B12 deficiency were recruited. Comprehensive central and peripheral neuro-diagnostic tests were performed, including whole spine MRI, and thermal quantitative sensory testing (QST). Specifically, paired motor and sensory nerve excitability tests were performed in order to obtain a complete picture of the sensorimotor axonal damage.

Results: The mean duration of N₂O exposure for the N₂O abuse patients was 17.13 ± 7.23 months. MRI revealed T2 hyperintensity in 87.5% of the N₂O abuse patients and 50% of the vitamin B12 deficiency patients. In N₂O abuse patients, the motor nerve excitability test showed decreased in peak response (7.08 ± 0.87 mV, *P* = 0.05), increased latency (7.09 ± 0.28 ms, *P* < 0.01), increased superexcitability (−32.95 ± 1.74%, *P* < 0.05), and decreased accommodation to depolarizing current [TEd (40–60 ms) 56.53 ± 0.70%, *P* < 0.05]; the sensory test showed only decreased peak response (30.54 ± 5.98 μV, *P* < 0.05). Meanwhile, motor test in vitamin B12 deficiency patients showed only decreased accommodation to depolarizing current [TEd (40–60 ms) 55.72 ± 1.60%, *P* < 0.01]; the sensory test showed decreased peak response (25.86 ± 3.44 μV, *P* < 0.05) increased superexcitability (−28.58 ± 3.71%, *P* < 0.001), increased subexcitability (8.31 ± 1.64%, *P* < 0.05), and decreased accommodation to depolarizing current [TEd (peak) 67.31 ± 3.35%, *P* < 0.001].

Conclusion: Compared to vitamin B12 deficiency, N₂O abuse patients showed prominent motor superexcitability changes and less prominent sensory superexcitability

changes, hinting a unique pathological process different from that of vitamin B12 deficiency. N₂O abuse might cause axonal dysfunction not only by blocking methionine metabolism but also by toxicity affecting the paranodal region.

Keywords: nerve excitability test, inhalant, nitrous oxide, vitamin B12, myeloneuropathy

INTRODUCTION

Recreational use of nitrous oxide (N₂O) is escalating in many countries, including the United States, the United Kingdom, and Taiwan (1, 2). With an unusually high lifetime prevalence in developed countries (38.6% in the UK and 29.4% in the US), N₂O consistently ranked the seventh most popular drug in the world in the Global Drug Survey (GDS) from 2016 to 2018 (3–5). Abuse of N₂O frequently leads to overexposure, and subsequently causing myeloneuropathy, subacute combined degeneration, psychosis, megaloblastic bone marrow changes, and pernicious anemia (6). As N₂O can inactivate methionine synthase that converts homocysteine to methionine via a methylation process, many medical consequences associated with N₂O overexposure, including peripheral neuropathy, are often attributed to vitamin B12 deficiency (7–9).

Although myeloneuropathy following N₂O abuse and vitamin B12 deficiency can both result in motor and sensory symptoms, experienced clinicians have often noted somewhat different clinical presentations between the two conditions, where N₂O abuse might cause severe motor deficits, especially in the lower limbs (10, 11). Johnson et al. have reported such a typical case of nitrous oxide abuse leading to significant distal limbs weakness, a clinical feature less likely to be observed in vitamin B12 deficiency from other causes (12).

Certain reports have suggested that N₂O neurotoxicity might cause neural injury on top of vitamin B12 deficiency, but the exact pathophysiology of N₂O-induced myeloneuropathy is still unclear (6, 13). The present study is the first that uses comprehensive neurodiagnostic tests covering central and peripheral nervous systems, including whole spine MRI, conventional nerve conduction study (NCS), thermal quantitative sensory test (QST), and the nerve excitability tests to study the myeloneuropathy. In particular, the nerve excitability test has been shown to be able to provide valuable data on the nodal, paranodal, and internodal regions of axons in various peripheral nerve diseases (14, 15), and reveal changes in axonal properties that cannot be detected using conventional NCS. It is hoped that paired motor and sensory nerve excitability test utilized in the study would provide a complete picture of the sensorimotor axonal damage, and further elucidate the pathophysiology underlying the inhalant abuse that could cause catastrophic consequences.

METHODS

A total of 18 patients with either N₂O abuse or vitamin B12 deficiency due to other causes were recruited for the study. Each

patient received clinical evaluation (including history taking, complete physical examination, and neurologic examination); laboratory examinations for levels of vitamin B12, folic acid, and homocysteine; conventional NCS; and paired motor and sensory nerve excitability tests; certain patients received a thermal QST. All patients received whole spine MRI, except one vitamin B12 deficiency patient.

All N₂O abuse patients had multiple exposures to N₂O inhalation (1). On the other hand, a patient was considered to have vitamin B12 deficiency if the measured serum vitamin B12 level was <150 pg/ml (9, 16–18). Patients with carpal tunnel syndrome, hyperkalemia/hypokalemia, or with other potential causes for sensory polyneuropathy such as diabetes mellitus, alcohol abuse, and uremia were excluded based on clinical assessments and NCS results. All patients enrolled in the study were recruited from Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan. Control nerve excitability data were obtained from 39 healthy control (HC) subjects that were divided into two age cohorts, HC1 with a mean age of 22.67 ± 0.21 years (*n* = 6) and HC2 with a mean age of 62.11 ± 7.51 years (*n* = 33). HC1 was age-matched to the N₂O abuse patients, and HC2 was age-matched to the vitamin B12 deficiency patients.

