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Diazepam and Learning: Assessment of Acquisition Deficits

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HINRICHS, J. V., S. P. MEWALDT, M. M. GHONEIM AND J. L. BERIE. *Diazepam and learning: Assessment of acquisition deficits.* PHARMAC. BIOCHEM. BEHAV. 17(1) 165–170, 1982.—Subjects treated with diazepam (0.3 mg/kg) showed significant reductions in performance on multiple-trial free recall, paired-associate learning, and serial learning tasks compared to placebo control subjects. The free recall task showed the largest drug effect with diazepam subjects failing in six acquisition trials to attain the level of performance achieved by placebo subjects on the first trial. Serial position curves in the serial learning task were changed by the diazepam treatment from their usual skewed form to symmetrical functions. Results indicate that diazepam exerts its greatest memory influence on the acquisition of new information.

Diazepam Human memory Free recall Paired-associate learning Serial learning Serial position effects Cognition

IN the past decade many studies have demonstrated that clinical administration of diazepam (Valium) has profound effects on human cognition, especially memory functioning. Although testing of memory performance in diazepam-treated subjects has most often used single-list presentation with immediate or almost immediate recall of the presented material, several studies suggest that the strongest influence of diazepam occurs in the acquisition or storage component of learning and memory [1,2]. However, few investigations of the effects of diazepam on learning beyond a single acquisition trial are available. If acquisition is most strongly affected by diazepam, it would be expected that learning paradigms employing several acquisition trials would be even more sensitive to the effects of diazepam than single-trial acquisition procedures.

One study [1] provides some support for this contention. On a "triple associate" learning task, control subjects essentially mastered the task in four trials while diazepam subjects attained only 50% success in the same number of attempts. Although the difference between the two groups was slight on the first recall attempt, performance diverged over successive trials until the control subjects approached asymptotic performance. Unfortunately, the "triple associate" task is not a common one and does not permit easy generalization to more familiar learning situations.

The primary goal of the present study was to examine the effects of diazepam compared to placebo controls on three different learning tasks. The three tasks chosen for investigation represent the major paradigms for studying human learning (serial learning, paired associate learning and multiple trial free recall) and each allows the evaluation of a different aspect of learning. The serial learning procedure requires subjects to acquire order as well as item information emphasizing the response integration component of learning. In contrast, the paired associate task permits the formation of separate stimulus-response associations with few demands for complex ordering or organization. The multiple-trial free recall task extends the usual memory test of immediately recalling a word list after a single presentation; the same words are presented in a different order over several trials, each followed by a new recall attempt. Delayed recall of the words was included to examine the effect of diazepam on retention as a function of the degree of prior learning. In addition, the effect of diazepam on the three tasks can be compared in order to determine the relative success of each task in discriminating between drugged and placebo subjects.

METHOD

To minimize interference among the three tasks the stimulus materials for each were chosen to be maximally different. Because subjects were tested in groups of five to seven individuals, all learning tests were conducted for a fixed number of trials rather than to a performance criterion.

Subjects

Twenty-four healthy paid volunteers (12 male and 12 female) were recruited through a local newspaper advertisement to serve as subjects. Their ages ranged from 18–30

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TABLE 1
SCHEDULE OF TASKS

Relative Time (min)	Tasks
−35	General Instructions
−20	Mood Evaluation 1
−5	Practice Multiple-trial Free Recall
0	Drug Administration
+5	Number Learning 1
+15	Practice Paired Associate Learning
+30	Mood Evaluation 2
+45	Multiple-trial Free Recall
+70	Number Learning 2*
+78	Paired Associate Learning*
+90	Delayed Free Recall
+100	Mood Evaluation 3

*The order of Number Learning 2 and Paired Associate Learning was counterbalanced. Two sessions had Number Learning first, followed by Paired Associate Learning; two sessions reversed the order.

years. At an initial interview, a detailed medical history was obtained and subjects were informed of the general nature of the drugs and tests to be employed in the study. Twelve subjects were randomly assigned to each of the two treatment conditions, diazepam or placebo, with six males and six females in each group. In order to avoid bias, the assigned treatment conditions were not revealed to subjects or to the experimenter conducting the test session until the end of the study.

