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The Effects of Scopolamine, Diazepam, and Lorazepam on Working Memory in Pigeons: An Analysis of Reinforcement Procedures and Sample Problem Type

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SAVAGE, L. M., M. A. STANCHFIELD AND J. B. OVERMIER. *The effects of scopolamine, diazepam, and lorazepam on working memory in pigeons: An analysis of reinforcement procedures and sample problem type.* PHARMACOL BIOCHEM BEHAV **48**(1) 183–191, 1994.—Two groups of pigeons were trained on a delayed-matching-to-sample (DMTS) task with both identity and symbolic problems, that had either a) specific outcomes correlated (differential group) or b) outcomes uncorrelated (nondifferential group), for each correct sample-choice sequence. After reaching a criterion of 90% correct at the 0 s delay, subjects were tested under saline, methylscopolamine (0.03 mg/kg), scopolamine (0.007, 0.015, 0.03 mg/kg), diazepam (0.0, 1.0, 1.75, 2.5 mg/kg), and lorazepam (0.0, 0.5, 0.75, 1.0 mg/kg) at delays of 0 to 8 s. Scopolamine, diazepam, and lorazepam at all doses impaired performance in the nondifferential group; however, in the differential group, the medium and high doses of both scopolamine and lorazepam, and only the high dose of diazepam impaired performance. The differential outcomes procedure, relative to the nondifferential procedure, enhanced retention in the non-drug state and under these amnestic drugs. Impairments observed in the differential group were a result of decreased performance only on samples correlated with a secondary reinforcer (flashing hopper light); there was no decreased performance on samples correlated with a primary reinforcer (grain). Neither group showed any differences in performance as a function of identity versus symbolic problems in a nondrug or drug state.

Scopolamine Diazepam Lorazepam Working memory Pigeons Differential outcomes Delayed-matching-to-sample

ANIMALS and humans commonly are called upon to make a choice among alternative possible acts based upon recently presented informative cues that are no longer present. For example, selecting specific items at a grocery store after recently surveying a home refrigerator or a honey creeper's behavior when gathering nectar from flowers. Buying too much milk or revisiting a flower devoid of nectar is a wasteful act. Use of such temporarily valid information has been designated "working memory" (18). Impairments of such short-term working memory are quite dysfunctional as can be seen, for example, in patients suffering from Alzheimer's and Korsakoff's diseases (4, 23). These disorders have multiple neurochemical abnormalities, one of which is disrupted cholinergic mediated neurotransmission (3,5,8). Hence, it would be useful to determine if there exist training regimes that a) improve the persistence of short-term working memory, b) render working memory less disruptable in the face of neurotransmitter disruption or therapeutic drug intake, and c) reduce working memory sensitivity to increases in cognitive load. These are the broad goals of the present research that uses an animal model of working memory and its assessment. Working memory has commonly been assessed in pigeons by using the delayed-matching-to-sample (DMTS) paradigm (18). In the DMTS task, the subject is given a choice between two or more response alternatives. The correct choice is determined by which of the sample stimuli was presented to the subject at

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the beginning of the trial. Memory is assessed by manipulating the time between the removal of the sample stimulus and the presentation of the choice stimuli.

In simple identity DMTS, the sample stimulus and the correct choice alternative are the same. Thus, for a pigeon, the correct response after seeing a red key as the sample is to peck the red key of the two or more choices alternative keys that appear after the delay. This is not the only relationship that pigeons can learn; they can also learn symbolic relationships. In the symbolic DMTS design, the sample stimulus stands for, or directs responses to, a unique choice alternative stimulus. For example, a sample of vertical lines may indicate that the subject should later peck at a green key, whereas horizontal lines indicate that the red key is the correct choice alternative. Pigeons are able to learn this task even though it may involve more complex cognitive processes than identity DMTS (13). It was assumed to be more complex because solving a symbolic DMTS task hypothetically involves a translation of the sample to the correct test stimulus (29).

In both types of DMTS noted, the typical observation is that accuracy of performance deteriorates dramatically over delays of even a few seconds. A procedure developed in the Minnesota labs for enhancing performance in DMTS is the differential outcomes procedure. In the standard laboratory procedure, all correct choices in the DMTS task are reinforced with the same reinforcer—typically food. The differential outcomes procedure involves consequating each sample stimulus/correct choice combination with a distinct reinforcement condition (34). For example, one sample/choice combination may always lead to the delivery of food at the end of the trial, whereas the alternative combination might result in a different outcome, say water or the sounding of a tone and then advancement into the next trial (28). This procedure allows for the learning of a predictive relation as to which reinforcer will be presented based upon the antecedent stimulus conditions. The common control procedure for such an experimental design is to use the same stimuli and reinforcers, but to randomize these relationships. Under these conditions, there is no predictive relation about which outcome will be given based on the antecedent stimulus conditions. The effect of using unique reinforcers correlated with each correct choice alternative, relative to either the traditional use of a common reinforcer for all correct choices or different but uncorrelated reinforcers, is that speed of learning the problem is increased and accuracy of memory-based performance across long delays is improved (24). This enhancement is called the differential outcomes effect (DOE). The DOE has been demonstrated using a variety of animal subjects such as rats, dogs, and pigeons, and a wide range of reinforcer relationships [see Goeters, Blakely, and Poling (15) for a review].