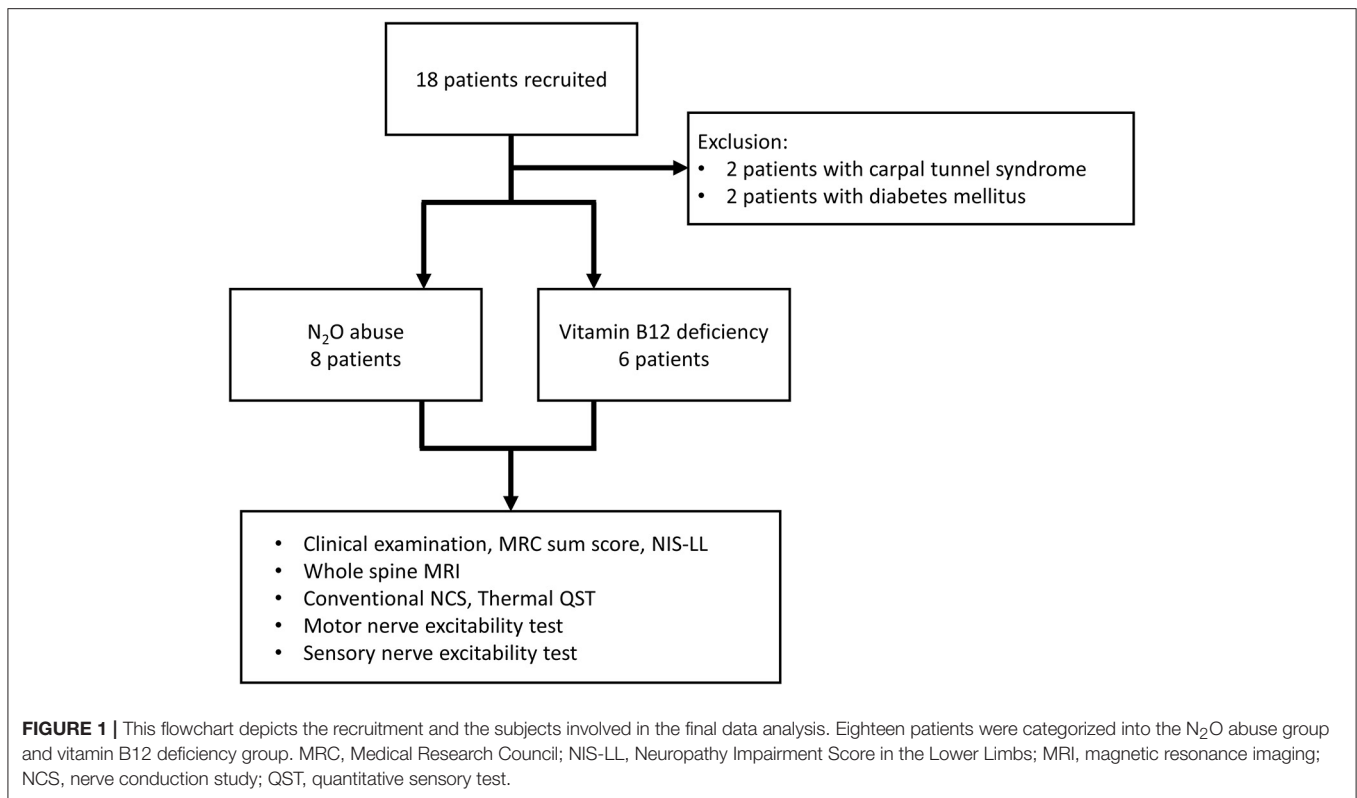
This study was carried out in accordance with the recommendations of the Joint Institutional Review Board of Taipei Medical University. The protocol was approved by the Joint Institutional Review Board of Taipei Medical University. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Clinical Evaluation, NCS, and QST

The Medical Research Council (MRC) strength score combined for 12 specified muscle groups (the MRC sum score; ranging from 0 [paralysis] to 60 [normal strength]) (19) and Neuropathy Impairment Score in the Lower Limbs (NIS-LL; ranging from 0 [normal] to 88 [total impairment]) (20) were obtained during neurological examination. Conventional NCS assessing the median, ulnar, peroneal, tibial, and sural nerves were performed in all subjects using standard clinical neurophysiology equipment. Cold and warm thermal QSTs were performed in the upper and lower limbs in certain patients.

Nerve Excitability Testing

Nerve excitability studies were performed by stimulating the nerve median at the wrist according to previously described protocols, with skin temperature over the wrist maintained at a minimum of 32.0°C (14, 15). Paired recordings of the motor and sensory nerve excitability indices were obtained for each subject. Compound muscle action potentials (CMAPs) were recorded from the abductor pollicis brevis muscle while sensory



nerve action potentials (SNAPs) were recorded from the index finger. Stimulation and recording were manipulated by software (QTRAC version 8/11/2014; Institute of Neurology, London, U.K.), and stimulus current was administered using an isolated linear bipolar constant-current stimulator (DS5; Digitimer, Welwyn Garden City, U.K.). The changes in current required to produce a target potential corresponding to predetermined target CMAP or SNAP (e.g., 40% of maximum) were tracked. Latency was defined as the time delay (ms) between stimulus onset and the peak of the CMAP or SNAP response. Stimulus threshold was defined as the current (mA) that is required to produce amplitudes of CMAP or SNAP response of the target amplitude.

The nerve excitability protocol incorporated the following recordings: (1) a stimulus-response (SR) curve; (2) strength-duration (SD) relationship, which determined strength-duration time constant (SDTC); (3) threshold electrotonus (TE) utilizing subthreshold 100-ms polarizing currents in both depolarizing (TE_d; +40%) and hyperpolarizing (TE_h; -40%) directions to change the potential difference across the internodal membrane; and (4) recovery cycle (RC) using a paired-pulse paradigm with a supramaximal conditioning stimulus followed by a test stimulus at interstimulus intervals from 2 to 200 ms. Superexcitability was measured as the maximal threshold reduction and subexcitability as the maximal threshold increase after an interstimulus interval of 10 ms.

Statistical Analysis

Nerve excitability recording data of N₂O abuse patients, vitamin B12 deficiency patients, and healthy controls were analyzed by

using unpaired *T*-tests or Mann-Whitney *U*-tests, depending on normality. Equality of variances was calculated with Levene's test. Correlation studies were performed with Pearson's *R*. Data analysis was performed using Statistical Package for the Social Sciences (SPSS) for Windows version 21 (SPSS Inc., Chicago, U.S.A.) or QTRAC software. All data are presented as the mean \pm standard error of the mean. *P*-values were considered significant if ≤ 0.05 .

RESULTS

Patient Clinical Profiles

Eighteen patients were recruited and categorized into N₂O abuse patients ($n = 8$) and vitamin B12 deficiency patients ($n = 6$). Two patients were excluded due to comorbid diabetes, and two for carpal tunnel syndrome (Figure 1). The clinical, MRI, and laboratory profiles for the patients were listed in Table 1. The average duration of N₂O exposure for the N₂O abuse group was 17.13 ± 7.23 months. The MRC sum score was 51.50 ± 2.87 for the N₂O abuse group, and 59.67 ± 0.33 for the vitamin B12 deficiency group; NIS-LL was 27.00 ± 5.83 for the N₂O abuse group, and 10.00 ± 4.48 for the vitamin B12 deficiency group.

The vitamin B12 level was 236.33 ± 52.80 pg/ml in the vitamin B12 deficiency group and 111.50 ± 15.46 pg/ml in the N₂O abuse group. The homocysteine level was elevated in both the N₂O abuse group (44.84 ± 12.00 μ mol/l) and vitamin B12 deficiency group (51.68 ± 25.52 pg/ml).

TABLE 1 | Patients clinical, MRI, and laboratory profiles.