Treatments

Diazepam or placebo was administered orally in identical gelatin capsules. All subjects received three capsules containing either diazepam or placebo. The dosage of diazepam was weight dependent, approximately 0.3 mg/kg. Combinations of three different capsules (2 mg, 5 mg, 10 mg) were used to administer the calculated dosage; in all cases the dose received was within 10% of the nominal value.

Procedure

Four 3-hr sessions were required to complete the study; two sessions were conducted in the morning between 9:00 to 12:00 noon and two sessions were conducted in the afternoon from 1:00 p. m. to 4:00 p.m. Approximately one-half of the subjects in each test group were drawn from each of the two treatment conditions. Subjects were advised to get a good night's sleep before the testing session and to abstain from food and beverages at least 4 hr prior to the testing session. (For those subjects participating in a morning session, subjects were required to abstain from breakfast while those subjects participating in an afternoon session were required to abstain from lunch.)

For each of the four testing sessions, the tests employed and their order of administration was constant with the exception of counterbalancing two learning tasks (see Table 1). Prior to administration of the drug and for the first 20 min afterwards, subjects practiced all tasks in order to insure an understanding of the procedures and to determine baseline performance on the tasks used in the study. Forty-five min after drug or placebo was administered, subjects repeated these same tasks as described in Table 1.

Mood Evaluation

Subjects rated their moods and feelings on ten subjective scales derived from Norris [4]. The ends of each of the seven-point scales were marked by adjectives representing the extremes of the dimension being rated, e.g., alert-drowsy. The positive end of the scale appeared on the right hand side of the page half of the time and on the left side half of the time. Subjects were asked to rate their mood on each dimension by circling the number on the scale that best represented their feeling on that dimension at the specific time of testing within the session. The mood evaluation was untimed and repeated three times over the course of the session (see Table 1). Subjects also completed a number of paper-and-pencil cognitive tasks shortly after each mood evaluation requiring about 10 min; these tasks were not relevant to the assessment of learning and memory and are not reported here.

Multiple-Trial Free Recall

In the practice multiple-trial free recall task, subjects were presented with a list of 16 words shown as slides. The practice list was composed of words of intermediate value on imagery and meaningfulness scales [5]. The slides were presented at a rate of 2 sec per word. Immediately after the list was presented, subjects were given 2 min to write in any order as many of the words as they could remember. Following the recall interval, the same list of words was presented again; however, this time the list of words was presented in a different random order. Again, after this second repetition, subjects were asked to write as many of the words as they could remember in any order.

Subjects viewed a new list of 32 words in the post-drug multiple-trial free recall task. These words were selected from the same source [5] and were classified as "easy" words, defined by ratings of imagery and concreteness greater than 5.0, ratings of meaningfulness greater than 5.97 and frequencies greater than 49 per million [6]. The list was repeated six times in one of four different random orders. Following each presentation, subjects were permitted 2 min to recall as many of the words as possible from the list in any order.

Finally, after the six repetitions of the 32-word list, and with an additional interval of approximately 20 min during which subjects completed a number learning sequence and a paired associate learning task (see Table 1), subjects were again asked to write in any order as many of the words as they could remember from the two lists (both practice and experimental) presented earlier in the session. Five min were permitted for this delayed free recall task.