The present study examined whether the persistence of working memory under an amnestic drug state would be compromised by a) increasing the duration of temporal delay, and b) using symbolic relationships; or would the differential outcomes procedure attenuate such disruptions in performance. Retention is assessed in the MTS by increasing the delay interval between the sample and the choice stimuli. If a drug impairs memory performance, its effect will be seen at longer delays (7). Symbolic relationships were originally thought to require more complex cognitive processes than identity relationships (13), although recent work suggests that pigeons learn and remember symbolic and identity discrimination problems to the same degree (36). However, taxing the memory system by use of an amnestic drug might reveal cognitive differences in solving these problems not seen under normal circumstances. It has been suggested that the higher accuracy obtained under the differential outcomes procedures, relative to the nondifferential procedure, reflects either greater stimulus control (1), or a different memory mediation process (24), and, therefore, should be more resistant to drug induced impairment.

Scopolamine, a cholinergic antagonist, and benzodiazepines, GABA facilitators, were chosen for the present study because both have shown to impair performance on some types of memory tasks (25). The mnemonic effects of scopolamine and benzodiazepines on working memory in pigeons have not been fully explored. The influence of the differential outcome procedure, or sample problem type on performance under these amnestic drugs has not been previously assessed. Previous research on working memory assessed by DMTS tasks without differential outcomes has shown that it is disrupted by scopolamine in the pigeon (30–32), in the rat (7, 12,17,21), and in the monkey (4,17,20). Likewise, benzodiazepines have also been shown to decrease DMTS accuracy in the pigeon (26), in the rat (17,21), and in the monkey (17,20,33). However, there is some uncertainty whether all benzodiazepines produce the same degree of disruption of working memory. Some human data comparing the effects of diazepam, clorazepam, and lorazepam found that only subjects treated with lorazepam were impaired at word-list recall (16). Thus, benzodiazepines may or may not be interchangeable in their efficacy in modulating memory; task, species, or both may be important determiners. Hence, this experiment included two different benzodiazepines.

METHOD

Subjects

Twelve adult female Carneaux pigeons (478–568 g) obtained from the Palmetto Pigeon Plant (Sumter, SC) served as subjects for this experiment. The pigeons were individually housed with unlimited access to water and grit in a colony room with a 14 L : 10 D cycle (onset at 0600 h). Prior to the experiment, the subjects body weights were gradually reduced to approximately 80% of their free-feeding weights by restricted feedings. Their weight was maintained at this level by supplemental feedings of mixed grain in their home cages at the conclusion of each experimental session. All the birds were experimentally naive at the initiation of the experiment. The subjects were randomly assigned to one of two groups (differential or nondifferential of six pigeons each). One pigeon in the nondifferential group died from unrelated causes.

Apparatus

The training and testing sessions were conducted in commercial small animal operant conditioning chambers (28 × 23.5 × 29 cm), each enclosed within a sound-attenuating chest (Coulbourn Instruments, Lehigh Valley, PA). The side walls of the chamber were made of Plexiglas, whereas the ceiling, back, and side walls were constructed of aluminum. The three response keys (2.5 cm diameter) were located in a horizontal line, 6 cm apart, on the front wall of the chambers. The centers of the response keys were positioned 9 cm from the ceiling of the chamber and 14 cm above the center of the food hopper. An aperture (5 × 5.5 cm) for accessing the food hopper was located centrally on the front wall, 3 cm off the grid floor. General illumination was provided by a houselight located in the middle of the ceiling. Background white noise

(80 dB) was presented throughout the experimental sessions by a speaker located behind the rear wall of each chamber. Experimental events were controlled and data collected using a PC computer (Zeos 286) and Med PC interface and software (Med Associates Inc., East Fairfield, VT).

Procedure

Pretraining. Training was initiated with 1 day of habituation to the experimental chamber. During this session, the subjects were placed in the chambers for 30 min with both the houselight and white noise activated. On the following day, the pigeons were trained to eat from the hopper which was illuminated when raised into the accessible position. This was accomplished by placing the pigeons in the chambers as before, but with the food-filled hopper lit and raised for the entire 30 min session. After the pigeons began to approach and eat from the food hopper regularly, they were placed on a program that initially provided 60 s of access to the hopper with a mean intertrial interval (ITI) of 24 s. The hopper access time was successively (20, 10, and 5 s) reduced to 5 s periods, again with an ITI of 24 s. Habituation and hopper training was completed in approximately 8 days.

Next, the birds were trained to peck the sample key using a standard autoshaping procedure (6). Prior to being given 5 s of access to the hopper, the pigeons were randomly presented with one of the four sample stimuli (green, red, vertical lines, or horizontal lines) for 8 s on the center key. After learning to peck the center key, they were then autoshaped to the side keys that were randomly lit red or green. All autoshaping sessions used an ITI of 60 s with 60 trials per session. This training lasted approximately 11 days.

After completion of the autoshaping sessions, the pigeons were given instrumental training on the side keys. These instrumental sessions also had 60 trials, but the ITI was reduced to 45 s. On the first day of this training, a single peck to the lit side key resulted in a 5 s access to the hopper. On the second and third days, three pecks were required before the hopper was raised. The food reinforcer used for all training and autoshaping sessions was pigeon chow.