	N₂O abuse	Vitamin B12 deficiency
Sex: male/female (number)	5/3	4/2
Age (year)	26.75 ± 2.59	60.5 ± 8.74
Duration of N ₂ O abuse (months)	17.13 ± 7.23	–
MRC sum score	51.50 ± 2.87	59.67 ± 0.33
NIS-LL	27.00 ± 5.83	10.00 ± 4.47
Vitamin B12 level (pg/ml)	236.33 ± 52.80	111.50 ± 15.46*
Homocysteine level (μmol/l)	44.84 ± 12.00*	51.68 ± 25.52*
UPPER LIMBS CLINICAL FINDINGS		
Weakness (%)	37.50	0
Upper limbs MRC sum score	27.50 ± 1.55	30.00 ± 0.00
Abnormal pain/temperature sensation (%)	75.00	16.67
Abnormal vibratory sensation/proprioception (%)	37.50	0
Hypo/hyperreflexia (%)	62.50	0
Ataxia (%)	0	0
LOWER LIMBS CLINICAL FINDINGS		
Weakness (%)	75.00	33.33
Lower limbs MRC sum score	24.00 ± 2.20	29.67 ± 0.33
Abnormal pain/temperature sensation (%)	100.00	66.67
Abnormal vibratory sensation/proprioception (%)	50.00	66.67
Hypo/hyperreflexia (%)	75.00	50.00
Ataxia (%)	12.50	0
Autonomic dysfunction (%)	25.00	33.33
MRI FINDING		
T2 hyperintensity on whole spine MRI (%)	87.50	50.00
MOTOR CONDUCTION VELOCITY		
Median nerve (m/s)	47.19 ± 2.83*	50.59 ± 2.58
Ulnar nerve (m/s)	46.31 ± 4.31*	52.50 ± 2.80
Tibial nerve (m/s)	17.00 ± 6.46*	33.33 ± 6.74*
Peroneal nerve (m/s)	15.69 ± 6.71*	34.25 ± 6.90*
ABNORMAL MOTOR CONDUCTION VELOCITY		
Median nerve (%)	87.50	40.00
Ulnar nerve (%)	87.50	100.00
Tibial nerve (%)	62.50	20.00
Peroneal nerve (%)	62.50	20.00

(Continued)

TABLE 1 | Continued

	N₂O abuse	Vitamin B12 deficiency
SENSORY CONDUCTION VELOCITY		
Median nerve (m/s)	47.38 ± 2.64*	51.92 ± 5.75
Ulnar nerve (m/s)	49.13 ± 2.09*	51.11 ± 4.69
Sural nerve (m/s)	24.06 ± 9.27*	35.17 ± 11.34*
ABNORMAL SENSORY CONDUCTION VELOCITY		
Median nerve (%)	75.00	40.00
Ulnar nerve (%)	50.00	80.00
Sural nerve (%)	50.00	40.00
THERMAL QST		
Upper limb warm threshold (Z-score)	4.05 ± 1.36*	2.95 ± 0.65*
Upper limb cold threshold (Z-score)	−2.00 ± 0.61*	−0.38 ± 0.80
Lower limb warm threshold (Z-score)	1.77 ± 0.93	3.10 ± 0.03*
Lower limb cold threshold (Z-score)	−1.76 ± 0.86	−4.21 ± 1.05*

The reported values of patient profiles, laboratory data, and thermal QST data represent the mean ± standard error. The reported values of abnormal nerve conduction velocity data, clinical findings, and MRI profile represent percentage.

MRC, Medical Research Council; NIS-LL, Neuropathy Impairment Score in the Lower Limbs; QST, Quantitative sensory test; MRI, magnetic resonance imaging; *mean data are out of the normal range for this laboratory.

A summary of the patients' neurological signs and symptoms are shown in **Table 1**. Weakness was more prevalent in the N₂O abuse group than in the vitamin B12 group, both in the upper (37.50 vs. 0.00%) and lower (75.00 vs. 33.33%) limbs. While sensory abnormalities are more prevalent in N₂O abuse group than in the vitamin B12 group in the upper limbs, the prevalence of sensory abnormalities of both conditions are similar in the lower limbs.

The whole spine MRI revealed T2 hyperintense lesions in the posterior columns in 87.5% of the N₂O abuse patients (100% were cervical spine lesions), and in 50% of the vitamin B12 deficiency patients (66.6% were cervical spine lesions and 33.3% were thoracolumbar lesions). **Figures 2A,B** shows MRI of cervical cord hyperintense changes in T2-weighted images from a case with N₂O abuse. Furthermore, **Figure 2C** showed a hyperpigmented maculopapular rash, a feature that could be observed in N₂O abuse patients, on one of the patients in the nitrous oxide abuse group.

NCS revealed motor and sensory slowing in conduction velocity in both N₂O abuse and vitamin B12 deficiency patients. Thermal QST in the upper limb (N₂O abuse warm threshold Z-score: 4.05 ± 1.36, cold threshold Z-score: −2.00 ± 0.61; Vitamin B12 deficiency warm threshold Z-score: 2.95 ± 0.65, cold threshold Z-score: −0.38 ± 0.80) and lower limb (N₂O abuse warm threshold Z-score: 1.77 ± 0.93, cold threshold Z-score: −1.76 ± 0.86; Vitamin B12 deficiency warm threshold

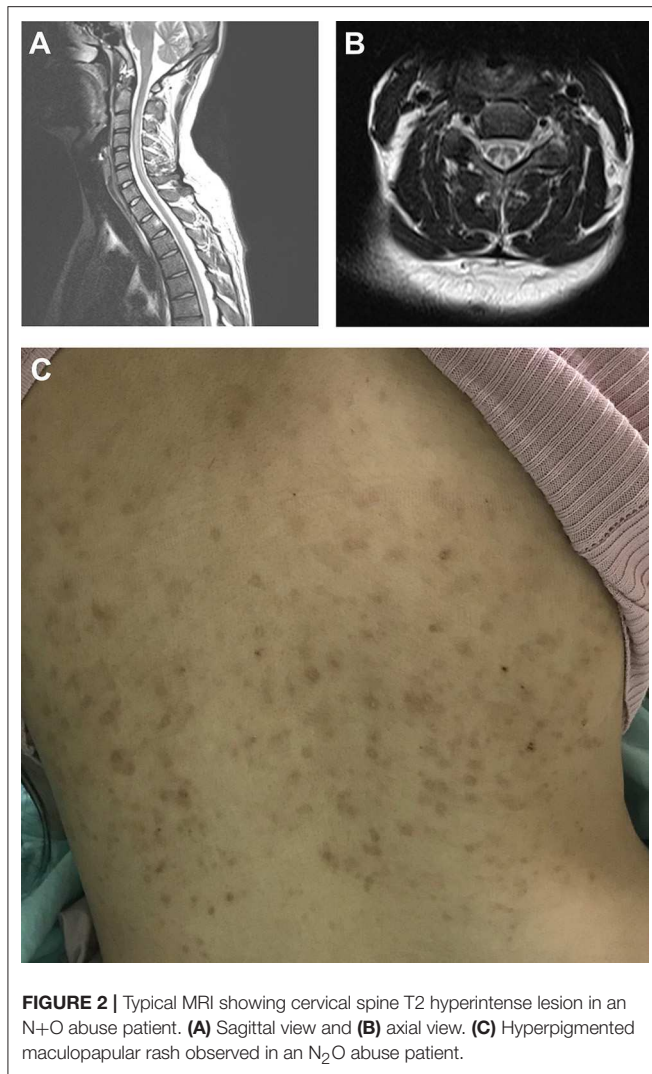


FIGURE 2 | Typical MRI showing cervical spine T2 hyperintense lesion in an N₂O abuse patient. **(A)** Sagittal view and **(B)** axial view. **(C)** Hyperpigmented maculopapular rash observed in an N₂O abuse patient.