Paired Associate Learning

In the practice paired-associate learning task, subjects were presented with six shape-number pairs. The items were presented as two slides appearing on a projection screen side by side at a rate of one pair every 2 sec. The shapes were line drawings of common geometric forms such as a circle or square. The numbers were three-digit numerals composed of the digits one to nine and were generated with the constraints that: (a) each number consisted of three different

digits, (b) each digit was used no more than twice in the list, and (c) no digit could appear more than once in any of the three possible ordered positions. After all six shape-number pairs were presented, subjects were required to recall the three-digit numbers given the shapes as cues. During the recall subjects were given an answer sheet containing each shape and were allotted 30 sec to recall the associated number. The procedure was repeated for two additional trials. On each trial a different random order of the items was employed in both the study and test phases.

In the experimental, post-drug, paired-associate learning task, subjects were presented with 12 animal picture-trigram (CCC's) pairs prepared as slides. The animal pictures were line drawings of familiar animals, e.g., elephant, dog, horse. The CCC items were consonant trigrams with 8% association values [7]. As described for the practice paired-associate learning task, subjects were presented with slides of the 12 animal picture-CCC pairs at a rate of one pair every 2 sec and following completion of the list were permitted 1 min to recall as many of the CCC's as they could using the animal pictures on the response sheet as cues. The same twelve pairs were presented six times in three different random orders. The dependent measure for both paired-associate learning tasks was the number of digits or letters correctly paired with the stimulus on each trial.

Serial Number Learning

Subjects were presented with two 15-digit serial lists on a cassette recorder. The numbers within each list were generated randomly with no constraints. Each sequence of numbers was presented six times at a rate of one digit per sec. Immediately after each presentation, subjects were given 30 sec to write as many of the digits as they could remember in their order of presentation on a sheet containing a row of 15 boxes. Subjects were not required to produce the digits in any order, but had to place each digit in its corresponding box. The number learning data was scored in terms of the number of digits correctly recalled in each of the 15 serial positions.

RESULTS

Before the three learning tasks are compared, results of each task are described separately. In addition, the subjects' impressions of their moods and feelings during the test session are examined. The analyses of variance reported below all used drug condition (diazepam vs placebo) and practice (trial number) as factors and included other factors as appropriate for each task. A relatively conservative alpha level of .01 was adopted for reporting statistical significance.

Mood Evaluation

The subjects' ratings of their feelings during the course of the session provide information about which aspects of behavior were subjectively most influenced by the administration of diazepam. As would be expected, diazepam subjects reported significant increases in drowsiness, clumsiness, and dreaminess (lower attention) compared to placebo subjects; subjects in both groups rated themselves as more bored as the session progressed. More surprising, diazepam-treated subjects did not report any change in scales measuring subjective impressions of mental ability: fuzzy vs clear-headed, mentally slow vs quick-witted, and incompetent vs capable.

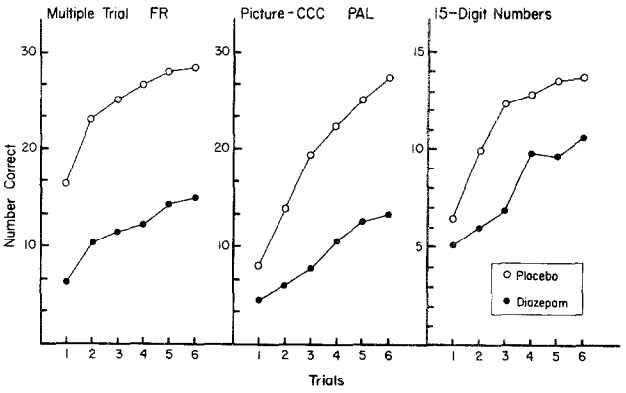

FIG. 1. Acquisition performance in three learning tasks.

Multiple Trial Free Recall

Subjects in the diazepam and placebo groups did not differ in performance on the two practice list trials administered prior to the drug. On the experimental list, however, the performance of diazepam subjects was markedly inferior to the placebo controls, $F(1,20)=61.75$. As shown in the left-hand panel of Fig. 1, even after six acquisition trials the drugged subjects did not attain the level of performance achieved by the control subjects on their first recall attempt. The drug by trial interaction was marginally significant by the conservative criterion used, $F(5,100)=2.48$, $0.01 < p<0.05$. Performance by the placebo subjects over the last few trials was constrained by the recall ceiling of 32 words reached by many subjects.