Conditional discrimination. The differential outcomes were introduced using a forced-choice procedure. These trials were initiated with the presentation of one of the four sample stimuli as before. In this case, three pecks to the sample would result in the withdrawal of the stimulus and the immediate presentation of the correct choice stimulus on one of the side keys which in turn had to be pecked three times. In the differential group, the red/red and the horizontal/red trials were reinforced with 5 s access to the primary reinforcer, mixed grain, whereas the green/green and vertical/green trials resulted in the secondary reinforcer, four rapid flashes of the hopper light and advancement to the next trial. In the nondifferential group, completion of each trial resulted in the random presentation of one of the two outcomes. Forced choice training was conducted for 10 days, one 60 trial session per day with a 45 s ITI.

After the forced-choice training, the true DMTS training began. Each trial began, as before, with the random presentation of one of the four sample stimuli. A single peck to this stimulus resulted in its removal and initiated the retention interval. The intervals were 0, 2, 4, and 8 s and were randomly determined for each trial. Following this delay period, the two choice stimuli were presented in a random configuration on the two side keys. Three pecks to the correct stimulus on the side key were required for the trial outcome to be presented. The relationships were the same as in previous training. In the differential outcomes group, the correct choices on red/red and horizontal/red trials resulted in 5 s access to grain from the lit hopper, whereas correct choices on the green/green and vertical/green trials resulted in four rapid flashes of the hopper light and advancement to the next trial. In the nondifferential group, these reinforcer outcomes occurred randomly. For both groups, an incorrect choice response resulted in a 3.5 s blackout period followed by a repeat of the same trial. Each experimental session consisted of 80 scheduled trials with a VT 25 s ITI. If, after 60 min, the pigeon had not correctly completed all 80 scheduled trials, the session was terminated. This design was used for all the remaining training and testing sessions.

Pharmacological testing. Once the birds reached a criterion level of performance of 90% correct at the 0 s delay interval on the DMTS procedure for three consecutive sessions, drug testing sessions began. Three drug agents with amnestic effects were used. All the injections were given intramuscularly into the pectoral muscle 30 min prior to the testing session, and were in a volume of 1.0 ml/kg of body weight. Two determinations were conducted for each condition. Injections were given in a mixed order; ascending then descending. Recovery days, on which no injections occurred, but on which behavioral sessions were conducted, intervened between drug testing days until performance recovered.

Saline baseline. Prior to any control or specific drug testing baseline performance was assessed in a nondrug context using saline injections. This was done to assess differences between the groups due to the injection manipulations before any pharmacological manipulations.

Scopolamine. The muscarinic antagonist scopolamine hydrobromide (Sigma, St. Louis, MO) and it's analog, methylscopolamine (Sigma), which does not cross the blood-brain barrier, were dissolved in saline. Subjects were tested under a) 0.03 mg/kg of methylscopolamine, and b) 0.03, 0.015, and 0.007 mg/kg of scopolamine.

Diazepam. The benzodiazepine diazepam (Elkin-Sinn, Inc., Cherry Hill, NJ) in a vehicle solution of 40% propylene glycol, 10% ethyl alcohol, 5% sodium benzoate, and 1.5% benzyl alcohol, was administered in four doses: 0.0 (vehicle), 2.5, 1.75, 1.0 mg/kg.

Lorazepam. The benzodiazepine lorazepam (Sigma) was mixed in the same vehicle solution as diazepam. Four doses were given: 0.0 (vehicle), 1.0, 0.75, and 0.50 mg/kg.

Data Analysis

This study was a split-plot repeated measures design that combined the between-subject factor of treatment (differential reinforcement vs. nondifferential reinforcement) and the within-subject factors of delay interval, sample problem type, and dose for each drug condition. Our interest was in observing the influence of psychological/procedural factors, rather than a direct test of the difference between the drugs. The data were analyzed with BMDP 4v software. Significance was accepted at $p < 0.05$. A priori orthogonal planned tests between group means were conducted using paired *t*-tests or linear contrasts (22). Fisher's LSD procedure was used for follow-up tests of significant effects if the overall F was significant (19).

RESULTS

As evidence of the DOE, all subjects in the differential group readily mastered the task while the subjects in the non-

differential group took an average of 151 more sessions to learn the task; furthermore, one subject in the nondifferential group never did master the task. The analysis for all conditions included six subjects with the differential training and four subjects with the nondifferential training.

Saline Baseline

Subjects in both groups performed the discrimination at the criterion level for the 0 s delay interval; however, the accuracy of choice performance of the nondifferential group was impaired as the delay interval increased. Figure 1 shows these differences in the two groups' performance as a function of reinforcement procedure and delay interval. To assess the components of the paradigm in a nondrug state, a groups (2) × delays (4) × sample problems (4) ANOVA was conducted for the saline condition. The interaction between group × delay was significant, $F(3, 24) = 4.51$. Linear trend functions were used to assess the pattern of performance across delays for each group. Performance declined significantly as delay interval increased for the nondifferential group, $F(3, 24) = 4.3$, but not in the differential group, $F(3, 24) = 0.3$.

Accuracy as a function of type of sample problem and delay interval for both groups for various conditions are portrayed in Fig. 2. No significant effects were found for sample problem, $F(3, 24) = 2.09$; sample × group, $F(3, 24) = 2.10$; sample × delay, $F(9, 72) = 1.81$; delay × sample × group, $F(9, 72) = 1.01$, indicating that the two sample problem types did not pose different memory demands for pigeons in a nondrug state.