Z-score: 3.10 ± 0.03 , cold threshold Z-score: -4.21 ± 1.05) confirmed abnormal temperature sensation in the patients.

Motor Axonal Dysfunction in N₂O Abuse and Vitamin B12 Deficiency Patients

A comparison of the motor and sensory nerve excitability parameters between groups are shown in **Table 2**. **Figure 3** shows the motor and sensory recovery cycles and threshold electrotonus in N₂O abuse and vitamin B12 deficiency patients.

Compared to those of HC, the motor nerve excitability of the patients with N₂O abuse showed a trend of decreased peak response (7.08 ± 0.87 mV, $p = 0.05$), increased latency (7.09 ± 0.28 ms, $p < 0.01$), increased superexcitability ($-32.95 \pm 1.74\%$, $p < 0.05$), and decreased TE accommodation to depolarizing current [TEd [40–60 ms] $56.53 \pm 0.70\%$, $p < 0.05$].

On the other hand, the motor nerve excitability test of vitamin B12 deficiency patients showed only increased stimulus for 50% CMAP (4.59 ± 0.73 mA, $p < 0.01$) and decreased TE

accommodation toward depolarization in TEd (40–60 ms) ($55.72 \pm 1.60\%$, $p < 0.01$).

More prominent motor superexcitability changes in N₂O abuse patients compared to those seen in vitamin B12 deficiency patients clearly suggested motor axonal dysfunction is more severe in N₂O abuse, possibly affecting the paranodal region.

Sensory Axonal Dysfunction in N₂O Abuse and Vitamin B12 Deficiency Patients

The sensory nerve excitability test of the patients with N₂O abuse showed increased stimulus for 50% SNAP (3.28 ± 0.60 mA, $p < 0.05$) and decreased peak response (30.54 ± 5.98 μ V, $p < 0.05$).

Sensory nerve excitability test of the vitamin B12 deficiency patients also showed increased stimulus for 50% SNAP (3.65 ± 0.78 mA, $p < 0.05$) and decreased peak response (25.86 ± 3.44 μ V, $p < 0.05$). Moreover, it revealed additional evidence of sensory axonal dysfunction including decreased SDTC (0.50 ± 0.03 , $p < 0.01$), increased superexcitability ($-28.58 \pm 3.71\%$, $p < 0.001$), decreased subexcitability ($8.31 \pm 1.64\%$, $p < 0.05$), and decreased TE accommodation toward depolarizing current (TEd[peak] 67.31 ± 3.35 , $p < 0.001$).

The fact that superexcitability and other TE & RC parameters showed significant changes in vitamin B12 deficiency but not in N₂O abuse suggested that vitamin B12 deficiency patients suffered from more severe sensory axonal dysfunction, compared to N₂O abuse patients.

Correlational study between peak response and latency for motor ($R = -0.65$, $p = 0.08$) and sensory ($R = -0.69$, $p = 0.06$) axon in N₂O abuse patients, as well as for sensory axon in vitamin B12 deficiency patients ($R = -0.11$, $p = 0.80$), suggested that decrease in peak response observed in the present study might be related to axonal loss instead of temporal dispersion.

Correlation Studies Between Clinical Parameters and Excitability Parameters

In order to clarify the cumulative effect of prolonged intermittent exposure of N₂O on the axon, the present study performed correlation analysis between the duration of intermittent N₂O exposure and motor and sensory excitability parameters in the N₂O abuse group. The analysis demonstrated that the duration of intermittent N₂O exposure is significantly correlated to both motor and sensory excitability parameters, specifically N₂O exposure is correlated to subexcitability ($R = 0.85$, $p < 0.01$) for motor and TEd (peak) ($R = 0.76$, $p < 0.05$) for sensory axons.

Also, vitamin B12 level was correlated with stimulus for 50% SNAP response ($R = -0.81$, $p < 0.05$) and sensory rheobase ($R = -0.90$, $p < 0.05$) of vitamin B12 deficiency patients. Interestingly, MRC strength score of tested muscle is correlated with TEh(overshoot) ($R = -0.82$, $p < 0.05$) in motor axonal study of N₂O abuse patients.

In the sensory axonal study of N₂O abuse patients, the present study found that upper limb warm threshold Z-score was correlated with refractoriness at 2.5 ms ($R = 0.97$, $p < 0.05$), superexcitability ($R = -0.98$, $p < 0.05$). Upper limb cold threshold Z-score was correlated with superexcitability ($R = 0.97$, $p < 0.05$) and latency ($R = -0.98$, $p < 0.05$). Lower

TABLE 2 | Comparison of sensory and motor nerve excitability parameters between groups.

Axonal properties	N ₂ O abuse vs. age-matched controls			B12 deficient vs. age-matched controls		
	N ₂ O abuse	HC1	p-value	B12 def.	HC2	p-value
MOTOR PARAMETERS						
Stimulus for 50% CMAP (mA)	3.58 ± 0.79	2.46 ± 0.17	NS	4.59 ± 0.73	2.76 ± 0.14	p < 0.01
Peak response (mV)	7.08 ± 0.87	9.7 ± 0.64	p = 0.05	8.14 ± 1.21	8.31 ± 0.46	NS
Latency (ms)	7.09 ± 0.28	5.59 ± 0.15	p < 0.01	7.11 ± 0.45	6.62 ± 0.17	NS
Motor SDTC (ms)	0.52 ± 0.04	0.49 ± 0.04	NS	0.48 ± 0.03	0.46 ± 0.01	NS
RRP (ms)	3.00 ± 0.17	2.99 ± 0.10	NS	4.14 ± 0.54	3.12 ± 0.09	NS
Superexcitability (%)	-32.95 ± 1.74	-25.98 ± 2.07	p < 0.05	-27.19 ± 5.71	-23.98 ± 0.98	NS
Subexcitability (%)	11.19 ± 1.01	12.63 ± 1.46	NS	12.83 ± 1.32	16.44 ± 0.94	NS
TEd (40–60 ms) (%)	56.53 ± 0.70	52.39 ± 1.77	p < 0.05	55.72 ± 1.60	50.04 ± 0.78	p < 0.01
TEd (peak) (%)	68.97 ± 2.30	68.12 ± 1.67	NS	70.58 ± 1.34	68.33 ± 0.69	NS
TEh (90–100 ms) (%)	-119.86 ± 6.84	-121.92 ± 8.00	NS	-127.76 ± 8.91	-129.87 ± 3.82	NS
SENSORY PARAMETERS						
Stimulus for 50% SNAP (mA)	3.28 ± 0.60	1.96 ± 0.13	p < 0.05	3.65 ± 0.78	2.24 ± 0.18	p < 0.05
Peak response (μV)	30.54 ± 5.98	55.48 ± 8.87	p < 0.05	25.86 ± 3.44	39.36 ± 2.47	p < 0.05
Latency (ms)	3.57 ± 0.30	2.9 ± 0.10	NS	3.45 ± 0.23	3.33 ± 0.06	NS
Sensory SDTC (ms)	2.58 ± 0.34	0.57 ± 0.03	NS	0.50 ± 0.03	0.63 ± 0.02	p < 0.01
RRP (ms)	3.52 ± 0.33	3.71 ± 0.10	NS	3.57 ± 0.26	3.44 ± 0.12	NS
Superexcitability (%)	-23.65 ± 3.50	-18.07 ± 1.03	NS	-28.58 ± 3.71	-16.61 ± 1.06	p < 0.001
Subexcitability (%)	10.88 ± 0.74	10.46 ± 0.71	NS	8.31 ± 1.64	12.39 ± 0.61	p < 0.05
TEd (40–60 ms) (%)	56.43 ± 6.25	47.98 ± 1.64	NS	54.60 ± 1.26	48.53 ± 0.62	p < 0.001
TEd (peak) (%)	68.56 ± 5.52	60.62 ± 1.03	NS	67.31 ± 3.35	59.36 ± 0.51	p < 0.001
TEh (90–100 ms) (%)	-130.08 ± 5.73	-133.05 ± 7.68	NS	-161.35 ± 13.79	-145.44 ± 4.35	NS