On the delayed free recall test (approximately 45 min and two interpolated tasks later) subjects were instructed to recall items from both the experimental (second) list and the practice (first) list. As would be expected, overall performance was better on the experimental list (19.75 words; 61.7% correct) than on the practice list (5.04 words; 31.5% correct) which had both fewer acquisition trials and a longer retention interval before the delayed test. However, an unexpected interaction was observed between recall list and drug condition. Placebo subjects maintained their superiority in the recall of the experimental list items, primarily reflecting their better performance at the conclusion of the six acquisition trials. Surprisingly, the diazepam subjects recalled more items from the practice list than the placebo subjects (5.83 vs 4.25 words). Although the score reversal on practice item recall was not significant, $t(22)=1.14$, $p>0.05$, the interaction of drug condition by list was, $F(1,22)=22.19$.

Performance on the delayed free recall task can be adjusted for acquisition performance by examining delayed retention as a function of the number of correct recalls of each word during the six learning trials. As shown in Table 2, most of the 384 observations (12 subjects $\times$ 32 words) for the placebo group were distributed across the upper range of performance (4–6 correct learning trials) and most of the diazepam responses were in the lower range, reflecting the acquisition differences described above. However, when delayed free recall was examined conditionalized on acquisition performance, both groups showed very similar trends. As would be expected, as learning performance increased so did delayed free recall ($r=.95$ in both groups); the relationship is virtually identical at the lower end of performance, diverging somewhat at the upper end.

TABLE 2

DELAYED FREE RECALL AS A FUNCTION OF THE NUMBER OF TRIALS A WORD WAS CORRECTLY RECALLED DURING ACQUISITION OF THE MULTIPLE-TRIAL FREE RECALL TASK

Number of Correct Responses	Group			
	Diazepam		Placebo	
	N*	Pr(Cor)*	N	Pr(Cor)
0	106	.066	2	.000
1	63	.111	9	.111
2	60	.383	22	.450
3	62	.500	48	.792
4	31	.774	71	.873
5	42	.810	109	.927
6	20	.750	123	.968

*N=Number of instances observed.
Pr(Cor)=Proportion of instances correctly recalled.

Paired Associate Learning

In order to evaluate partial learning, performance was scored in terms of the number of individual letters correctly paired with the stimulus, rather than requiring all three letters in the trigram to be recalled for a correct response to be scored. As in the other tasks, placebo and diazepam subjects did not differ over three trials in their performance on the practice paired associate list (geometric forms paired with digit trigrams). Performance was so similar that on each trial the difference between the two groups was less than the observed standard error of the mean (1.20). On the second list, under the influence of diazepam, subjects' acquisition of the picture-trigram paired-associate list was markedly inferior to the acquisition of placebo control subjects, $F(1,20)=27.20$. Furthermore, a significant interaction was observed between drug condition and the trial, $F(5,100)=7.64$, such that over the six test trials the difference between the two groups became progressively larger (see Fig. 1, middle panel).

Serial Number Learning

Because the same task was used in pre-drug practice as in the post-drug experimental comparison, a time-of-testing factor was added to the other analysis of variance factors. Serial position was also incorporated into the analysis by dividing the 15-digit recall protocols into five blocks of three digits each. The within-subject variance yielded an estimate of the standard error of the difference among means of the three-digit recall scores of 0.119, equivalent to about 0.6 items over the 15-item sequence, or approximately 4% of overall performance.