Pharmacological Tests

For each drug condition a groups (2) × dose (4) × delays (4) × sample problems (4) repeated measures ANOVA was conducted on percent accuracy scores. This was done to determine if different doses of each drug differentially affected performance as a function of outcome contingencies, delay, sample problem, or an interaction of those variables. For all three drugs each independent variable produced a significant main effect, group, Fs(1, 8) > 11.21, dose, Fs(3, 24) > 10.29, delay, Fs(3, 24) > 6.89, sample, Fs(3, 24) > 15.36,

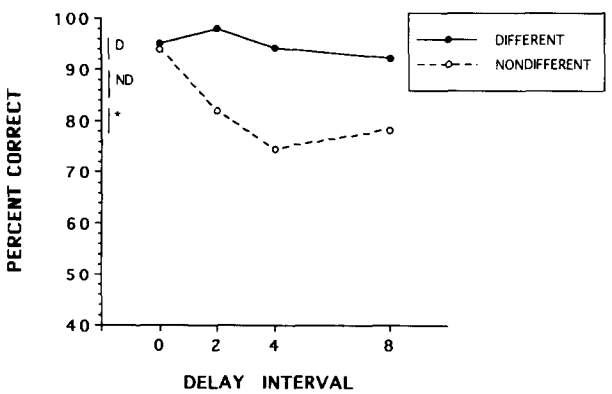

FIG. 1. Mean percent correct as a function of group and delay interval under the saline baseline [D indicates the standard error of the within differences for the differential group; ND is the standard error of the within differences for the nondifferential group; asterisk (*) is the standard error of differences between group means].

but these also interacted with each other in complex ways. Because these interaction modulate the possible meaning of the main effects, for each separate drug, our analysis focused upon the components of the interaction to identify the locus of the effects. The data are best parsed, however, according to relevant issues of the experiment: a) dose, b) delay interval, and c) problem sample type.

Scopolamine. For scopolamine, methylscopolamine was used as the reference condition. Relative to this reference condition, increasing doses of scopolamine decreased performance accuracy in both groups, but in different ways. The two-way interactions of group with delay and with sample (both Fs(3, 24) > 5.16), a three-way interaction of group with dose and sample, $F(9, 72) = 2.50$, and a four-way interaction of group × dose × delay × sample, $F(27, 216) = 1.61$, were all significant.

A priori contrasts were used to assess differences in performance between the two groups as a function of drug dose. Performances obtained on all doses of scopolamine were compared to performances under methylscopolamine. The group treatment effect is clear; the differential group was more resistant to the debilitating effects of scopolamine, being impaired only at the two higher doses [both ts(48) > 6.35], whereas the nondifferential group had significantly reduced performances at all three doses [all ts(48) > 2.46]. Looking at Fig. 3, it is apparent that when administered 0.015 mg/kg the differential group has higher accuracy than the nondifferential group at the 0 s, $t(72) = 2.25$, and longer delays [all ts(72) < 3.03].

The overall group × delay interaction arises because there is no effect of delay on performance in the differential group either under the methylscopolamine control condition or under the doses of scopolamine, whereas there is a such a delay effect under all drug treatments in the nondifferential group. Memory performance, assessed by percent correct as a function of increasing delay intervals, was assessed using linear trend analyses. Linear trends were found for the data collapsed across doses and sample problems for the nondifferential group, $F(3, 24) = 5.95$, but not for the differential group, $F(3, 24) = 0.56$.

The data obtained at the high dose of scopolamine best illustrates the influence of sample problem type and are shown in Fig. 2. It was expected that accuracy performance on the symbolic samples would be lower than that obtained for the identity samples—and perhaps especially in the nondifferential group; however, that was not the case. The group × sample effect was not a result of performance differences in the nondifferential group, but a result of the differential group's decreasing performance on a particular type of trial—the secondary reinforced (green and vertical) trials relative to the primary reinforced trials (red and horizontal), $F(3, 24) = 8.89$. This difference was not obtained between these samples for the nondifferential group $F(3, 24) = 0.01$.

The higher order interaction among group, dose, delay, and sample arise because the differential group's performance decreased significantly on the secondary reinforced sample stimuli but not on the primary reinforced sample stimuli for the high dose of scopolamine, $F(9, 72) = 11.85$—a similar effect was also observed at the middle dose, $F(9, 92) = 14.22$, and at the low dose of scopolamine, $F(9, 72) = 2.51$—but not under the reference condition, $F(9, 72) = 0.56$. In contrast, no effect of sample problem type was detected in the performance of the nondifferential group under any condition, all Fs(9, 72) < 0.22.

