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Nitrous Oxide and Human State-Dependent Memory

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MEWALDT, S. P., M. M. GHONEIM, W. W. CHOI, K. KORTTILA AND R. C. PETERSEN. *Nitrous oxide and human state-dependent memory*. PHARMACOL BIOCHEM BEHAV 30(1) 83–87, 1988.—State-dependent effects of nitrous oxide on human memory were examined by administering serial and paired-associate learning tasks to subjects receiving 20 and 30% nitrous oxide or placebo. Nitrous oxide in 30% concentration impaired learning of both tasks. In addition, it produced an atypical form of asymmetric state-dependent memory; subjects who learned while receiving placebo and recalled while receiving nitrous oxide displayed the worst recall.

Nitrous oxide State-dependent memory Learning Retrieval

MEMORIES formed in one drug state (e.g., while intoxicated) will be better recalled in the same drug state (e.g., while reintoxicated) than in a different drug state. This phenomenon, called "state-dependent memory," has been demonstrated with a variety of centrally active drugs [4, 6, 11, 14, 19, 20].

The most common design for assessing state-dependent memory in humans is a 2×2 design in which subjects learn material in either a drug or nondrug state and later try to recall the information in either the same or opposite drug state. Overton [15] has outlined problems associated with interpreting results from this design and suggests a more rigorous discrimination learning design which involves testing over many days.

Unfortunately, the discrimination designs employed with animals are often impractical with humans. Repeated changes in drug states would be expensive and likely to produce a high subject drop out rate. In addition, there are the possible problems of drug accumulation and drug tolerance which may occur with repeated administration of many drugs. Such problems can be overcome, however, by using a drug which does not accumulate and allows a rapid transition between drug states so that subjects can receive more than one drug treatment in a single testing session. Korttila *et al.* [12] found that subjects receiving nitrous oxide reach a steady behavioral state after establishing an endtidal concentration of 30% and return to their predrug performance levels about 20 min after ending inhalation. In addition, they found no evidence of the development of tolerance to the drug. Consequently, if nitrous oxide produces state-dependent effects, it could be ideal for studying state-dependent memory in humans.

In addition, because subjects receiving nitrous oxide quickly reach a drug state which remains constant during its inhalation, it is possible to have subjects learn material to a specified criterion. With many drugs one must be concerned with the timing of tests following drug administration. In part because drug level varies with time since administration, experimenters have typically chosen to equate the amount of time subjects spend in learning and have not been able to assure that equal learning has occurred in the drug and nondrug states. With nitrous oxide, on the other hand, because the drug state remains constant throughout inhalation, the number of learning trials can be varied in order to assure more equal learning. The present study employed nitrous oxide to test these advantages in investigating state-dependent memory in humans. The study employed a three stage transfer design. In Stage 1 subjects learned information to criterion while receiving either nitrous oxide or placebo. In the second stage, they were tested for recall of that information in the same or different drug state and they then learned some interfering material in this second drug state. In Stage 3, subjects were returned to their original (Stage 1) drug state and were tested for retention of all the material they had learned.

METHOD

Subjects

The subjects who completed the experiment were 26 male

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TABLE 1
SEQUENCE OF TREATMENTS AND NUMBER OF SUBJECTS FOR EACH GROUP

Group†	Stage 1 N_2O Concentration	Stage 2 N_2O Concentration	Stage 3 N_2O Concentration	Number of Subjects in Final Group	Subjects Dropped for Failing to Reach Criterion*
PPP	0%	0%	0%	8	0
PNP	0%	30%	0%	8	0
NPN	30%	0%	30%	8	6
NNN	30%	30%	30%	7	1
LLL	20%	20%	20%	5	1
LPL	20%	0%	20%	5	0

†P=placebo; N=30% nitrous oxide; L=low dose (20%) nitrous oxide.
*Three other subjects were dropped because of side effects.

and 15 female paid healthy volunteers whose ages ranged from 19 to 30 years. Most were college students with at least one year credit. Subjects were to get a good night's sleep and to abstain from marijuana and alcohol for at least 24 hours before their test session. They were also asked to skip the meal immediately preceding their session. Subjects were tested individually beginning at 8:00 a.m. or 1:00 p.m.

