Psychomotor function during mild narcosis induced by subanesthetic level of nitrous oxide: individual susceptibility beyond gender effect

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ABSTRACT

Objective: We investigated the effect of narcosis induced by subanesthetic concentrations of nitrous oxide (N_2O), a behavioral analogue for hyperbaric nitrogen, on psychomotor performance. In particular, we assessed individual susceptibility to narcosis.

Methods: The participants were 12 female and 12 male undergraduate students. Psychomotor assessment was conducted with a computerized Visual Simple Reaction Time (VSRT) test, and Trail Making Tests Part A (TMT-A) and Part B (TMT-B). The tests were conducted on two separate occasions in the following order: VSRT, TMT-A, TMT-B. On the first occasion participants conducted the tests breathing room air (air trial), and during the second test they conducted the tests while breathing a normoxic mixture containing 30% N_2O (N_2O trial).

Results: Males had significantly ($p = 0.036$) shorter VSRT in the air trials. There was no effect of gender on psychomotor performance in the N_2O trials. Overall, mean performance in the N_2O trials degraded significantly ($p = 0.004$) only in VSRT. Performance of individual participants exhibited different and inconsistent direction of change in the N2O trials.

Conclusion: N_2O-induced alterations in psychomotor function are primarily dependent on the individual susceptibility to narcosis (*i.e.*, concentration threshold).

INTRODUCTION

In diving, the most common type of inert gas narcosis is nitrogen narcosis, which is caused by the raised partial pressure of the nitrogen in compressed air [1]. In the broadest sense, the term "narcosis" refers to the reversible depression of function of an organism [2]. The term "inert gas narcosis" refers to the narcotic effects of inert gases. Inert gases are a subgroup of the gaseous and volatile anesthetics, some of which exert a narcotic effect under higher pressure (argon, nitrogen, hydrogen). For practical purposes, neon and helium are considered to be non-narcotic, whereas other gases (nitrous oxide – N_2O, cyclopropane and ethylene) are narcotic at atmospheric pressure [3].

We investigated individual susceptibility to narcosis induced by subanesthetic concentrations of N_2O through testing psychomotor performance. This is a valid approach for diving research because hyperbaric nitrogen and normobaric N_2O exert similar effects on cognitive performance and behavior [4].

The signs and symptoms of nitrogen narcosis in diving are first noticed at approximately 30 meters (100 feet) during compressed-air breathing [3]. Compressed-air diving elicits several effects on human performance. Nitrogen narcosis produces significant impairment by decreasing both speed and accuracy of processing in the majority of performance tests [5]. It may also interfere with encoding and/or retrieval of verbal information [6]. Loss of memory during deep air dives has long been well documented [3,7,8]. Narcosis-induced overconfidence and impaired performance represent an important, and probably underestimated, threat to diver safety [9].

Human behavioral studies have concluded that N_2O in the concentration range from 20% to 30% depresses psychomotor function [10], cognitive performance [11], learning and memory [12]. It is also apparent from these studies that there is a substantial degree of intersubject variability regarding the magnitude of the narcosis-induced effects on psychomotor performance. This is most likely due to differences in the N_2O concentration threshold for inducing detectable narcotic effects among subjects, as well as differences in the dose-dependent effects of narcosis on psychomotor function [13].

The majority of the studies mentioned above included only male subjects, and those that included both genders did not focus on gender differences. Although it is well established that there is no difference between adult males and females in general intelligence level [14], some distinctions have been reported regarding different aspects of verbal and performance intelligence [15]. Men usually outperform women in mathematical problem-solving [16-18], visual-spatial ability [17,19-23], map reading [20,24] and targeted motor skills [25,26]. Women generally excel in verbal fluency [14,27,28], memory for object location [29,30], fine motor skills [31] and perceptual speed tasks [21,27]. These differences may be attributed to sensory nerve action potential amplitude [32-35], volume of white and gray matter and cerebrospinal fluid [36,37], global and regional cerebral glucose metabolism and blood flow [38-41], and activation pattern of the central nervous system [39,41-43].

