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Triazolam Impairs Delayed Recall but Not Acquisition of Various Everyday Memory Tasks in Humans

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A double-blind test battery was administered to 24 human subjects (8 control, 16 drug) to assess the effects of 0.125 mg triazolam (oral) on memory encoding and retention across delay intervals ranging from seconds to 1 week after presentation. Although the drug reduced immediate psychomotor performance, it did not impair recall of previously learned information, nor did it significantly impair encoding of new information. The drug enhanced immediate recall of the location and identity of playing cards, without affecting 4-h delayed recall. The drug treatment impaired correct recall of object names after a delay of 20 min. At 4 h delay, the drug impaired olfactory recognition and free-recall of object names. At both 1 day and 1 week delay, the drug impaired recall of biographical information and correct identification of picture–photographer pair associations. The drug also impaired the daily improvement of the drug group as compared with the control group in a geometric puzzle solving task. The time course of these memory impairments compares well with the known effects of triazolam on long-term potentiation (LTP), a candidate biological mechanism underlying telencephalic memory formation and expression. © 1997 Academic Press

INTRODUCTION

Benzodiazepines have well-known anterograde amnesic effects on humans and animals, predominantly affecting memory consolidation (2, 17, 5). Immediate recall is usually spared, as is memory of previous events. Triazolam was chosen as the experimental treatment for this study based upon its short half-life of 4.5 h (14), and its reported lack of residual effects on subsequent days after administration (8). Doses of 0.25 and 0.5 mg are typical for studies primarily concerned with the hypnotic properties of triazolam, so a lower dose of 0.125 mg was chosen to reduce the sedative effect of the drug. Long-term potentiation (LTP), which has been proposed to be a primary mechanism underlying many forms of telencephalic-dependent memory (for review, see 12), is also impaired by benzodiazepines (4, 13). The specific effect of triazolam on LTP is to spare LTP induction while preventing permanent potentiation. The induced potentiation decays back to baseline in 15–30 min, consistent with the observed effects of the drug sparing immediate recall and impairing delayed recall in both rats (3) and humans (16, 18, 20, 8).

Curran (2) has stated that many traditional memory tests used in previous benzodiazepine impairment studies are "unrepresentative of everyday memory requirements" of normal human subjects. The test battery reported here was designed to include a range of tasks that are more directly related to "everyday" memory, using a variety of sensory systems for the mnemonic stimuli. The time courses chosen for retention testing were intended to provide information on memory acquisition and delayed recall at both immediate (seconds–minutes), short (minutes–hours), and long (days–week) delay intervals, as well as to facilitate comparisons with previous studies on the effects of triazolam on LTP. Several of these tasks have also been further used in a battery to assess the effects of a member of a class of centrally active DL-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) type glutamate receptor enhancers ("Ampakines") on memory (9).

MATERIALS AND METHODS

Subjects

The subjects were 24 healthy volunteers (14 male, 10 female), recruited from the University of California, Irvine, campus and paid for their participation. The mean subject age was 23.7 ± 1.2 (mean ± SE) with range 18 to 45 years. Only 12 of the 24 subjects listed English as their native language, reflecting the cultural diversity of the Irvine campus. The subjects were given detailed medical and psychological questionnaires, along with blood and urine tests. Subjects with either a history of head trauma, brain damage, depression, schizophrenia, epilepsy, liver dysfunction or other metabolic disorders, history of alcohol or drug abuse, those currently taking prescription medicine, or female sub-

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jects currently pregnant or nursing were excluded from the study. Subjects were instructed to refrain from smoking, drinking alcohol, or consuming caffeinated beverages during the course of the study.

Drug Treatment Schedule

The subjects were divided into control ($n = 8$) and experimental ($n = 16$) groups. Each group was further subdivided so that half of the subjects in each group received version A of the test battery and half received test version B. The testing schedule consisted of an interview/screening day (session S0), five consecutive test days (sessions S1–S5), with an additional follow-up session (S6) 7 days after session S5. Each of the five daily sessions S1–S5 commenced with medication administration, a "morning" set of tasks given 1 h postmedication and an "afternoon" set of tasks given 5 h postmedication. The medication was administered in a double-blind manner, with the experimental group subjects receiving 0.125 mg triazolam capsules on sessions S2 and S3 and visually identical placebo capsules on sessions S1, S4, and S5. The control group received placebo capsules on all of the daily sessions S1–S5. The test administrators were kept blinded of both the subject treatment group assignment and the daily drug/placebo schedule. As a self-assessment measure, at the end of the afternoon part of session S5, each subject was asked if they believed that they had received drug treatment on any of the sessions S1–S5.

