Toxicology, 49 (1988) 449–457
Elsevier Scientific Publishers Ireland Ltd.

EXPERIMENTAL NITROUS OXIDE EXPOSURE AS A MODEL SYSTEM FOR EVALUATING NEUROBEHAVIORAL TESTS

F. CARL MAHONEY[a], PAUL A. MOORE[b], EDWARD L. BAKER[c] and RICHARD LETZ[d,*]

[a]Harvard School of Public Health, 665 Huntington Ave., Boston, MA 02115, [b]University of Pittsburgh, School of Dental Medicine, Pittsburgh, PA 15261, [c]National Institute for Occupational Safety and Health, 1600 Clifton Road, Atlanta, GA 30333 and [d]Division of Environmental and Occupational Medicine — Box 1057, Mount Sinai School of Medicine, 10 East 102nd Street, New York, NY 10029 (U.S.A.)

SUMMARY

A recent study reported that a minimally suprathreshold dose of 20% nitrous oxide significantly affected performance on 3 of 9 computer-administered neurobehavioral tests. Performance decrements were observed in that study on 3 tests of psychomotor speed while other neurobehavioral functions such as visuospatial ability, verbal learning and mood were not significantly affected. The current study was undertaken to assess the reproducibility of these earlier results. Its experimental design was expanded to include an additional dose of the anesthetic and a more complex reaction time task implemented by the authors since the earlier study. Fifteen males aged 24—34 years were tested with the Neurobehavioral Evaluation System (NES) test battery on 4 separate occasions. An initial training session was followed by randomly presented control, 20%, and 40% drug sessions. Drug-induced decrements in performance were observed in the current study at the 20% dose for 2 of the 3 tests of psychomotor speed which had shown effects in the earlier study, and average decrement in performance on the third approached statistical significance in the current study ($P = 0.055$). Performance on a complex reaction time task was significantly affected at the low dose. The higher dose of nitrous oxide impaired performance on 8 of the 9 tests administered, and impairment on the ninth test was nearly significant ($P = 0.055$). Overall, these data are consistent with those in the previous study and with other reports that higher doses of nitrous oxide produce impairments in more cognitive CNS functions. Owing to its relative safety at low dosages, ease of administration, and ready acceptance by experimental subjects, nitrous oxide appears to be a useful model for evaluating the validity of neurobehavioral tests.

*To whom all correspondence and reprint requests should be addressed.

0300-483X/88/$03.50

Key words: Computerized neurobehavioral testing; Nitrous oxide; validation

INTRODUCTION

The utilization of microcomputers in acquiring systematic neurobehavioral data in epidemiologic neurotoxicity research is an area of considerable promise for the allied disciplines of occupational medicine, behavioral toxicology, and neurotoxicology. The advantages of computerized administration of neurobehavioral tests have been detailed in recent reports on the development and rationale of a neurobehavioral evaluation system (NES) and this system has been successfully applied in field studies of populations exposed to known neurotoxins [1,2]. The challenges for this approach toward evaluating the neurobehavioral effects of exposure to environmentally occurring agents are numerous and complex [3]. Two central concerns, common to the construction and development of any psychometric test, are the issues of reliability and validity [4]. In a previous study, the NES was employed to examine the effects of nitrous oxide (N_2O). The rationale for choosing N_2O as a model test compound was that its functional effects on the central nervous system are well documented [6,7]. Nitrous oxide is a commonly employed anesthetic gas that is itself the subject of concern as an occupational hazard in dental offices and operating rooms [8].

The current study was designed to address several objectives. Foremost was putting the NES to a test of replicability. Demonstration of the reproducibility of results obtained in an earlier study would provide evidence of the reliability of NES in the detection of pharmacologically-induced CNS impairment [8]. Toward this end, experimental conditions and methods used in a recent study were carefully duplicated. Additionally, since only 1 dose of nitrous oxide was used in the previous study, 2 nitrous oxide conditions were used in the current study to further explore dose-response relationships. Other research has demonstrated that higher doses of nitrous oxide impair performance on tasks of higher order CNS functions such as mathematical problem solving ability [9]. Finally, a complex choice reaction time task similar to one developed for another computer-administered test system had been implemented in NES and needed a test of its sensitivity to detect functional impairment in the CNS [10].