Three subjects did not finish the study and were excluded; one female became nauseous and tried to vomit and two males became excited, uncomfortable, and refused to proceed. All three were inhaling 30% nitrous oxide. Data from eight volunteers who received nitrous oxide during Stage 1 (seven were inhaling 30% and one 20%) were also excluded because they were unable to attain the required criteria of learning (see below).

Design and Procedure

Subjects inhaled either 30% nitrous oxide in oxygen, 20% nitrous oxide in oxygen or placebo (100% oxygen) in a double-blind procedure. Neither the subjects nor the assistants who adminstered the test knew which gas mixture was inhaled.

Subjects performed all tests while sitting on a bed at a 50 degree angle with a writing board in front of them. Prior to drug administration they were given instructions and practice on each task. Gases were administered through a semiclosed circuit from an anesthesia machine situated behind the subject. Attached to the mouthpiece was a Rahn endtidal sampler which collected endexpired air. The concentration of nitrous oxide in endexpired gases was monitored by an infrared meter (Beckman medical gas analyzer LB-2). (Under normal circumstances, alveolar gas anesthetic concentrations are approximately equal to arterial and brain concentrations.) A clip was fastened to the nose to prevent contamination of the administered gases by atmospheric air.

Subjects were assigned randomly to one of six treatment groups. Each group was tested in 3 stages, 0.5 hours apart. Four of the groups received either 30% nitrous oxide or placebo during Stages 1 and 2 in order to examine the four possible combinations of drug states during these stages (nitrous oxide-placebo, placebo-nitrous oxide, nitrous oxide-nitrous oxide, and placebo-placebo). The other two groups received 20% nitrous oxide during Stage 1; then one group received 20% nitrous oxide and the other placebo during Stage 2. During Stage 3, subjects in all groups received the same drug treatment they had been given during Stage 1. The sequence of treatments and number of subjects for each group is summarized in Table 1.

The following two tests were administered:

Serial learning task. During Stage 1 subjects were presented with a list of 13 nouns each having imagery and concreteness values greater than 6 [16]. The words were presented by a slide projector at a rate of one word every 5 sec. After the last word was presented subjects recalled the words by writing them on a sheet of paper containing 13 lines. They were to write each word on the line corresponding to its position in the list. After 1 minute, the sheet was scored. If the whole list was recalled in order, the test was completed and the subject was presented with the second task. Otherwise, the whole list was presented again after 20 sec after the end of the recall period. The procedure was repeated until the subject learned the list or failed to learn it after 20 trials. During Stages 2 and 3, subjects were asked to recall the list in order.

Paired-associate task. During Stage 1 subjects were presented with an eight-item paired-associate list. The pairs consisted of an adjective paired with a two digit number, e.g., Bland-91. Each pair was presented on a slide for 5 sec. After the last pair had been presented, subjects were given a sheet of paper listing the adjectives in a different order from that in the slide presentation. They were given 1 min to write the number associated with each adjective. If the whole list was recalled correctly the task was terminated. Otherwise, the procedure was repeated until learning was achieved or the subject failed during 20 trials. A series of four lists consisting of different random orderings of the pairs and corresponding different answer sheets were used, after which the sequence was repeated.

Subjects were tested during the second stage for recall of the pairs they had learned in Stage 1. They were then required to learn another list of adjective-number pairs in which the adjective was the same as in the first list, but which were associated with different numbers, e.g., Bland-65. Subjects were required to learn the second list to the criterion of one perfect recall. During Stage 3, subjects were asked to recall the numbers from each list which had been associated with each adjective. They were then asked to relearn the first list to criterion.