Diazepam. Diazepam also decreased performance accu-

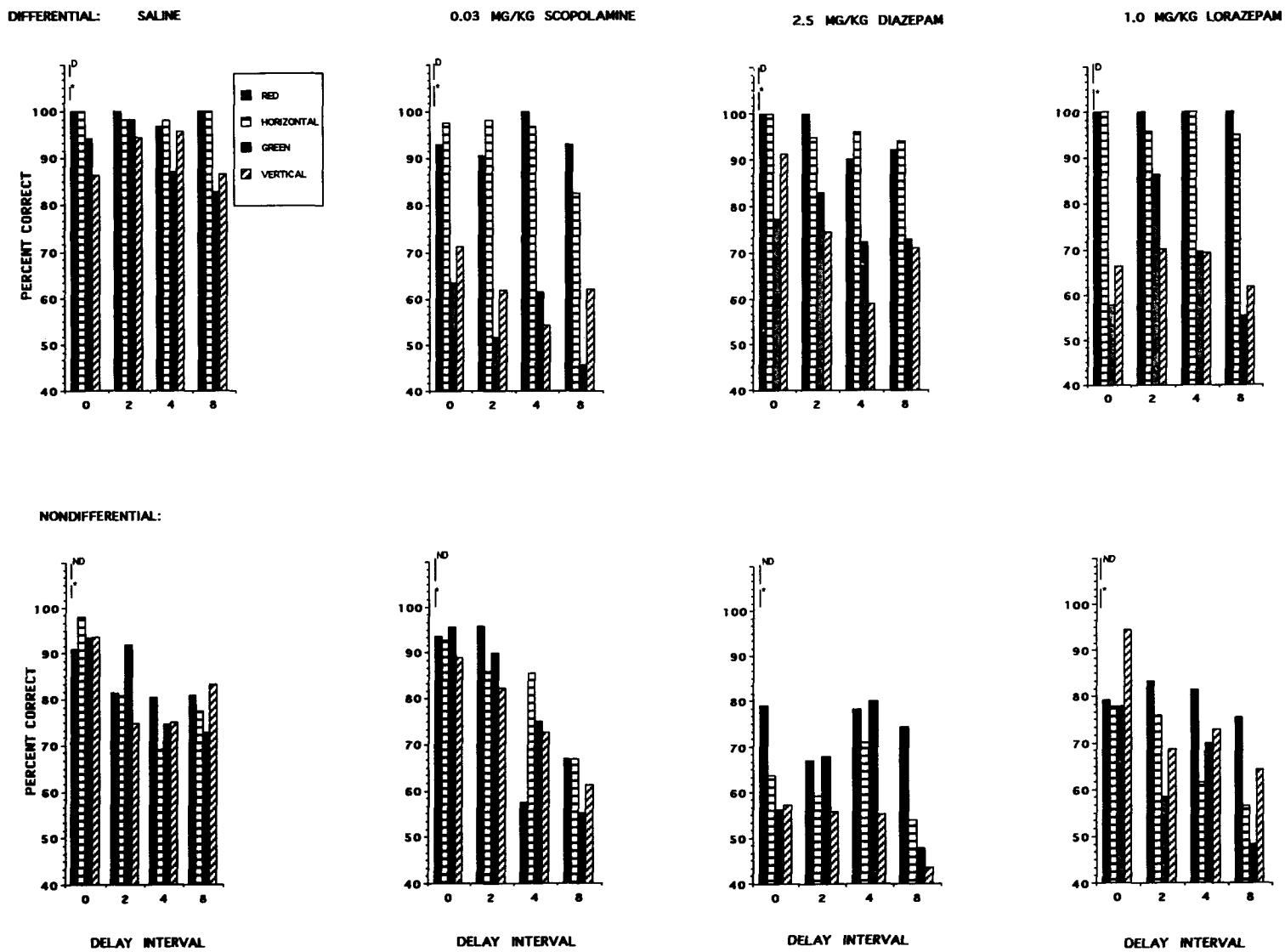

FIG. 2. Mean percent correct as a function of sample problem and delay interval for both groups under the saline baseline, 0.03 mg/kg scopolamine, 2.5 mg/kg diazepam, and 1.0 mg/kg lorazepam [D indicates the standard error of the within differences for the differential group; ND is the standard error of the within differences for the nondifferential group; asterisk (*) is the standard error of differences between group means]. The top row depicts data for the differential group, whereas the bottom row depicits data from the nondifferential group.