MATERIALS AND METHODS

Subjects

Fifteen white males, aged 24–34, volunteered for paid participation. The intent of the study was thoroughly explained and each subject was counseled with respect to nitrous oxide effects by the attending clinician (P.A.M.). They were encouraged to halt any testing session if they experienced discomfort (e.g. nausea, dizziness) and also to try as hard as they could on the computer tests. Informed consent given by each subject also assured that no penalty was associated with declining further participation at any point in the study [11].

Prior to each test session a brief questionnaire was administered which probed the subject's general state of health on the day of and in the days immediately previous to the test session. Information was also obtained regarding use of alcohol, caffeine, and tobacco. All subjects were native English speakers. Mean number of completed years of education was 18.5 reflecting the preponderance of graduate dental students in the sample.

Procedures

The current study emphasized reconstructing the testing conditions and protocol of a previous study as precisely as possible [8]. Testing occurred on 4 separate days over 2 weeks. Schedules were drawn up so that sessions for each subject were run at the same hour of the day. Test sessions were conducted in a large room free of visual and auditory distractions. Overhead fluorescent fixtures were disabled to reduce glare on the computer screen. For all subjects the initial session was a training period during which they were fitted with a nasal inhalation mask so that they could acclimate both to the computer tests and wearing the mask. Subjects were invited to use the training session to determine an approach toward the tests that would ensure their best possible performance in subsequent sessions, e.g. joystick positioning, physical distance from the video screen. Subjects and the neurobehavioral test administrator (F.C.M.) were kept blind to the treatment conditions. The order of exposure conditions was balanced according to a 3 × 3 Latin square design. The dose was supplied to the test administrator at the conclusion of all experimental sessions.

Neurobehavioral Evaluation System (NES) software developed by Baker et al. (1985) was run on a portable COMPAQ microcomputer (Compaq Computer Corp.). NES is a collection of menu-driven computer administered neurobehavioral tests selected for their applicability to computer implementation and developed specifically to collect standardized neurobehavioral data with minimal examiner interaction with individuals taking the tests. Subjects respond to the automated tests by pressing numbered keys on a restricted region of the keyboard or with simple external manipulanda (joystick controller, 2 buttons). Nine NES tests selected for inclusion in the current study are detailed below according to the order of their administration in this experiment.

Neurobehavioral tests

Continuous performance test. Subjects are asked to press a button in response to a block letter "S" which appears randomly as part of a 5-min series of other block letters (B,C,E,A) that flash at the center of the video screen at the rate of 1/s. Reaction times in ms to the target stimuli are recorded along with errors of commission and omission.

Hand-eye coordination test. Using a joystick controller (Commonwealth Environmental, Inc.), subjects trace a sine wave pattern by moving a small cursor in the vertical while its rate of movement across the screen is constant. Vertical deviations from the set line are recorded in this test of coordination and dexterity.

Serial digit learning. In this task, which probes short-term memory and attention, a series of 10 digits is displayed at the rate of 1/s. Subjects' instructions are to type the same series of numbers when the display finishes. The series is presented until 2 successive trials are performed correctly up to a maximum of 8 trials. The series is changd between sessions.

Symbol-digit substitution test. Nine pairs of matched symbols and digits are painted at the top of the screen constituting a key for solving the task which is to type in numbers associated with a set of scrambled symbols at the bottom of the screen. The response measure is latency per symbol and is intended to evaluate psychomotor speed and coding ability.

Pattern recognition. This task requires that subjects discriminate among 3 similar 10 × 10 block stimulus arrays. One of the 3 stimuli is slightly different from the remaining 2 with differences becoming increasingly subtle over 15 trials. Subjects key a number (1, 2, or 3) corresponding to their choice for each trial. Response latencies and errors (expressed as negative latencies) are recorded.

Pattern memory. This test of short term visual memory involves presentation of a single block stimulus array as described above for 5 s. A brief retention interval follows before 3 different arrays are presented simultaneously. One of these is the original while the other 2 have a small number of the array elements changed. Correct and incorrect response latencies are recorded.