RESULTS

The data from each test stage were analyzed separately

TABLE 2

DRUG EFFECTS ON LEARNING (MEAN NUMBER OF TRIALS TO CRITERION AND CORRESPONDING STANDARD ERRORS)

Group‡	Serial List Stage 1	Paired Associate Learning, Stage 1	Paired Associate Learning (New List), Stage 2
PPP	3.5 ± 0.4	4.0 ± 0.7	4.1 ± 0.7
PNP	3.6 ± 0.7	3.0 ± 0.6	11.9 ± 2.2†
NPN	9.5 ± 1.5*	9.3 ± 1.7†	3.9 ± 0.5
NNN	8.9 ± 0.9*	4.4 ± 1.2	6.9 ± 1.5
LLL	5.2 ± 1.0	3.4 ± 0.7	5.8 ± 1.2
LPL	5.6 ± 1.8	4.8 ± 1.8	3.0 ± 0.7

*Differs significantly from placebo and 20% nitrous oxide groups.
†Differs significantly from all other groups.
‡P=placebo; N=30% nitrous oxide; L=low dose (20%) nitrous oxide.

using a two-way multivariate analysis of variance with drug group and sex as between-subject factors. However, as there were no interactions between any drug condition and sex, sex was dropped as a factor. In addition, a separate analysis was performed comparing recall in Stages 2 and 3. Univariate follow-up analyses reported below were calculated if a multivariate contrast was significant at the $p<0.05$ level.

Analyses were computed for only those subjects who completed the experiment. This may have introduced a bias in the data in that the slowest learners in the nitrous oxide groups were lost from the experiment. The effect of this attrition is to underestimate the size of the drug impairment. However, it should not affect the direction of the reported findings.

Learning

Before examining the state-dependent effects of nitrous oxide, it is useful to examine the drug's effects on learning. All subjects were required to reach the same criterion of learning in order to minimize confounding of drug effects on recall (used to assess state-dependency) with drug effects on learning. Learning was evaluated by determining the number of trials needed to reach the specified criterion for each task.

Serial Learning. As Table 2 reveals nitrous oxide had a devastating effect on serial learning for subjects receiving the higher concentration of drug. Subjects receiving 30% nitrous oxide during Stage 1 learned almost three times more slowly than the placebo subjects, $p<0.001$. Subjects receiving 30% nitrous oxide also learned more slowly than the 20% nitrous oxide groups, $p<0.01$. However, these latter two groups did not differ significantly from placebo, $p>0.05$. There were no significant differences between any of the pairs of groups which received the same drug treatment.

Paired associate learning. Paired-associate learning in both Stages 1 and 2 also revealed marked impairment due to nitrous oxide (see Table 2). However, the adverse effects of 30% nitrous oxide were milder in the group that received this concentration in both the first and second stages than in the two groups that received it only during the first or second stage. Since this difference was already present in Stage 1, before these subjects were treated differently, this result seems more likely to reflect random differences among groups than true drug effects. No learning differences between the two groups receiving placebo or between the two groups receiving 20% nitrous oxide were found.

TABLE 3

DRUG EFFECTS ON RECALL (MEANS AND CORRESPONDING STANDARD ERRORS)

Task	Group‡	Stage 2 Recall	Stage 3 Recall
Recall of Information Learned During Stage 1			
Serial List Task	PPP	10.9 ± 0.7	10.4 ± 1.0
	PNP	7.9 ± 1.4*	12.3 ± 0.3†
	NPN	11.6 ± 0.7	10.4 ± 0.8
	NNN	11.6 ± 0.3	10.4 ± 1.0
	LLL	12.4 ± 0.2	11.6 ± 0.8
	LPL	12.0 ± 0.3	11.0 ± 1.0
Paired Associate Task	PPP	6.6 ± 0.6	3.8 ± 0.8
	PNP	7.1 ± 0.3	3.8 ± 0.9
	NPN	6.9 ± 0.5	2.9 ± 0.6
	NNN	6.6 ± 0.5	3.7 ± 0.7
	LLL	7.6 ± 0.4	2.8 ± 0.9
	LPL	6.8 ± 0.5	5.0 ± 0.8
Recall of Information Learned During Stage 2			
Paired Associate Task (New List)	PPP		6.0 ± 0.5
	PNP		6.8 ± 0.5
	NPN		4.3 ± 0.6
	NNN		6.3 ± 0.5
	LLL		5.2 ± 1.2
	LPL		7.2 ± 0.5

*Differs significantly from all other groups in Stage 2.
†Only group to recall significantly more of the serial list in Stage 3 than in Stage 2.
‡P=placebo; N=30% nitrous oxide; L=low dose (20%) nitrous oxide.