HC, healthy control; CMAP, compound muscle action potential; SNAP, sensory nerve action potential; SDTC, strength-duration time constant; RRP, relative refractory period; NS, not statistically significant. The reported values represent the mean ± standard error and the p-value from t-tests or Mann-Whitney U-tests of patient groups vs. age-matched healthy controls, depending on normality.

limb warm threshold Z-score was correlated with subexcitability ($R = -0.98$, $p < 0.05$).

Also, in the sensory axonal study of vitamin B12 deficiency patients, the present study found that upper limb cold threshold Z-score was correlated with peak response ($R = 0.93$, $p < 0.05$) and TEd (40–60 ms) ($R = 0.89$, $p < 0.05$). Lower limb warm threshold Z-score was correlated with refractoriness at 2.5 ms ($R = 0.94$, $p < 0.05$). Lower limb cold threshold Z-score was correlated with peak response ($R = 0.95$, $p < 0.05$), TEd (10–20 ms) ($R = 0.9149$, $p < 0.05$), TEd (40–60ms) ($R = 0.91$, $p < 0.05$), and TEd (peak) ($R = 0.96$, $p < 0.05$).

DISCUSSION

In the present study, the spinal MRI, motor and sensory nerve excitability tests, and thermal QST revealed evidence of myeloneuropathy in both the N₂O abuse and vitamin B12 deficiency patients. Nevertheless, the pattern of myeloneuropathy appears to be different between the two groups (Figure 4). Thermal QST revealed that temperature sensation could be affected by both N₂O abuse and vitamin B12 deficiency.

While spinal MRI abnormalities have been previously reported in both vitamin B12 deficiency (21) and N₂O overexposure (12, 22, 23), we have not found studies that

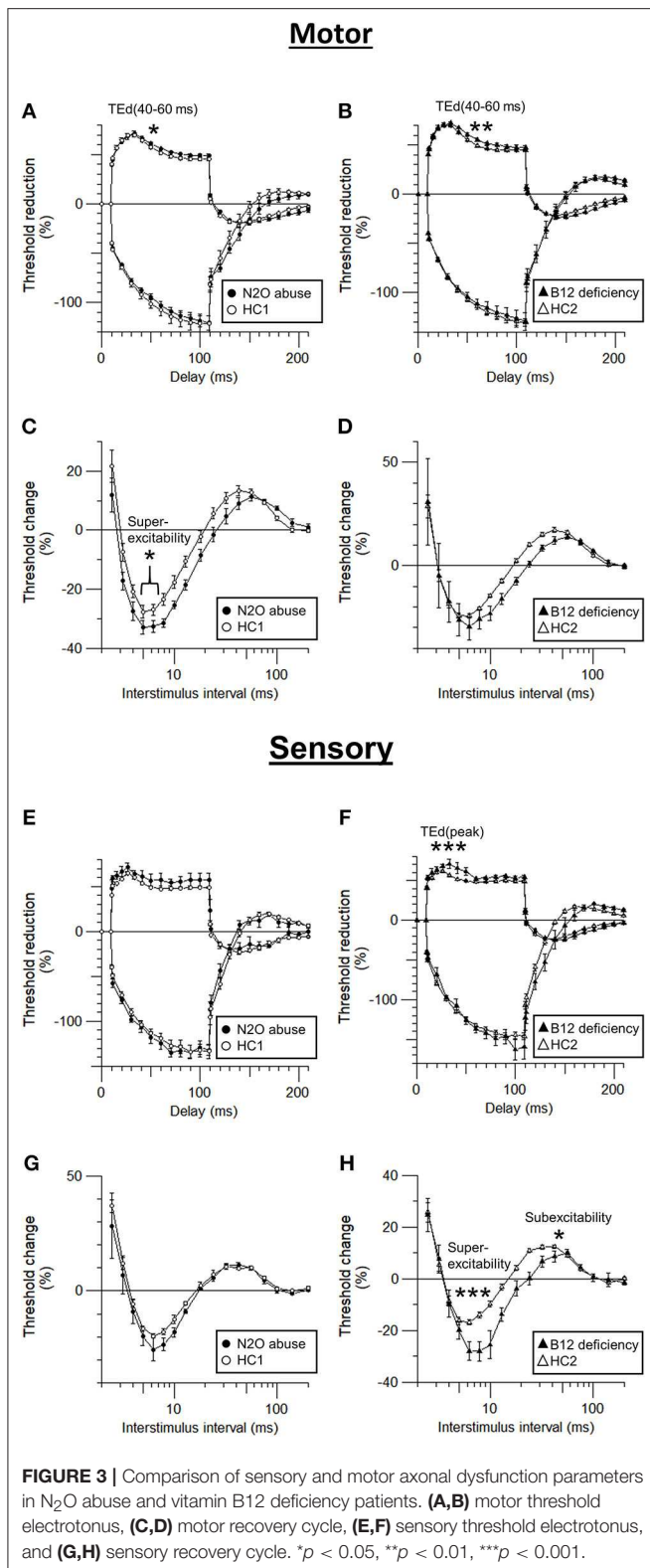
compare spinal MRI abnormalities in the two conditions; the present study revealed that spinal cord T2 hyperintense lesions were more prevalent in the N₂O abuse group. Nerve excitability test can reveal evidence of axonal dysfunctions that cannot be detected by conventional NCS, allowing us to gain insights into the unique pathophysiology associated with N₂O abuse.