To investigate individual susceptibility to N_2O-induced mild narcosis, we selected psychomotor tests that are simply and quickly administered, widely accessible and have good metric characteristics. Because of the gender differences in information processing described above and because of the cerebral vasodilatory effects of N_2O [44], we also investigated gender differences in susceptibility (in terms of psychomotor performance) to N_2O-induced mild narcosis. As already emphasized, the main premise of our study is that the findings obtained using N_2O can be generalized to nitrogen narcosis.

METHODS

The study protocol was approved by the National Committee for Medical Ethics of the Ministry of Health (Republic of Slovenia). Written informed consent was obtained from each participant before participating in the study. All participants attended a screening interview, during which their medical status was assessed to determine whether there were any contraindications to their participation in the study. None of the participants had any previous experience with N_2O. No female participant was pregnant. Twelve female and 12 male undergraduate physiotherapy students participated in the study. The mean (SD) age of the participants was 21.7 (1.7) years, 21.6 (2.2) years for females and 21.8 (1.2) years for males. For the psychomotor assessment, two timed psychomotor tests were administered, which differed in complexity. The first one, the computerized Visual Simple Reaction Time (VSRT) test, is a sustained attention task that measures attention and response speed to an easily discriminated but temporally uncertain visual signal.

The task is to press a key on the mouse as quickly as possible when the stimulus is presented on the display. The stimulus was a circle; it was triggered by the experimenter; the random latency range between stimuli was two to 10 seconds. After one familiarization trial (five stimuli), five stimuli were presented within one session, and the average was taken as the test result. The temporal resolution of reaction time recording was 1 millisecond. Reliability of the measurement procedure was assessed (using intraclass correlation coefficient – ICC, average measure version, two-way random model for absolute agreement [45]) and was found to be very high (ICC = 0.998 (95% confidence interval 0.996-0.999), and ICC = 0.987 (0.975-0.993) under air and N_2O trials, respectively; see below for explanation).

Psychomotor speed and executive control were assessed with the Trail Making Test Part A (TMT-A) and Part B (TMT-B) [46]. The task in the TMT-A test is to connect 25 circled numbers by lines in sequence; in TMT-B, each circle contains either a letter or a number, and the task is to draw lines alternating from a number to a letter in increasing order (*e.g.*, 1-A-2-B . . .). The participants performed a short practice trial (TMT-A with eight numbers and TMT-B with four numbers and four letters) followed by the test proper. The accepted cutoff value for TMT is 40 seconds for task A and 91 seconds for task B [47,48].

Each individual experiment lasted approximately 30 minutes. It was conducted at the same time of the day for each subject. The subjects were instructed not to eat food or drink coffee, tea or alcohol drinks four hours prior to the experiment. Before the start of the experiment, each participant's height and weight were measured in order to calculate the body mass index (BMI). The three psychomotor tests were performed under two experimental conditions: breathing room air (air trial) and breathing a normoxic mixture containing 30% N_2O (N_2O trial), whereby the same sequence was followed for all participants. N_2O is a non-volatile, gaseous, inhaled anesthetic; the dose of 30% was selected because previous studies showed that subanesthetic concentrations (*i.e.*, FN_2O from 0.2 to 0.3) produce marked

TABLE 1: ***Descriptive statistics and results of statistical tests for Visual Simple Reaction Time (VSRT) and Trail Making Test (TMT)***

Test	Trial	Subjects			Regression model (*p*-values)			N_2O effect in pooled sample**
		Females	Males	Pooled sample	Model as a whole	Effect of gender*	Effect of BMI*	
VSRT (ms)	**air**	244 (36)	214 (17)	229 (31)	0.050	0.036	0.895	0.004
	N_2O	272 (44)	223 (38)	247 (47)	0.232	0.091	0.410	
TMT-A (s)	**air**	17.41 (5.34)	15.74 (3.80)	16.58 (4.62)	0.614	0.594	0.627	0.156
	N_2O	18.35 (6.02)	17.14 (3.55)	17.75 (4.87)	0.565	0.819	0.307	
TMT-B (s)	**air**	34.72 (10.61)	39.66 (14.46)	37.19 (12.65)	0.030	0.781	0.014	0.202
	N_2O	37.40 (22.48)	47.51 (23.67)	42.46 (23.16)	0.720	0.434	0.606	

Data are reported as mean (SD); **p*-values in the N_2O rows refer to the change score (air – N_2O);
***p*-value from paired t-tests; see the Methods section for details