Recall

Stage 2. In Stage 2 subjects were first asked to recall the serial list. The results were scored by a free scoring method in which a word was scored as correct if it belonged to the list regardless of its recall position. A strict serial position and relative scoring procedure produced results similar to those reported below. The analyses indicated that the only significant differences involved the group which originally learned the list while receiving a placebo and then recalled it while receiving 30% nitrous oxide (Group PNP). This group recalled significantly fewer words than any other group, $p<0.01$, in this case (see Table 3). Analysis of Stage 2 recall of the paired-associate list revealed no significant differences among the groups, $p>0.5$.

Stage 3. In Stage 3 subjects were returned to the drug state they had been assigned to in Stage 1 and attempted to recall all material learned earlier. The multivariate analysis revealed no significant differences among the groups.

Stage 2 vs. Stage 3.A critical question in testing for state-dependent memory in the present experiment deals with how recall of material learned in Stage 1 compares in Stages 2 and 3. Half the subjects were switched to a new drug state in Stage 2. They were then returned to their original drug state in Stage 3. If these subjects displayed better recall of the original material during Stage 3 than during Stage 2 one would have fairly strong evidence for state-dependent memory.

Analysis of the serial recall data revealed a strong Drug Group × Stage interaction, $p<0.001$. Follow-up analyses confirm a pattern which is apparent in Table 3. For all groups except group PNP, recall was slightly lower during Stage 3 than Stage 2, probably reflecting memory loss due to the passage of time. For Group PNP, on the other hand, the pattern was reversed. Mean recall of the serial list in Stage 2, when the drug state was different from that of original learning, was 7.9 words compared with 12.3 in Stage 3, when subjects were returned to their original drug state. In contrast, analysis of the paired-associate data indicated no interaction between test stage and drug condition. However, recall of the first list was much worse in Stage 3 than in Stage 2, $p<0.001$, probably due to forgetting and the influence of the interfering list which had been learned after the recall test in Stage 2.

A final set of analyses was performed to determine the effects of item difficulty on state-dependent memory. In the serial learning task it was possible to identify words which were responded to correctly on almost every trial and those which were only learned on the final learning trials. Using the procedure of dividing the items into "easy" and "difficult" halves based on number of correct responses per item in learning, analyses as reported above were calculated adding item difficulty as a within-subject factor. Similarly, for the paired-associate task we examined the number of correct responses on the first two learning trials of each stage as a possibly more sensitive dependent variable. None of these analyses revealed significant effects different from those reported above. However, the direction of results suggest that in future research one might want to look further at whether state-dependent effects are stronger on more poorly learned material.

DISCUSSION

The present study was designed to examine the state-dependent memory effects of nitrous oxide. Support for the existence of state-dependent recall comes from the performance of Group PNP on the serial learning task. Subjects in this group, who had learned the list in Stage 1 while receiving placebo and then recalled it in Stage 2 while receiving nitrous oxide, recalled fewer words in Stage 2 than any other group. In addition, the deficit displayed by these subjects in Stage 2 disappeared in Stage 3 when they were returned to placebo, their drug condition during learning. In other words, subjects who learned material on placebo only had difficulty retrieving it while receiving nitrous oxide. That this is a state-dependent memory effect and not just a drug-induced retrieval deficit is supported by the fact that subjects who had originally learned the material in Stage 1 while receiving nitrous oxide had no difficulty in retrieving it in Stage 2 while once again receiving nitrous oxide (Group NNN).