A Different Pattern of Axonal Dysfunction in N₂O Abuse and Vitamin B12 Deficiency

Compared to vitamin B12 deficiency patients, N₂O abuse patients showed a unique nerve excitability pattern showing prominent motor superexcitability changes and less prominent sensory superexcitability changes. This provides evidence for the underlying pathological mechanism that affects the two conditions might be different, as discussed below.

Obvious Motor Axonal Dysfunction in N₂O Abuse

The nerve excitability test in patients with N₂O abuse showed decreased peak response, increased latency, decreased accommodation toward depolarizing current, and increased superexcitability. The increase in superexcitability may correspond to an alteration in fast K⁺ channels in the axonal paranodal region, while decreased accommodation toward depolarizing current signifies an alteration in nodal



and internodal slow K⁺ channels (24). Similar changes in superexcitability and increased TE accommodation toward depolarizing current have been noted in other demyelinating

diseases in previous studies. In particular, “fanning-out” of TE and increased superexcitability were seen in demyelinating diseases such as chronic inflammatory demyelinating polyneuropathy and multifocal motor neuropathy and was thought to possibly be related to compensatory Na⁺/K⁺ pump action in measured nerve segments (25–27). Such changes observed in patients with N₂O abuse may similarly be related to demyelination of proximal axons. The decrease in peak response noted in the patients may signify motor axonal loss.

Patients with vitamin B12 deficiency similarly showed motor axonal dysfunction, particularly, decreased accommodation toward depolarization. Although the changes are still in accordance with demyelination, the motor axonal damage appears to be less prominent than those in our N₂O abuse patients, as there are no significant changes in the RC. Moreover, no significant reduction in peak response is observed.

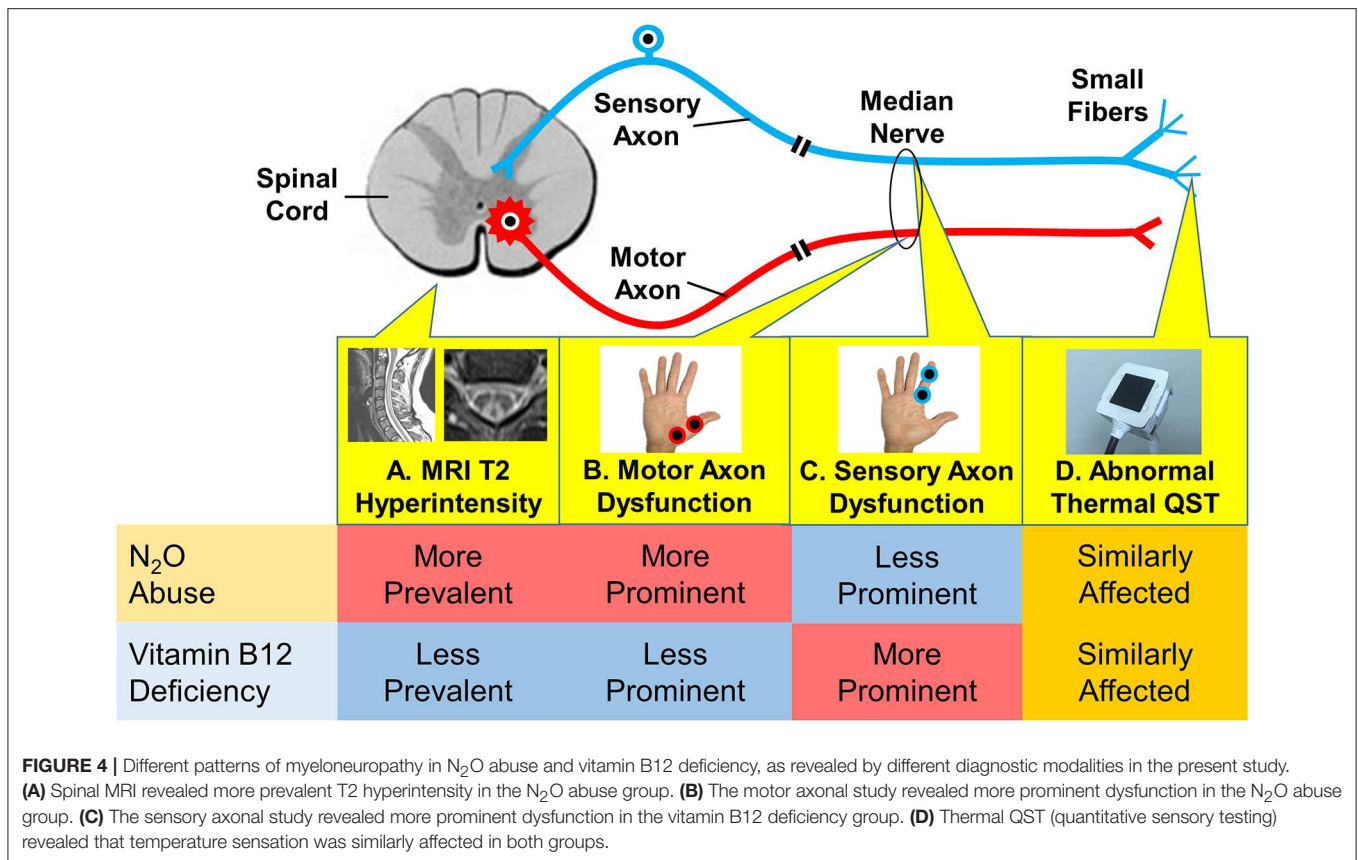
The motor axonal dysfunction pattern in both patients with N₂O abuse and vitamin B12 deficiency is compatible with motor demyelination. However, patients with N₂O abuse appear to have more prominent dysfunction in motor axons. The finding is in accordance with the clinical findings, in which patients with N₂O abuse show prominent motor deficits compared to those with vitamin B12 deficiency. Previous reports also described disproportionate motor amplitude reduction in the lower limbs in NCS of N₂O abuse patients (1, 12).

Traditionally, neuropathy induced by N₂O abuse was attributed to the effect of N₂O on the inactivation of vitamin B12 through blockage of methionine synthase, which converts homocysteine to methionine via a methylation process. The blockage could lead to the reduction of the vitamin B12 level, which then leads to hypomyelination and abnormal myelination (9, 28). It would concurrently lead to elevation of the homocysteine level, which might cause inflammation, oxidative stress, and microvascular disease (9, 29–31). Nevertheless, previous reports have also mentioned that N₂O might exert its neurotoxic effect independent of vitamin B12 deficiency, via mechanisms such as antagonism of N-methyl-D-aspartate (NMDA) receptors (6, 13). Certain reports reported ischemic pathological changes associated with nitrous oxide use (32).

While the exact pathophysiology of N₂O-induced myeloneuropathy remains poorly understood, as mentioned in the discussion above, the prominent changes in motor superexcitability observed in the present study might be an opportunity to elucidate further whether N₂O abuse causes unique pathophysiological changes in motor axons, especially in the paranodal region.