[49,50] and consistent effects on performance [13]. The participants breathed through a low-resistance T-shaped Hans Rudolph (Hans Rudolph Inc., Kansas City, USA) respiratory valve. They inspired the breathing mixtures via respiratory tubing subsequent to it being humidified by passing the gas through a water bath at room temperature (21-25° C). During the air trial, the participant inspired air for 10 minutes and then executed the tests. Assessment in the N_2O trial commenced after 10 minutes of breathing the N_2O mixture, to ensure a stable N_2O blood saturation of approximately 95% in the brain [51]. In each trial, the VSRT was followed by TMT-A and then TMT-B. Before each test, the procedure was explained to the subject, and a familiarization test performed.

Statistical analysis

First, an *a priori* comparison of BMI between genders (using independent samples t-test) was performed. Because it revealed a statistically significant difference, the analyses of potential gender effect on psychomotor performance had to account for the potential confounding effect of BMI. For this purpose, baseline (*i.e.*, air trial) psychomotor tests results and their change due to mild narcosis (*i.e.*, change scores computed as the difference between N_2O and air trials, with positive values reflecting a worsening, and negative values an improvement in function under mild narcosis) were analyzed using linear regression as outcome variables with gender and BMI as predictors. Regression diagnostics (probability plots of residuals, standardized residuals *vs.* standardized predicted values scatterplots, variance inflation factors) were derived and none indicated any substantial departure from the regression model's assumptions.

Because no significant gender effect on change score was found in any of the regression models, simple comparisons between means of the air and N_2O trials were subsequently performed using paired t-tests on the pooled sample. Since psychomotor tests can be considered as having very high reliability, and thus any test score change as exceeding the minimum detectable change (also known as minimal real difference [52]), participants with zero or negative change score were considered as not having reached the concentration threshold. These binary data were compared between genders using Fisher's exact test.

Statistical analyses were performed using IBM SPSS Statistics 19 software (SPSS Inc., an IBM Company, 2010). Statistical significance was set at $p \leq 0.05$.

RESULTS

BMI differed statistically significantly between genders: on average, females had lower BMI (mean [SD] (22.0 (2.6) kgm^{-2}) than males (24.9 [3.4] kgm^{-2}, $p = 0.023$).

The results of the psychomotor tests are summarized in Table 1 *(above)*. During the air trials, mean VSRT