Switching attention. This complex choice reaction time test measures motor and perceptual processing speed and employs 3 separate conditions. In the first condition, subjects categorize a solid block stimulus by evaluating its position on the screen and pressing 1 of 2 corresponding response buttons (left side, right side). In the second condition, an arrow appears within the block which is presented at the center of the screen. Response criteria are button presses corresponding to the direction the arrow is pointing. In the final condition the subject switches back and forth between categorizing the stimuli by position or direction according to a prompt flashed on the screen just prior to any given stimulus. Response latencies and errors within each of the 3 conditions are recorded.

Finger tapping. This measure of simple motor quickness and accuracy requires subjects to tap a button as many times as possible in 10 s with 3 conditions (dominant index finger, non-dominant index finger, alternate 2-button tap with dominant index finger). Data are recorded as number of taps per trial. The measure used in the current study was the combined number of taps for both alternate tapping trials.

Mood scales. A series of 25 adjectives is presented to which the subjects rate themselves on a scale of 1—5 regarding their feelings during the testing session. Responses contribute equally to 5 mood scales (tension, depression, anger, fatigue, and confusion).

Inhalation apparatus

During each of the 4 sessions subjects were fitted with a Brown

Scavenging Mask (Narco Corp.) and instructed to breathe only through their noses. The experimental gases were administered through McKesson Analor 3C inhalation sedation apparatus. Before commencing the computer tests a 10-min saturation period ensured that subjects were comfortable and breathing appropriately and that a stable nitrous oxide level in the blood was achieved [7]. At the conclusion of each experimental session, subjects were observed for 15–20 min before the attending clinician permitted them to leave.

Data analysis

Analyses of these data were performed to match those carried out in a previous study [8]. Personal computer statistical software (SPSS–PC+, 1985) was used to perform statistical tests [12]. Each behavioral measure was subjected to a one-tailed Student's *t*-test for paired comparisons. Performance within each condition was compared with performance during the 0% nitrous oxide session.

RESULTS

Response measure results are presented in Table I. Nitrous oxide at 20% concentration significantly impaired performance on symbol-digit and finger tapping and approached statistical significance for continuous performance test response latency ($P = 0.055$). These were the 3 NES tests on which Greenberg et al. (1985) found impairment in performance at 20% nitrous oxide. Additionally, the current study noted a significant drug effect on the Confusion items contained in the NES Mood Scales at the low dose.

The 40% dose of nitrous oxide impaired performance on virtually all NES measures. The serial digit learning task was the only NES test not significantly affected although statistical significance was approached ($P = 0.055$). Also, as seen in the lower nitrous oxide dose, only 1 of the 5 self-rating scales within NES Mood Scales (Confusion) was significantly affected.

In addition to the effects of nitrous oxide exposure, significant improvement in performance was observed between training and control sessions when no nitrous oxide was administered. Significant "practice" or "learning" effects were observed for continuous performance test, symbol-digit, hand-eye coordination, switching attention and finger tapping.

Internally consistent performance and effects of nitrous oxide were observed on the Switching Attention task. No impairment was noted with 20% nitrous oxide in the first 2 'simple' conditions, but 2 separate variables associated with the 'complex' condition were significantly affected (switching-side and switching-direction). In Fig. 1, results on all 4 performance measures (in mean response times) of the Switching Attention task are plotted against the experimental conditions and the training session employed in the current study. Significant improvement in performance was observed between training and control sessions for both switching conditions while response times for the simple conditions, side and direction, remained relatively stable across all sessions. The lowest order of stimulus complexity

TABLE I

EFFECTS OF EXPERIMENTAL NITROUS OXIDE EXPOSURE ON NES PERFORMANCE IN NORMAL SUBJECTS (N = 15)