Test Battery Tasks

There were seven tasks included in this test battery, chosen to test a variety of sensory modalities (e.g., olfactory, visual, verbal, spatial, etc.) and lengths of memory duration (seconds, minutes, hours, days, week). A problem-solving task and a psychomotor task were also added to test for general cognitive and motor effects of the drug treatment. These tasks were combined to produce two versions of the test battery, test version A and test version B, with an equal number of subjects from each treatment group assigned to each test version. These test versions were counterbalanced across the testing sessions and delay times for each task to help control for presentation ordering effects by testing the same material in sessions where placebo or drug treatment is administered on the alternate test version. Unless specifically mentioned, the tester did not provide any feedback as to the correctness of the subject's responses on any task. The test battery consisted of the following tasks: odor recognition task, misplaced objects task, card layout task, photographer biography task, picture–photographer association task, Raven puzzle task, and digit canceling task.

Odor Recognition Task

The odor samples for this task consisted of five odorants chosen from standard olfactory testing reagents (four from International Flavors & Fragrances, one from Tropico Enterprises, Ltd.) with the criteria that they were subjectively not easily identifiable and were all mixed to similar relative strengths. These odors were mixed into an equal volume of colorless medium (glycerin) and stored in identical sealed flasks. During the interview session S0, each subject was asked to sample each odor for 20 s and verify that the odors were readily distinguishable, which also served to familiarize each subject with the odors. During the morning segment of each of the daily test sessions S1–S5, the subject was given two unlabeled flasks from which to sample the odors, one at a time for 20 s each, and required to later identify the two sampled flasks in the reverse order from a randomly ordered lineup of the five similar odor flasks, with a delay time of either 30 min (sessions S1, S2, and S5) or 4 h (sessions S3 and S4). The specific pairs of odors used each day were chosen to minimize odor repetition across sessions and to counterbalance the delay times for test groups A and B. The number of correct choices (correct odors chosen in the correct order) was recorded to indicate the subject's performance. This measure minimizes the possible contribution of guessing, giving a random expected score of 0.41 of a possible 2.00.

Misplaced Objects Task

This task was modeled after the misplaced objects test of Crook *et al.* (1). During the morning segment of each of the daily test sessions S1–S5, the subject was shown a diagram (80×50 cm) representing a house with 12 rooms of various sizes and asked to imagine that this represented the rooms in their house. The subject was then sequentially given 20 cards, each with an approximately 6×6-cm ink drawing representing a common household object, told what each object represented (e.g., "a toothbrush" or "an umbrella"), and then asked to place the object into a room and remember its location. The tester recorded the object's position on a score sheet, removed the card from sight, and then presented the subject with the next object (this differs from the procedure detailed in Crook *et al.* (1), in which all objects remained in the rooms until completion). The object placement was further restricted so that no more than two objects could be placed into any particular room. If the chosen room already contained two objects, the subject was informed that the room was already filled and asked to choose another room to place the object in. The number of these "room collision errors" was recorded as a measure of immediate memory. On each session, a new house diagram was used, along

with 20 new objects, counterbalanced by presentation session between test groups A and B.

After a delay time of either 20 min (sessions S3 and S4) or 4 h (sessions S1, S2, and S5), the subject was shown the house diagram and asked to recall the names of the objects from that session in a free-recall format. The number of correct objects identified in the verbal free-recall and the number of incorrect extra names were recorded. Next the subject was shown each of the 20 object cards sequentially (in a different ordering from the presentation ordering) and asked to point to the room in which that object had been placed earlier. The number of correctly placed objects was recorded as a measure of retention, having a random expected score of 1.7 of 20.

Card Layout Task

The subject was presented with a box containing 16 playing cards (excluding aces and "royal face" cards) taped face-down in a 4 × 4 arrangement. The tester then sequentially showed the subject 8 of the card faces, for 4 s each, and told the subject to try to note the number value (2–10) and color (black or red) of each card shown, as well as the order in which the 8 cards were shown. For each session, a different card set and ordering of the 8 card locations was presented, counterbalanced between test groups A and B.