Analysis of variance of overall learning performance revealed a significant interaction between drug condition and time of testing, $F(1,20)=26.74$. When subjects learn the 15-digit number sequence 5 min after drug administration and before the diazepam could become effective there was no difference in performance between diazepam and placebo groups. Over the six-trial sequence, placebo subjects were correct on 72.3% of their responses and diazepam subjects were correct on 78.5% of their responses. Approximately an hour later on the second test, the comparable overall performance for the placebo subjects was 76.3% and for the diazepam subjects 53.6%. Only the reduced performance for the drugged subjects at the time of the second test was significantly different from the other three performance scores. The right panel of Fig. 1 shows the trial by trial changes in second list performance for the placebo and diazepam subjects. From a difference of approximately 1.5 items on the first recall attempt the difference increased to more than 5 items on Trial 3 before narrowing as the placebo group asymptotically approached maximum performance.

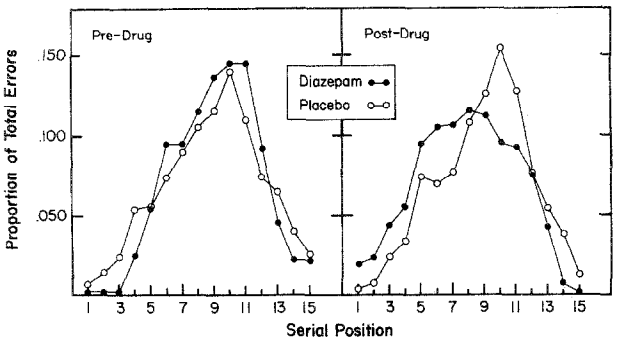

FIG. 2. Relative proportion of errors across serial position.

There was also a significant block serial position effect, $F(4,80)=63.97$, and a marginally significant interaction between condition, time, and serial position, $F(4,400)=3.04$, $p<0.025$. Inspection of the serial position performance curves reveals not only a significant decrease in correct performance for diazepam subjects under the influence of the drug but also a relative shift in performance so that the serial position curve was more symmetrical in the diazepam-treated subjects. The shift in relative performance is even more apparent when performance is plotted in terms of proportion of total errors for each group [3], a transformation which eliminates differences in relative performance and usually reduces differences in serial position curves. As shown in Fig. 2, the two groups of subjects did not differ in relative error performance before diazepam took effect, but the diazepam-treated subjects exhibited almost perfectly symmetrical serial position curves in the post-drug test.

Task Comparison

Given the similar pattern of results across all three learning tasks, it is not surprising to observe relatively high and significant correlations between tasks and across individual subjects. Combining drug and placebo conditions, using the data from all 24 subjects (a comparison that capitalizes on the experimental manipulation as well as intrasubject consistencies), the highest observed correlation was between mean performance on the multiple-trial free recall task and the paired associate learning task, r=.80. The corresponding correlation between number learning and paired associate learning was .70 and between free recall and number learning was .47. As would be expected, the correlations were less when the comparisons were restricted to subjects within each condition, but remained high in most cases. In the diazepam group the observed correlations reported in the same order as above were .41, .59, and .19. Within the placebo control group the same values were .73, .70, and .52.

Of the three tasks, the multiple-trial free recall performance was most successful in discriminating between groups. Only one diazepam-treated subject was able to exceed the number of words recalled by the poorest performing placebo subject over the six acquisition trials. The largest number of words recalled in six trials by a subject in the diazepam group was 129 (the next largest was 94; the mean was 69). The fewest words recalled by a placebo subject was 113 (the next fewest was 121; the mean was 148). Hence, setting a criterion of correctly recalling about one-half of the words presented over the six trials (equivalent to 96 words summed over trials) would have misidentified only one diazepam subject as a placebo subject. Similar comparisons using the number learning and paired associate tasks misidentified approximatley one-third of the subjects in both cases. Using multiple criteria across the three tasks did not improve identification beyond that provided by free recall performance.