NES Test (measure)	Experimental condition (% N_2O)			
	Training	0%	20%	40%
Finger Tapping (mean no.)	112.60	117.47**	112.00*	109.60*
	(14.79)	(12.53)	(14.52)	(13.20)
CPT Latency (msec)	364.67	354.76*	360.44	384.24*
	(33.82)	(39.90)	(40.91)	(44.94)
Pattern Memory (No. correct)	13.00	12.93	13.07	11.53*
	(1.46)	(1.33)	(1.39)	(1.46)
Handeye Coordination (RMS)	3.24	2.89**	2.85	3.26*
	(0.61)	(0.56)	(0.47)	(0.67)
Symbol Digit (s/symbol)	1.97	1.83**	1.95*	2.22*
	(0.29)	(0.31)	(0.34)	(0.93)
Switching Attention (msec)				
(latency side)	295.02	289.43	311.26	318.18*
	(47.52)	(51.36)	(62.35)	(62.59)
(latency dir)	460.93	465.47	469.33	476.93*
	(49.09)	(70.37)	(74.81)	(125.03)
(latency swside)	496.07	442.67**	514.20*	573.84*
	(108.26)	(102.89)	(148.28)	(209.74)
(latency swdir)	617.07	563.53**	625.87*	663.47*
	(100.49)	(121.15)	(182.68)	(195.64)
Mood Scales				
(Confusion)	1.87	1.68	2.17*	2.72*
	(0.64)	(0.55)	(0.51)	(0.66)
(Tension)	2.05	1.85	1.89	1.88
	(0.75)	(0.51)	(0.61)	(0.55)
(Depression)	1.44	1.33	1.40	1.33
	(0.38)	(0.21)	(0.29)	(0.21)
(Anger)	1.08	1.05	1.13	1.03
	(0.18)	(0.12)	(0.26)	(0.10)
(Fatigue)	2.36	2.31	2.43	2.43
	(0.75)	(0.87)	(0.68)	(0.63)
Serial Digit Learning	3.13	2.47	2.73	4.80
	(3.58)	(2.92)	(1.71)	(3.86)

Data are Means (S.D.).
*Significant drug effect compared to 0% N_2O (Control).
**Significant "practice" effect. Control session compared to training session.

in the Switching Attention task (which requires subjects simply to respond to the location of a large rectangle) produced the lowest mean response time of 4 variable measures. Adding to the perceptual complexity of the stimulus in the second condition, i.e. determining the direction of an inset arrow, raised the response time. The same relationship was also observed when comparing the stimuli used in both switching conditions, that is, the switching-side respose latency was substantially lower than switching-

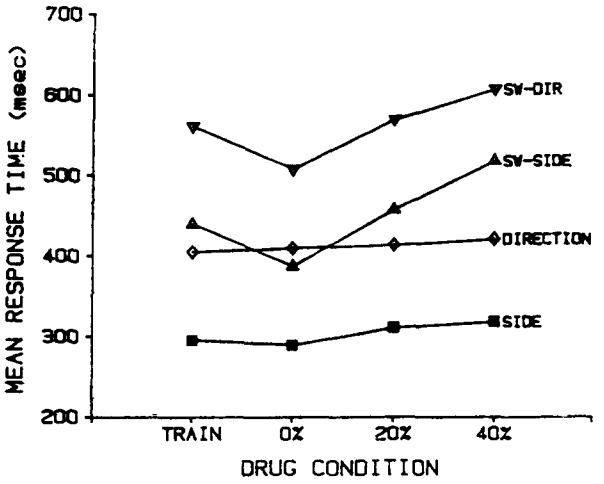

Fig. 1. Switching attention performance under nitrous oxide.

direction latency. In addition, the added cognitive load of switching repeatedly from one response criterion to the other increased response latencies above those for the corresponding simple response conditions.

DISCUSSION

Results of the current study were completely consistent with those found previously [8], and with other investigations of the effects of trace concentrations of nitrous oxide on psychomotor performance [13]. The pattern of scores across sessions was again similar for the behavioral measures, i.e. control condition performance was faster and more accurate than in either of the drug conditions. We found, again consistent with the previous study [8], that there was an improvement in performance when comparing the training session with the control session. Such "practice" or "learning" effects will need to be taken into consideration in designing studies employing repeated measurements with NES.

From a strictly statistical viewpoint, the use of multiple one-tailed *t*-tests in assessing the statistical significance of the current results carries a high risk of Type I errors. However, we could not use a single multivariate test because the number of dependent variables is as great as the number of subjects tested. Correction of the significance level by using the Bonferroni multiple comparison method would not be appropriate because the dependent variables are not independent, and the corrected significance levels would end up being far too conservative. In addition, all of the effects obtained showed changes in the predicted direction even when they were not statistically significant, a result that would be extemely rare by chance

alone. Finally, this study was a replication of a previous one, and replicated experimental results argue against chance findings more persuasively than calculated significance levels.