Prominent Sensory Axonal Dysfunction in Vitamin B12 Deficiency Patients

Although the results of the sensory nerve excitability test of patients with N₂O abuse showed a decreased peak response, they showed only a trend of increased superexcitability and “fanning-out” toward depolarizing and hyperpolarizing current. These axonal changes are less pronounced than those of vitamin B12 deficiency patients, which not only showed peak reduction but also showed obvious signs of demyelination such



as decreased accommodation toward depolarizing current, increased superexcitability, and decreased subexcitability. Decreased sensory SDTC in vitamin B12 deficiency patients, signifying alterations in persistent Na channels, is likewise compatible with hyperpolarization.

Sensory axonal dysfunction patterns in both N₂O abuse and vitamin B12 deficiency patients are similarly compatible with demyelination. Prominent changes in patients with vitamin B12 deficiency reflected significant sensory axonal dysfunction. These findings are in accordance with the clinical situation, in which vitamin B12 deficiency patients have more significant sensory complaints than N₂O abuse patients.

Previous nerve biopsy reports have shown that vitamin B12 deficiency could cause a combination of axonal degeneration and demyelination in vitamin B12 deficiency (33). A sural nerve biopsy report of N₂O abuse revealed evidence for both axonal degeneration and demyelination. Specifically, it found prominent evidence for axonal degeneration such as axonal swelling, shrunken axon, and varying degrees of myelin ovoid formation. However, it also presented evidence for focal demyelination and subsequent remyelination, including the occasional focal area of myelin loss, focal sites of denuded myelin, and short internodal segments of myelin (34).

Decreased peak response, together with prominent TE and RC changes seen in motor axonal study of N₂O abuse patients and sensory axonal study of vitamin B12 deficiency patients probably reflect a combined effect of axonal loss and focal

demyelination, in accordance with previous pathology report (33, 34). Prominent motor superexcitability changes in N₂O abuse patients might be related to toxicity involving paranodal region, possibly affecting fast K⁺ channel.

Less prominent TE and RC changes observed in the sensory axonal study of N₂O abuse patient and motor axonal study of vitamin B12 deficiency patient probably indicate that focal demyelination and toxicity involving paranodal region has less effect on the sensory axon of N₂O abuse patients and motor axon of vitamin B12 deficiency patient.

Potential Future Roles of Nerve Excitability Test in the Diagnosis of Myeloneuropathies

The ability of the nerve excitability test to detect axonal dysfunction in both N₂O abuse and vitamin B12 deficiency suggests that the tool could play a role in the diagnosis of neuropathy in both conditions. A previous study has described the axonal dysfunction pattern in myelopathy and radiculopathy, and the present study has further elucidated axonal dysfunction pattern in N₂O abuse and vitamin B12 deficiency myeloneuropathy, further clarifying axonal dysfunction patterns that could be observed when injury affects the spinal cord, root, and/or peripheral nerve (35). The present study has shown that the tool can distinguish motor and sensory nerve injury much more clearly than the

NCS, and may pave the way for the nerve excitability test to become another useful neurophysiologic diagnostic tool in the evaluation of myeloneuropathies, alongside with NCS. In particular, for patients presenting with a myeloneuropathy with unclear history, a nerve excitability test result showing the unique motor predominant axonal dysfunction as unveiled by the present study could support the diagnosis of N₂O abuse.

The fact that motor subexcitability and sensory TE_d (peak) are correlated with N₂O exposure duration, and that motor TE_h (overshoot) are correlated with MRC strength score of tested muscle in N₂O abuse, suggested that nerve excitability parameters may have the potential to monitor axonal damages caused by N₂O exposure, and may further add to the value of the nerve excitability test as an evaluation tool for the myeloneuropathy. Correlation between vitamin B12 level with numerous sensory axonal study parameters suggested that the parameters reflect axonal dysfunction related to vitamin B12 deficiency. Correlational study results also showed that RC and TE parameters are correlated with sensory perception threshold in the patients, but the interpretation of the results should be very careful, as the nerve excitability test was not specifically designed to evaluate small fiber function.

In summary, the present study has revealed that the nerve excitability test could detect motor and sensory axonal dysfunction in both N₂O abuse and vitamin B12 deficient patients. Follow-up studies with more cases would be beneficial to confirm the unique axonal dysfunction pattern in N₂O abuse and to explore treatment strategies for the abuse of the inhalant based on its axonal pathophysiology.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Joint Institutional Review Board, Taipei Medical University with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Joint Institutional Review Board, Taipei Medical University.

AUTHOR CONTRIBUTIONS

JT and J-YS contributed to the study design. JT, H-JC, T-SC, H-YW, and J-YS contributed to the data collection. JT, T-SC, J-YS, and CL contributed to the data analysis and interpretation. JT, J-YS, and CL contributed to the manuscript preparation. All authors approved the final version of the manuscript.

FUNDING

This work was supported by Taipei Medical University, Wan Fang Hospital, Taipei, Taiwan (grant no. 105TMU-WFH-02), Ministry of Science and Technology, Taiwan (MOST104-2314-B-038-012-MY3 and MOST107-2314-B-038-078), and Ministry of Education, Taiwan (DP2-107-21121-01-N-03).