The serial number learning task permits a pre-post comparison of performance within individual subjects because the same task (but with different digit sequences) was used before and after drug administration. In the placebo group, the subjects averaged about a one-half item improvement in recall scores on each post-drug trial compared to pre-drug acquisition. By comparison, diazepam-treated subjects showed about a four-item decline in the number of digits recalled on each post-drug trial. With only one exception, subjects whose average post-drug performance on each trial was 1.5 items below pre-drug recall scores were administered diazepam. (One placebo subject declined an average of three items per trial on the post-drug test.) Therefore, although between-subject variability was sufficiently high to preclude use of absolute number learning performance as an index of drug impairment, the within-subject changes from pre-drug to post-drug number learning almost perfectly identified subjects receiving diazepam.

DISCUSSION

All three learning tasks were remarkably consistent in both the pattern and the magnitude of the differences between the placebo and the diazepam-treated subjects. These similarities can be described in several ways: First, all tasks showed a difference on the very first acquisition trial that increased over at least the next two trials. The point of maximum difference was generally achieved on the third trial primarily because control subjects beyond that point were exhibiting decreasing gains in performance as they approached maximum performance levels (except on the paired associate task). Second, the level of performance achieved after six trials for the drug subjects was approximately equivalent to that obtained on the first or second trial for the placebo subjects. Third, if one measures the rate of learning by the amount of increase from the first to the sixth trial, the rate of gain was 50 to 200% faster in the control subjects than in the drug subjects. If performance is considered only over the first three trials, the discrepancy between the two groups was even greater.

Although the similarities in performance are the most striking, some differences among the tasks in the pattern of learning were observed. Each of the three tasks manifested a different pattern of trial by trial changes in the placebo and drug groups. The multiple-trial free recall task showed almost the maximum effect on the first trial with the difference in performance between the two groups increasing only slightly over the succeeding five acquisition attempts. The difference between the two groups increased steadily for the paired associate learining tasks because initial performance levels were low in both groups and neither was able to approach maximum performance in the allotted six trials. Finally, the number learning task shows an increasing, then decreasing, difference in performance between the two groups as the placebo group rapidly approached maximum performance. The minor differences in the pattern of results can be explained by the different demands that the three tasks place upon short-term memory. First-trial performance, at least in the drug subjects, can primarily be attributed to short-term memory recall. Additional gains in performance require more enduring associations to be formed and retrieved. The diazepam-treated subjects were at a great disadvantage in making these additional gains, typically adding only one to two items on each acquisition trial while placebo control subjects were adding five to six items on each trial until they began to approach maximum performance levels.

Differences in delayed free recall of the word lists can be most easily attributed to differences in original learning. When adjusted for number of correct recalls during acquisition, later recall was quite similar for both diazepam and placebo subjects. Recall of items learned before drug administration did not differ significantly.

An unexpected and surprising result was the effect of diazepam on the shape of the serial position curves. One of the most stable characteristics of human learning is the skewed serial position function observed in serial list learning. Over many independent variables and wide variation in accuracy, when adjusted for level of performance, the relative serial position curve is virtually invariant [3]. The shift to an almost perfectly symmetrical serial position curve suggests a strong intrusion into the normal process of learning a serial list. Even the slight discrepancy from symmetry—better performance on the last two items than on the first two—is in the opposite direction of the usual asymmetrical serial position function and may provide a clue to the reason for the shift. If the normal acquisition process is impaired by diazepam, subjects under its influence may be forced to rely more on short-term memory to aid performance, producing the observed increase in recall of the last few items in the list, and reducing the usual performance skew.

Finally, it must be noted that diazepam subjects' performance was in marked contrast to their own subjective evaluation of their behavior. Although drugged subjects reported greater physical clumsiness and reduced attention, demonstrating their awareness of some of the effects of diazepam, they reported no significant change in mental abilities. Even after performing three learning tasks including one (number learning) which was identical to a task performed in the pre-drug state, diazepam subjects rated their mental abilities as no different than before the drug was administered and no lower than the impressions of the placebo subjects. Such results suggest that diazepam-treated patients may not notice or be appropriately concerned about even fairly large impairments in learning and cognitive abilities.

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