FIGURE 1 – *BMI vs. VSRT*

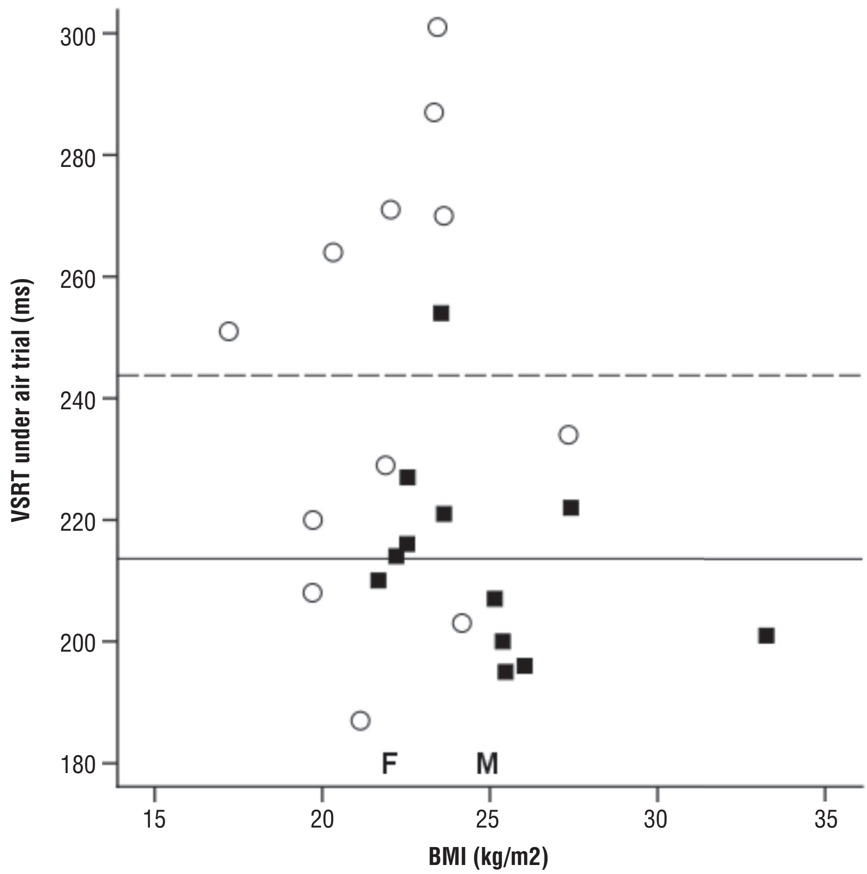

Figure 1. Relation between body mass index (BMI) and computerized visual simple reaction time (VSRT) test, depicted separately by gender. Open circles – female subjects; closed squares – male subjects; horizontal lines – VSRT means (dashed line for females, solid line for males); letters M and F on the horizontal axis – mean BMI for males and females, respectively.

was statistically significantly shorter in males than in females (p = 0.036). A similar trend was observed in TMT- A, but the gender effect was not statistically significant. Conversely, the observed mean execution time for the more demanding TMT-B was shorter in females, albeit not significantly. There was no statistically significant effect of either gender or BMI on change of performance in the N_2O trials. Higher BMI was statistically significantly associated with longer TMT-B time during air trials (the raw and standardized regression coefficient, not reported in Table 1, was 2.196 and 0.558, respectively). Overall, N_2O statistically significantly worsened performance in VSRT (p = 0.004), but not in TMT-A (p = 0.156) or TMT-B (p = 0.202), though the observed mean execution times were prolonged in both TMT parts as well.

The gender difference in the air trial VSRT is depicted in Figure 1 *(above)*. The vertical lines show that the mean VSRT was shorter in males than in females. Additionally; the letters on the horizontal axis clearly indicate that males had higher BMI on average. However, it is also apparent that confounding was not an issue in this comparison, because neither the groups nor the pooled samples exhibited a correlation between BMI and VSRT. This can also be illustrated, albeit not properly statistically tested, by computing the three correlations, none of which was statistically significant (r = 0.155, p = 0.630 for females; r = 0.371, p = 0.235 for males; r = 0.250, p = 0.239 for the pooled sample).

Eight participants (three women and five men) performed VSRT equally or faster during the N_2O trials.

Six participants (four women and two men) performed equally or better under N_2O in TMT-A, and 11 participants (seven women and four men) in TMT-B. None of the differences between genders in the proportion of (non-) responders was statistically significant (p=0.667, 0.640 and 0.414 for VSRT, TMT-A and TMT-B, respectively). Only four participants performed worse (and could therefore be considered as having reached the concentration threshold) in all three tests in the N_2O trial, and only one performed equally or better on all three tests in the N_2O trial (and could therefore be considered as not having reached the concentration threshold in any of them). During the N_2O trials, none of the participants reached the cutoff time for TMT-A and only two exceeded the cutoff time for TMT-B.

DISCUSSION

The observed gender difference in the air-trial VSRT is in agreement with the finding that males have faster reaction times than females in almost every age group [53,54]. Almost all of the gender differences appear to be accounted for by the lag between the presentation of the stimulus and the beginning of muscle contraction [55]. However, there is some disparity in the results of studies investigating the concentration threshold of N_2O at which such changes are detected. The initial conclusion [56] that the threshold concentration of N_2O for an effect on psychomotor performance (as assessed by choice reaction times) probably lies between 8% and 12% is not supported by more recent studies [57,58], which do not report any significant differences in simple reaction time between N_2O (25% of N_2O and 75% O_2) and control (air) sessions. Our findings of prolonged visual reaction times are similar to those [59] who observed a prolongation of auditory reaction time under sub-anesthetic concentrations of various inhalation anesthetics (including N_2O).

TMT scores are affected by age, education [60-62] and general intelligence [60]. The influence of these factors was minimized in the present study, because the gender groups were age-balanced and the participants were studying in the same university department, at the same level, and had been admitted to the department by meeting the same strict high-school grade criterion for admission (which can be taken as a rough proxy for intelligence). In some studies, gender correlated neither with raw TMT scores [62] nor with derived indices [63]. Our results confirmed the observation [60] that females tend to take a longer time to complete Part A than males.