That results were replicated provides further evidence of the reliability of NES in detecting the effects of a subclinical dose of a known neuroactive agent. This is in keeping with several original design considerations of the NES which focused on implementing a system of standardized neurobehavioral tests to be used in epidemiological studies of occupational neurotoxic hazards.

The current study also demonstrated that the trends towards impairment in more highly integrated CNS functions, e.g. visual memory, reported earlier were significant when a higher dose of nitrous oxide was administered [8]. This result was particularly evident when analyzing performance on the Switching Attention test which places substantial attention and cognitive demands on subjects.

The need for standardized methods in neurobehavioral assessment is essential to ensure that future research has the benefit of comparable results [14]. A suggestion emerging from the current study is that nitrous oxide should be considered as a standard against which to measure the sensitivity of neurobehavioral tests in general. Furthermore, this study demonstrated that the Switching Attention task implemented in NES appears to hold particular promise as a specific neurobehavioral test to be used in evaluating the effects of neurotoxic exposure. Taken together, the evidence reported in this study supports the validity of NES in documenting functional status in the CNS.

ACKNOWLEDGEMENTS

This research was funded in part by a grant from the National Institute of Dental Research (No. DE 07011) and a cooperative agreement with the U.S. Environmental Protection Agency (No. CR 81189601-0). The manuscript was not subjected to either agency's peer and policy review and therefore does not reflect the views of either agency, nor should any official endorsement be inferred.

REFERENCES

1 E.L. Baker, R. Letz, A.T. Fidler, S. Shalat, D.L. Plantamura and M.L. Lyndon, A computer-based neurobehavioral evaluation system for occupational and environmental epidemiology: Methodology and validation studies. Neurobehav. Toxicol. Teratol., 7 (1985) 369.

2 E.L. Baker, R. Letz and A.T. Fidler, A computer-administered neurobehavioral evaluation system for occupational and environmental epidemiology: Rationale, methodology and pilot study results. J. Occup. Med., 27 (1985) 206.

3 H. Hänninen, Twenty-five years of behavioral toxicology within occupational medicine: A personal account. Am. J. Ind. Med., 7 (1985) 19.

4 A. Anastasi, Psychological Testing (5th edn.), New York, Macmillan Publishing Co., 1982.

5 T.L. Cook, M. Smith, J.A. Starkweather, P.M. Winter and E.I. Eger, Behavioral effects of trace and subanesthetic halothane and nitrous oxide in man. Anesthesiology, 49 (1981) 419.
6 B.D. Snyder, R.S. Thomas and Z. Gyorky, Behavioral toxicity of anesthetic gases. Ann. Neurol., 3 (1978) 67.
7 P.A. Moore, Psychomotor impairment due to N_2O exposure. Anesthes. Prog., 30 (1983) 72.
8 B.D. Greenberg, P.A. Moore, R. Letz and E.L. Baker, Computerized assessment of human neurotoxicity: Sensitivity to nitrous oxide exposure. Clin. Pharmacol. Ther., 38 (1985) 656.
9 K. Kortilla, M.M. Ghoneim, L. Jacobs, S.P. Mewaldt and R.C. Petersen, Time course of mental and psychomotor effects of 30 per cent nitrous oxide during inhalation and recovery. Anesthesiology, 54 (1981) 220.
10 D. Eckerman, J. Carroll, D. Foree, C. Gullion, M. Lansman, E. Long, M. Waller and T. Wallsten, An approach to brief field testing for neurotoxicity. Neurobehav. Toxicol. Teratol., 7 (1985) 387.
11 American Psychological Association, Ethical Principles in the Conduct of Research with Human Participants, Washington, DC: Author, 1984.
12 SPSS/PC + for the IBM PC/XT/AT. SPSS, Inc., Chicago, 1986.
13 G. Smith and A.W. Shirley, A review of the effects of trace concentrations of anaesthetics on performance. Br. J. Anaesth., 50 (1978) 701.
14 R. Letz and E.L. Baker, Computer-administered neurobehavioral testing in occupational health. Sem. Occup. Med., 1 (1986) 197.