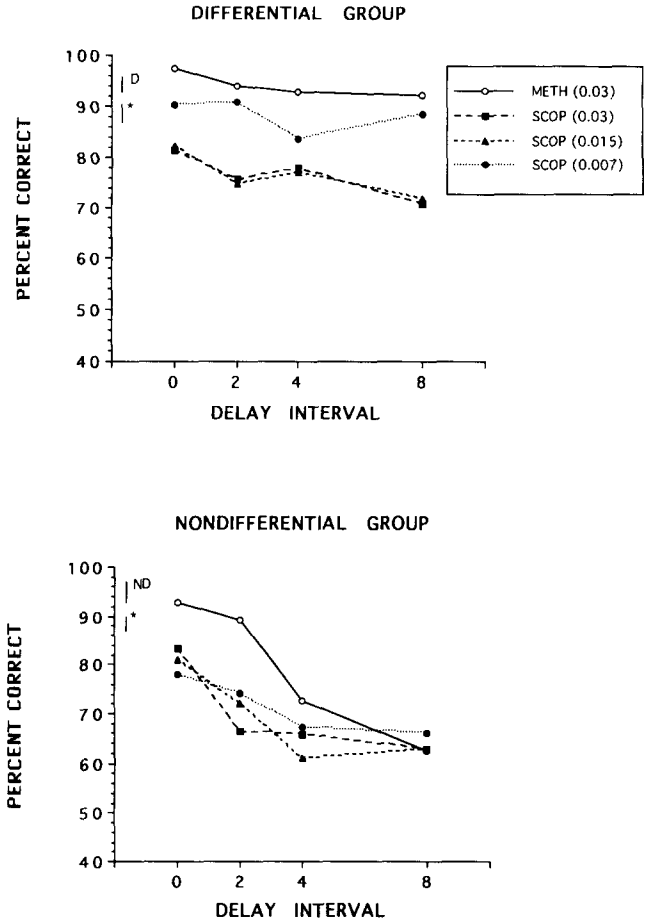

FIG. 3. Mean percentage correct choices under the scopolamine treatments. Panel A presents performance of the differential group as a function of dose and delay, panel B presents performance of the nondifferential group as a function of dose and delay. [In the figure, D indicates the standard error of the differences between means within the differential group; ND indicates the standard error of the differences between means within the nondifferential group; asterisk (*) indicates the standard error of the differences between means for between-group comparisons.]

racy in both groups, but the effects were different for each group sample interacted with group, $F(3, 24) = 3.69$, and dose, $F(9, 72) = 2.03$, and jointly with dose and delay, $F(9, 72) = 2.68$. Group also interacted jointly with dose and delay, $F(9, 72) = 2.68$. As before, these data are best understood when parsed in accordance with the relevant experimental issues.

As expected, the differential and nondifferential groups were differentially sensitive to the diazepam drug treatment. Pair-wise contrasts revealed the differential group has significantly impaired performance, relative to that obtained under vehicle, only at the highest dose, $t(48) = 3.63$. Performances in the nondifferential group, however, were impaired at all three drug doses, all $ts(48) > 1.84$.

Figure 4 shows the percent accuracy for each group as a function of delay for the various doses of diazepam; these relations are clearly different for the two group treatments. The group × dose × delay interaction was examined using linear trend analyses at each dose. There was a significant linear trend in the differential group at the high dose, $F(9, 72) = 2.07$, but not at the other doses, all $Fs(9, 72) < 0.41$. In the nondifferential group, there was significant linear trends in the vehicle condition, $F(9, 72) = 2.69$, and the 1.0 mg/kg, $F(9, 72) = 2.05$, but not at 2.5 mg/kg or 1.75 mg/kg, all $F(9, 72) < 1.43$. Furthermore, with the exception of the vehicle condition, the differential group had higher accuracies than the nondifferential group at the 0 s delay intervals under all doses (all $ts(72) > 2.79$).

Figure 2 displays the data obtained at the high dose of diazepam for both groups. Further analysis of the group × sample interaction revealed that the differential group had decreased performance only on the secondary reinforced samples compared to the primary reinforced samples, $F(3, 24) = 5.43$; whereas no differences in performance were observed between the samples in the nondifferential group, $F(3, 24) = 0.005$.

Lorazepam. Lorazepam influenced accuracy of performance in both groups at all doses tested, but again, in differ-

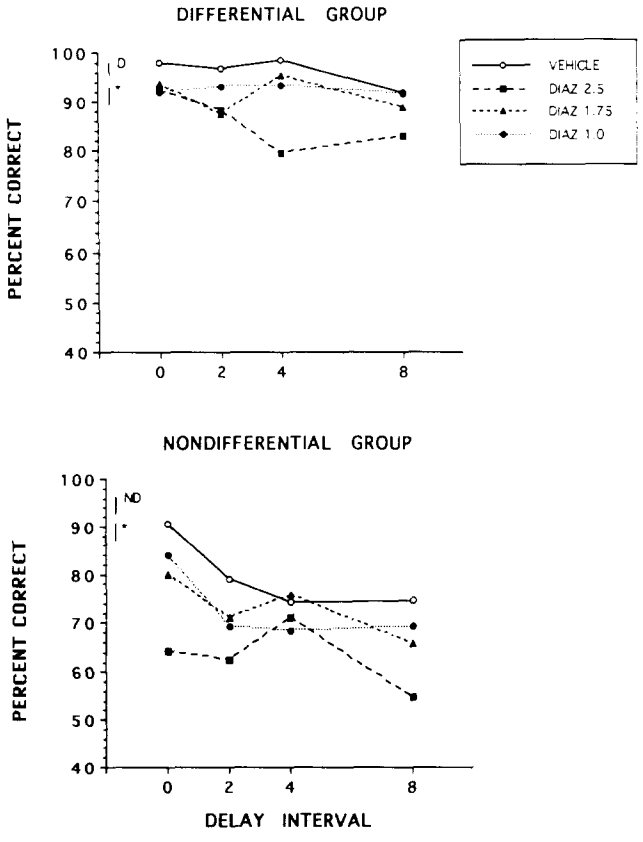

FIG. 4. Mean percentage correct choices under the diazepam treatments. Panel A presents performance of the differential group as a function of dose and delay, panel B presents performance of the nondifferential group as a function of dose and delay. [In the figure, D indicates the standard error of the differences between means within the differential group; ND indicates the standard error of the differences between means within the nondifferential group; asterisk (*) indicates the standard error of the differences between means for between-group comparisons.]

ent ways. Group treatment interacted with delay, $F(3, 24) = 4.22$, sample, $F(3, 24) = 8.37$, and dose × sample, $F(9, 72) = 3.52$. Again, the data will be presented as a function of the important experimental issues.