We observed a clear overall decline in performance in the N_2O trial only regarding VSRT. Previously, significant decrease in Digit-symbol Substitution Test scores has been observed in subjects who inspired 30% N_2O as compared to placebo (air) at five and 15 minutes of the inhalation period [64]. Similarly, inhalation of a normoxic mixture containing as little as 15% N_2O produced significant impairments in cognitive tasks (as measured by the Digit-symbol Substitution Test and the Sentence Verification test) [65]. In contrast, 25% N_2O was used in a study establishing that performance regarding accuracy of digit vigilance between N_2O and control sessions did not differ [58].

The dose-response profiles of various tests used to date reveal substantial differences [13]: no measure showed evidence of a change at the lowest concentrations (5% N_2O), several measures (digit-symbol substitution, choice reaction time – latency and total, tapping, and continuous attention) showed significant impairment at 10% N_2O, and all tests except critical flicker fusion showed substantial effects at the highest dose (40% N_2O). These results indicate that comparisons of profiles of drug-induced changes must take into account the variable effects of dose before interpretations in terms of specific drug effects can be made [13].

There were substantial differences among the participants in our study regarding their responses to N_2O in terms of psychomotor performance. Only about one-fifth of the participants could be classified as responders on all three tests, and only one as a non-responder on all three tests. Given such inter-individual differences, it is not surprising that the results of the three tests could not provide a good indication of a gender effect regarding the proportion of "responders" and "non-responders." As further explained in the Appendix, it may therefore be very difficult, or at least unproductive, to try to separate gender differences from effects of body stature.

The main effects of inhaling subanesthetic concentrations of N_2O are observed in the central nervous system. The global increase in cerebral blood flow induced by N_2O is distributed unevenly, with the main increases observed in the frontal, temporal, parietal cortex, basal ganglia, insula and thalamic regions [44]. The flow pattern suggests that inhalation of N_2O augments flow through regions associated anatomically with the limbic system, most likely due to selective activation of these areas [44]. In contrast, N_2O deactivates the posterior cingulate, hippocampus, parahippocampal gyrus, and visual association cortices in both hemispheres; the former two regions are known to mediate learning and memory [66]. This may, in part, explain why subjects who reached concentration threshold were not able to execute psychomotor tests equally or better.

A recent animal study provided evidence that N_2O impairs information processing by altering at least the stage of motor adjustment [67]. Since N_2O spared the sensory processes implemented during the stimulus pre-processing stage, the authors concluded that at some concentrations, N_2O displays opposite effects on reaction time and movement time. These results further preclude any universal and dose-independent conclusions regarding psychomotor functioning under nitrogen narcosis, including any straightforward gender differences.

To summarize, we believe that the most likely reason for the lack of agreement between the results of different studies involving N_2O-induced narcosis is the disregard for individual concentration thresholds of the participants. In other words, it seems that some experiments (or groups) may have involved mainly participants who did not reach the concentration threshold, while the opposite goes for the others. The solution to this problem appears to be twofold. The first option may be to carry out all such experiments in a dose-response manner; a second option may be to use a single concentration of N_2O but incorporate a subject inclusion criterion based on preliminary psychomotor tests to select susceptible participants.

APPENDIX – *Some statistical considerations*

It should be emphasized that conducting either an analysis of variance (mixed-model ANOVA with gender as between-subject and air vs. N_2O trial as within-subject factor, thus ignoring the confounding effect of BMI) or an analysis of covariance (ANCOVA; in an attempt to "adjust" for BMI) on our data would not be valid.

The reason for the latter deserves special emphasis, because in addition to the assumption of homogeneity of regression slopes, ANCOVA also requires that the groups do not differ on the covariate [68]. This is a well-known and widespread issue in quasi-experimental research (i.e., comparison of pre-existing groups, observational studies, non-randomized studies), known as Lord's paradox [69,70], for which no simple solution exists.

In our analysis we used regression modeling of change scores, since it is arguably the most appropriate approach in such studies [71,72].

Conflict of interest statement

The authors have no conflict of interest to declare. ■

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