State-dependent learning is designated "symmetric" if both directions of change in drug state from learning to recall (i.e., drug to placebo or placebo to drug) produce deficits relative to no change in drug state (i.e., drug-drug or placebo-placebo). When one direction of change in drug state produces deficits but the other does not, as in the present study, state-dependent learning is designated "asymmetric." Asymmetric state-dependency has previously been reported with a number of drugs [4, 11, 20]. However it has usually been the reverse of the present asymmetry; that is, subjects who learn material while under the influence of a drug, later show better recall of that material after receiving the drug again than after receiving placebo. On the other hand, subjects who learn following placebo administration are able to recall equally well in either drug state. This asymmetry has typically been attributed to the fact that learning in the drug state is often much poorer than in the placebo state, and therefore similarity of the learning and recall conditions may be much more important in enabling subjects to recall these weaker memory traces [15]. The reversal of the asymmetry in the present study may reflect at least two factors. First, unlike previous state-dependent memory studies, we required subjects to attain a specified criterion of learning, so there should have been little difference in the degree of learning by subjects in the various groups. Second, studies demonstrating the typical asymmetry pattern may reflect the effects of drugs which impair learning but not retrieval. Unlike many other drugs which affect memory (e.g., alcohol [1], diazepam [13], marijuana [5], scopolamine [8]) nitrous oxide appears to impair retrieval [9].

The present results can be explained if one assumes that nitrous oxide impairs memory by interfering with retrieval from long-term memory, but that with practice subjects can learn to compensate for this debilitating effect. By interfering with retrieval nitrous oxide should impede learning, as observed here. However, once drugged subjects had successfully learned a list, they would have obviously learned to retrieve those items despite the handicap. Subsequently, they might be able to recall the items in either the placebo or drug state. On the other hand, subjects who had originally learned the material while receiving placebo would have no experience in overcoming the retrieval deficit produced by the drug. Therefore, when they were tested for recall while receiving nitrous oxide they should display relatively poor retention compared to all other groups, thereby producing the asymmetric state-dependent memory pattern of the present experiment.

If this account is accurate it remains to be determined whether learning to retrieve information while receiving nitrous oxide involves a process specific to the particular associations acquired or whether the learning effect generalizes to other associations. That in Stage 2 the group which had experience with nitrous oxide learned the new paired-associate list faster than the group receiving it for the first time supports the latter interpretation, but as mentioned earlier these data are not clear. Future studies should examine this and the more general questions of the importance of degree of learning and retrieval deficits in state-dependent memory.

In contrast to the serial learning task, the paired-associate task proved insensitive for detecting state-dependent memory. This was true even though we employed measures which should have been more sensitive than traditional recall, i.e., transfer and relearning. The results support suggestions that paired-associate tasks do not typically reveal state-dependent memory [6,7]. Apparently the strong retrieval cue provided by the stimulus term of a paired-associate overrides the effects of state-dependency. We agree with suggestions [6,7] that in testing for state-dependent memory the less structured the task, i.e., the fewer retrieval cues present, the better. A free recall task should therefore be among the most sensitive tasks.

The 30% concentration of nitrous oxide produced a powerful debilitating effect on memory, consistent with previous findings [2, 3, 9, 12, 19]. The higher concentration of drug was found to impede acquisition of both the serial and paired-

associate lists. Even when the slowest learners were removed from the nitrous oxide groups, acquisition of the serial list took almost three times longer for the drugged subjects. When one considers that eight subjects were dropped from the nitrous oxide groups for failure to learn the serial or paired-associate list in 20 trials the devastating effects of the drug are even more apparent. One dropped subject in the 30% NPN group failed to learn the eight-item paired-associate list in 40 trials while under the influence of the drug (Stage 1 and Stage 3). However he learned the competing paired-associate list in Stage 2 in seven trials while receiving placebo.

In contrast to the strong impairment produced by 30% nitrous oxide, the 20% concentration did not significantly affect performance. While some investigators have found a small effect on memory at a 20% concentration [3], others have not [10]. Apparently nitrous oxide exhibits a very steep dose-effect curve which is typical for anesthetic drugs [17]. Twenty percent nitrous oxide may not be an effective dose for studying memory or for use clinically to produce amnesia (see also Smith and Shirley [18]).

In summary, the present results suggest that a 30% concentration of nitrous oxide exerts a powerful debilitating effect on learning. This effect may be due to its influence on retrieval. In addition, it appears that nitrous oxide produces an asymmetrical state-dependent memory effect which differs from the asymmetry typically observed with other drugs. The results suggest that nitrous oxide may be a very useful drug for further explorations of human state-dependent memory, especially with regard to the effects of degree of learning and retrieval versus acquisition deficits.

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