Mean percent correct at each delay interval and each dose of lorazepam for both groups are portrayed in Fig. 5. A priori contrasts revealed that both the differential and nondifferential groups performances were impaired at all doses of lorazepam relative to performance at vehicle, all $ts(48) > 2.28$. However, if performance is examined at the 0 s delay it is apparent that accuracy in the differential group is higher than the nondifferential group at the low and medium doses (both $ts(72) > 4.25$).

The significant interaction of group × delay was examined using linear trend functions for each group. Collapsing across doses and sample types no linear trend was found in the differential group, $F(3, 24) = 0.31$; however, there was a significant linear trend for the nondifferential group, $F(3, 24) = 3.16$.

The effect of the interaction between group × sample was due to a) decreased performance by the differential group on the secondary reinforced samples, but sustained performance on the primary reinforced samples, $F(3, 24) = 3.74$, and b) no difference in performance based on sample type in the nondifferential group, all $Fs(3, 24) < 0.41$. Subsequent analysis of the group × dose × sample interaction demonstrated that the differential group made significantly more errors on the secondary reinforced samples at the low, $F(9, 72) = 6.54$, medium, $F(9, 72) = 9.37$, and high, $F(9,72) = 10.89$, doses. No significant effects of sample by dose were found in the nondifferential group, all $Fs(9, 72) < 0.81$; rather, performance on all samples were decreased in a dose- and delay-related fashion.

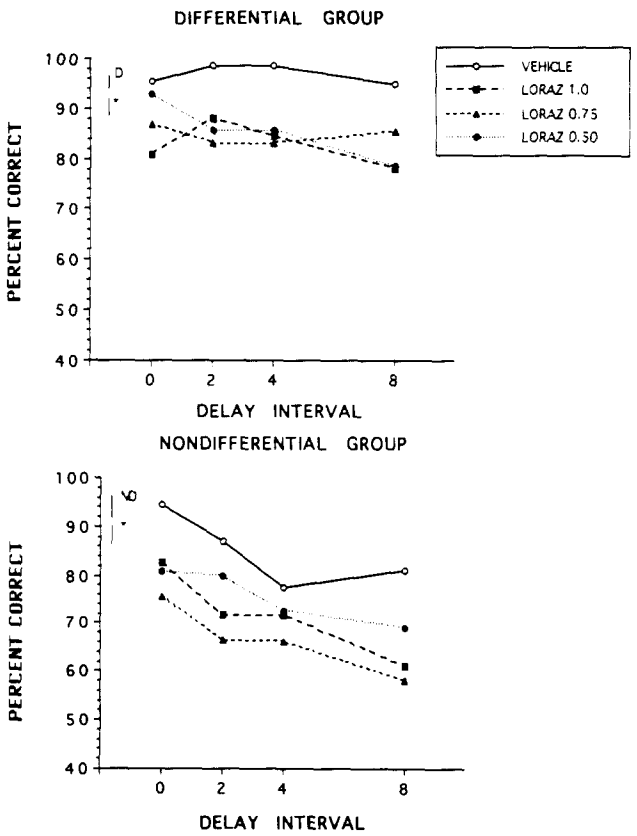

FIG. 5. Mean percentage correct choices under the lorazepam treatments. Panel A presents performance of the differential group as a function of dose and delay, panel B presents performance of the nondifferential group as a function of dose and delay. [In the figure, D indicates the standard error of the differences between means within the differential group; ND indicates the standard error of the differences between means within the nondifferential group; asterisk (*) indicates the standard error of the differences between means for between-group comparisons.]

DISCUSSION

The expected difference in performance between the differential and nondifferential groups was obtained under saline condition, the noncentral acting reference drug, and vehicle conditions: the differential group sustained high levels of accuracy at the 0, 2, 4, and 8 s delay intervals, whereas the nondifferential group only retained a high level of accuracy at the 0 s delay interval. This confirms that the differential reinforcement procedure in which each stimulus sample-correct choice relation has its own unique reinforcer establishes learned relations that improve memory based performance—and it does so equally well for identity DMTS and for symbolic DMTS problems. Furthermore, in the nonchallenged (undrugged) state, this was true both for trials that were followed by the primary reinforcer and for those that were followed by the secondary reinforcer.

Scopolamine, diazepam, and lorazepam did disrupt nonspatial working memory; however, the nondifferential and differential groups were impaired by these drugs in different ways. Analysis of dose effects on performance indicated that the groups differed in their sensitivity to the doses of scopolamine, diazepam, and lorazepam. Tasks like ours that have both no-delay and delay intervals can be used to evaluate separately the effects of a drug on encoding and retention functions (7,25). When administration of a drug produces decreased performance at the 0 delay intercept, relative to the control condition, it suggests that encoding is being impaired, whereas an increase in slope, indicative of performance at longer delays being deteriorated, suggests that retention is impaired. One drawback to this analysis occurs in cases where encoding is impaired to a large degree. Under such circumstances there is little opportunity to detect additional drug effects on retention.

When administered scopolamine, the differential group, at the medium and high dose, and the nondifferential group, at all doses, have decreased intercepts, relative to the intercept obtained under methylscopolamine. This suggests that scopolamine has a primary effect on encoding processes. The slope of the curves for either group did not increase under any dose of scopolamine, suggesting scopolamine had no effect on retention. The differences between the groups seen at the low dose, is a result of the differential group having higher accuracy scores at all delays, suggesting this procedure improves both encoding and rentention.