REFERENCES

- 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) 127:3288–93. doi: 10.1016/j.clinph.2016.08.005
- Randhawa G, Bodenham A. The increasing recreational use of nitrous oxide: history revisited. *Br J Anaesth.* (2016) 116:321–4. doi: 10.1093/bja/aeV297
- Kaar SJ, Ferris J, Waldron J, Devaney M, Ramsey J, Winstock AR. Up: the rise of nitrous oxide abuse. An international survey of contemporary nitrous oxide use. *J Psychopharmacol.* (2016) 30:395–401. doi: 10.1177/0269881116632375
- Barratt MJ, Ferris JA, Zahnow R, Palamar JJ, Maier LJ, Winstock AR. Moving on From Representativeness: testing the utility of the global drug survey. *Subst Abuse.* (2017) 11:1–17. doi: 10.1177/1178221817716391
- The Lancet. Changing the conversation to make drug use safer. *Lancet.* (2018) 391:1965. doi: 10.1016/S0140-6736(18)31075-4
- 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) 25:358–69. doi: 10.1111/ajad.12372
- Chanarin I. Cobalamins and nitrous oxide: a review. *J Clin Pathol.* (1980) 33:909–16. doi: 10.1136/jcp.33.10.909
- Van Amsterdam J, Nabben T, Van Den Brink W. Recreational nitrous oxide use: prevalence and risks. *Regul Toxicol Pharmacol.* (2015) 73:790–6. doi: 10.1016/j.yrtph.2015.10.017
- Green R, Allen LH, Bjorke-Monsen AL, Brito A, Gueant JL, Miller JW, et al. Vitamin B12 deficiency. *Nat Rev Dis Primers.* (2017) 3:17040. doi: 10.1038/nrdp.2017.41
- 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) 51:614–6. doi: 10.1002/mus.24482
- Kaski D, Kumar P, Murphy E, Warner TT. Iatrogenic B12-deficient peripheral neuropathy following nitrous oxide administration for functional tonic leg spasm: a case report. *Clin Neurol Neurosurg.* (2017) 160:108–10. doi: 10.1016/j.clineuro.2017.07.006
- Johnson K, Mikhail P, Kim MG, Bosco A, Huynh W. Recreational nitrous oxide-associated neurotoxicity. *J Neurol Neurosurg Psychiatry.* (2018) 89:897–8. doi: 10.1136/jnnp-2017-317768
- Maze M, Fujinaga M. Recent advances in understanding the actions and toxicity of nitrous oxide. *Anaesthesia.* (2000) 55:311–4. doi: 10.1046/j.1365-2044.2000.01463.x
- Bostock H, Cikurel K, Burke D. Threshold tracking techniques in the study of human peripheral nerve. *Muscle Nerve.* (1998) 21:137–58. doi: 10.1002/(SICI)1097-4598(199802)21:2<andgt;137::AID-MUS1andgt;3.0.CO;2-C
- Kiernan MC, Lin CS, Andersen KV, Murray NM, Bostock H. Clinical evaluation of excitability measures in sensory nerve. *Muscle Nerve.* (2001) 24:883–92. doi: 10.1002/mus.1085
- Stabler SP. Vitamin B12 deficiency. *N Engl J Med.* (2013) 368:2041–2. doi: 10.1056/NEJMcp1113996
- Hannibal L, Lysne V, Bjorke-Monsen AL, Behringer S, Grunert SC, Spiekerkoetter U, et al. Biomarkers and algorithms for the diagnosis of vitamin

- B12 deficiency. *Front Mol Biosci.* (2016) 3:27. doi: 10.3389/fmolb.2016.00027
18. Khan A, Shafiq I, Hassan Shah M. Prevalence of vitamin B12 deficiency in patients with type II diabetes mellitus on metformin: a study from khyber pakhtunkhwa. *Cureus.* (2017) 9:e1577. doi: 10.7759/cureus.1577
 19. Kleyweg RP, Van Der Meche FG, Schmitz PI. Interobserver agreement in the assessment of muscle strength and functional abilities in Guillain-Barre syndrome. *Muscle Nerve.* (1991) 14:1103–9. doi: 10.1002/mus.880141111
 20. Bril V. NIS-LL: the primary measurement scale for clinical trial endpoints in diabetic peripheral neuropathy. *Eur Neurol.* (1999) 41(Suppl. 1):8–13. doi: 10.1159/000052074
 21. Marelli C, Salsano E, Politi LS, Labauge P. Spinal cord involvement in adult-onset metabolic and genetic diseases. *J Neurol Neurosurg Psychiatry.* (2018) 90:211–8. doi: 10.1136/jnnp-2018-318666
 22. Beltramello A, Puppini G, Cerini R, El-Dalati G, Manfredi M, Roncolato G, et al. Subacute combined degeneration of the spinal cord after nitrous oxide anaesthesia: role of magnetic resonance imaging. *J Neurol Neurosurg Psychiatry.* (1998) 64:563–4. doi: 10.1136/jnnp.64.4.563
 23. Sotirchos ES, Saidha S, Becker D. Nitrous oxide-induced myelopathy with inverted V-sign on spinal MRI. *J Neurol Neurosurg Psychiatry.* (2012) 83:915–6. doi: 10.1136/jnnp-2012-303105
 24. Boerio D, Bostock H, Spescha R, Z'graggen WJ. Potassium and the excitability properties of normal human motor axons *in vivo*. *PLoS ONE.* (2014) 9:e98262. doi: 10.1371/journal.pone.0098262
 25. Kiernan MC, Guglielmi JM, Kaji R, Murray NM, Bostock H. Evidence for axonal membrane hyperpolarization in multifocal motor neuropathy with conduction block. *Brain.* (2002) 125:664–75. doi: 10.1093/brain/awf041
 26. Kaji R. Physiology of conduction block in multifocal motor neuropathy and other demyelinating neuropathies. *Muscle Nerve.* (2003) 27:285–96. doi: 10.1002/mus.10273
 27. Sung JY, Tani J, Park SB, Kiernan MC, Lin CS. Early identification of 'acute-onset' chronic inflammatory demyelinating polyneuropathy. *Brain.* (2014) 137:2155–63. doi: 10.1093/brain/awu158
 28. Green R, Kinsella LJ. Current concepts in the diagnosis of cobalamin deficiency. *Neurology.* (1995) 45:1435–40. doi: 10.1212/WNL.45.8.1435
 29. Maler JM, Seifert W, Huther G, Wiltfang J, Ruther E, Kornhuber J, et al. Homocysteine induces cell death of rat astrocytes *in vitro*. *Neurosci Lett.* (2003) 347:85–8. doi: 10.1016/S0304-3940(03)00655-4
 30. Troen AM, Shea-Budgell M, Shukitt-Hale B, Smith DE, Selhub J, Rosenberg IH. B-vitamin deficiency causes hyperhomocysteinemia and vascular cognitive impairment in mice. *Proc Natl Acad Sci USA.* (2008) 105:12474–9. doi: 10.1073/pnas.0805350105
 31. Caterino M, Pastore A, Strozzi MG, Di Giovamberardino G, Imperlini E, Scolamiero E, et al. The proteome of cblC defect: *in vivo* elucidation of altered cellular pathways in humans. *J Inherit Metab Dis.* (2015) 38:969–79. doi: 10.1007/s10545-014-9806-4
 32. Thompson AG, Leite MI, Lunn MP, Bennett DL. Whippits, nitrous oxide and the dangers of legal highs. *Pract Neurol.* (2015) 15:207–9. doi: 10.1136/practneurol-2014-001071
 33. Kalita J, Chandra S, Bhoi SK, Agarwal R, Misra UK, Shankar SK, et al. Clinical, nerve conduction and nerve biopsy study in vitamin B12 deficiency neurological syndrome with a short-term follow-up. *Nutr Neurosci.* (2014) 17:156–63. doi: 10.1179/1476830513Y.0000000073
 34. Sahenk Z, Mendell JR, Couri D, Nachtmann J. Polyneuropathy from inhalation of N2O cartridges through a whipped-cream dispenser. *Neurology.* (1978) 28:485–7. doi: 10.1212/WNL.28.5.485
 35. Sung JY, Tani J, Hung KS, Lui TN, Lin CS. Sensory axonal dysfunction in cervical radiculopathy. *J Neurol Neurosurg Psychiatry.* (2015) 86:640–5. doi: 10.1136/jnnp-2014-308088

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Tani, Weng, Chen, Chang, Sung and Lin. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.