Diazepam influenced the two groups differently. Only the nondifferential birds had dramatic dose-related reduced intercepts, as seen clearly at the high and medium doses of diazepam. The differential birds had relatively minor nonsignificant changes in intercepts as a function of dose of diazepam. However, for the differential group, the high dose of diaze-

pam caused the slope to increase dramatically, which is reflected in the significant linear trend obtained at that dose. This suggests that retention of information was impaired by this dose of diazepam in this group. In the nondifferential group, the slope at the low dose approximates that for vehicle, but at the medium and high doses the curves are flatter and more variable, as was shown by the lack of linear trends at those doses. These flatter curves, normally indicative of no retention effect, reflect the enormous effect diazepam has on encoding in this nondifferential group.

Lorazepam caused decreased intercepts at the high and medium doses in the differential group and at all doses in the nondifferential group, suggesting lorazepam, like scopolamine, impairs encoding in both groups. No linear trends were detected in the differential group, and the slope did not increase significantly in the nondifferential group, suggesting that lorzepam did not impair retention.

These results suggest that where in the cognitive process a drug exerts an influence is dependent on the dosage, the procedures, and the parameters of the task. These results concur with some of the research examing the influence scopolamine (7,9,12,17,20,30) and diazepam (17,20,21,33)—that the disruption these drugs produce primarily involves encoding rather than retention of information.

The differential outcomes procedure sustains memory performance under all drugs. This is reflected in the graphs in which the differential group has relatively flatter delay functions (with the exception of diazepam 2.5 mg/kg) than the nondifferential group, even when they have the similar intercepts. Furthermore, encoding seems relatively enhanced at the low doses of scopolamine and lorazepam, and at all doses of diazepam under differential procedure.

The influence of cognitive load, as tested in our comparison of symbolic vs. identity samples, was not found to influence performance in drugged or undrugged states. This is best seen in the nondifferential group in Fig. 2. When performance was decreased as a function of sample, it was in the differential group, and it was not a function of symbolic or identity problems, as we originally hypothesized. What was revealed in the differential group was a difference in performance based on hedonic load, primary vs. secondary reinforced trials. In the differential group, encoding was disrupted only on the secondary reinforced trials: in fact, there was no effect of any drug on encoding on the primary reinforced trials (see Fig. 2). In the nondifferential group, the impairment can not be linked to any specific sample problem.

A number of explanations can account for the phenomenon obtained in the differential group. It is likely that the processing of the stimuli and the two outcomes used in this study require different cognitive mechanisms. A representation of a specific food event is distinctive (35,36), but a representation of a conditioned stimulus may not be as distinctive (11). Although this nondistinct representation can be supported under normal conditions, it is seriously challenged under a amnestic drug state (see Fig. 2).

A representation of a food event may have motivational properties that are different than the no-food situation, and that factor may make the behavior more resistant to disruptions. Although we did not record response rate or response latency, the pigeons may have behaved differently to the samples that were paired with food (primary reinforcer), compared to samples that were paired with only the hopper light (secondary reinforcer). Alling et al. (1,2) recently reported that pigeons have higher response rates for samples that are paired with food relative to those paired with a conditioned stimulus (flashing hopper light). A similar effect has also been observed by Nevin and Grosch when contrasting samples followed by a "large reinforcer" to those followed by a "small reinforcer" (27). This behavioral difference suggests an increased response strength for samples paired with a reinforcer of greater magnitude, relative to samples paired with a reinforcer of less magnitude. However, Nevin and Grosch (27) did not find that samples followed by the "larger reinforcer" were more resistant to drug disruption (phenobartital), and Alling et al. (1) did not mention such a difference either.

Why we obtained performance differences based on the type of outcome that was correlated with a specific sample and Nevin and Gosch and Alling et al. did not, is likely due to the different drugs used, scopolamine and benzodiazepines vs. phenobarbital. Although phenobarbital (1), scopolamine (30–32), and diazepam (26) all have been reported to disrupt accuracy on DMTS in the pigeon, the mechanisms for the disruptions are likely to be different.

It could be possible that scopolamine and benzodiazepines disinhibit responding. In any situation, an animal must decide from a wide array of response alternatives. The cholinergic system has been proposed to modulate this process by antagonizing activation (10,14). When cholinergic activity is decreased, activation is increased, and the net result is an increase in the responses elicited in a given context. This hypothesis was proposed to account for the disinhibition of nonreinforced responses; but in our case, when the sample was green or horizontal, it may be the disinhibition of primary reinforced responses (choose red) that compete with the correct response (choose green). The net result is impaired accuracy.

Although our design has demonstrated differences in memory performance as a function of the drug and reinforcement procedures used, it does not allow us to determine the exact causal mechanism of those effects. Assessing the reinforcer magnitude-mediated theory would involve using outcomes of similar preference that do not require or elicit different response topographies (13). The disinhibition issue would require testing a larger sample of drugs that impair memory.

In sum, however, it is clear that the easily implemented differential outcomes training regime is one that can be used to reduce encoding deficits and preserve short-term memory function for reinforced behaviors even in the face of CNS challenge, including impaired CNS cholinergic function. Hence, this research suggests that the differential outcomes training procedure may prove a useful training strategy for behavior therapists trying to teach patients suffering from the personal challenge of short-term memory dysfunctions that accompany CNS diseases such as Alzheimer's and Korsakoff's.

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