After a delay time of either 1 min (sessions S1, S2, and S5) or 4 h (on the afternoon session of S3 and S4), the subject was first asked to point out (without turning over) the locations of the eight cards that had been shown, in the same order as they had been presented. The subject's responses were recorded and used to compute the number of cards pointed to in the correct order before the first mistake was made, as well as the number of previously shown cards identified without respect to the original presentation order. Next the subject was asked if any of six verbally specified cards had been shown (e.g., "black 7" or "red 2"), and if so, the subject was further asked to point out the location of that card in the box. On each session, exactly three of the above six cards had actually been shown, so the subject could potentially give at most three correct location responses. Next the tester pointed sequentially to six specific cards and asked if each one had been shown, and if so, what was the number and color of the card at that location. On each session, exactly three of the six cards pointed to had actually been shown, so the subject could potentially identify at most three correct card face values. Finally, the subject was asked to point out the eight cards that had not been previously shown. The total number of correct responses to all questions was computed as a composite performance index, with a maximum of 42 possible and 12.7 for the random expected score.

Photographer Biography Task

The subject was shown a diagram with portraits of six faces of various ethnicity, gender, and age and told that these were photographers employed by a popular magazine. On each session S0–S5, the subject was told the name of one of the photographers and the tester recited a short (approximately 100-word) story containing biographical information about that photographer (the initial 10 subjects, 4 control and 6 drug, did not receive a new biography on sessions S0 and S5). The six stories were composed in such a way as to have approximately equivalent content (the same number of place names, dates, education information, hobbies, etc.), so that the same set of 12 questions (e.g. "What was the photographer's age?") could be used for each biographical story. The order of presentation of the specific biography for each session was counterbalanced between test groups A and B.

After a 7-min delay (during which new pictures from the picture–photographer association task, described below, were presented), the subject was asked a set of 4 verbal questions about the photographer, which served as an initial acquisition estimate of the subject's successful encoding of the content of the story. In the next session (1 day later for the biography presented on sessions S1–S4, approximately 3 days later for session S0, and 4 h later for session S5), the photographer's portrait was shown to the subject and the subject was asked to answer a set of 4 new questions about the photographer. This served as an estimate of the retention of the biographical information after the given delay interval. A final set of 4 different questions about the photographer was asked on a later session in order to get estimates of 3-day retention from the biographies presented on sessions S1 and S2 and estimates of week-long retention (7–9 days) for the biographies presented on sessions S0, S3, S4, and S5.

Picture–Photographer Association Task

The subject was shown a diagram with six faces of various ethnicity, gender, and age and told that these were photographers employed by a popular magazine (these were the same faces as used in the photographer biography task detailed above). The data consisted of 216 digitized color photographs taken from magazines with diverse subject matter. Each picture was randomly assigned to one of the six "photographers," representing a picture taken by that photographer. The digitized picture was rendered onto a high resolution computer image with a small portrait of the photographer in the upper right corner (picture size 762 × 762 pixels; photographer portrait size 255 × 255 pixels; 256 colors), producing a picture–photographer pair collage. The images were displayed on a 14″ SVGA personal computer screen (1024 × 768 pixel resolution). On each

session S0–S5, the subject was presented with 36 new picture–photographer pairs (6 pictures per photographer, randomly ordered) and told to remember which photographer took each picture (the initial 10 subjects, 4 control and 6 drug, did not receive new picture–photographer pairs on sessions S0 and S5). Each pair was displayed on the screen for 5 s, with a neutral gray screen displayed for 3 s until the next image was revealed. The order of presentation of the specific sets of 36 pictures used for each session was counterbalanced between test groups A and B.

After a 2-min delay (during which time questions were asked from the photographer biography task described above), the subjects were presented with a selection of 12 of the previously shown pictures (2 pictures per photographer, randomly ordered, comprising test set 1 for this session) and asked to identify which photographer took each picture. Each test image was rendered in a similar manner to the picture–photographer pair collages, except that small images of all six photographers were arranged in a randomized order in the upper right corner of the image (picture size 762 × 762 pixels; each photographer portrait size 128 × 190 pixels; 256 colors). The subject was required to point to the portrait of the correct photographer, guessing if necessary, in a forced-choice manner. The number of correct choices of 12 possible was used as an estimate of the amount of the original data set of 36 picture–photographer pairs that was successfully encoded during that session. Note that the random expectation for this score was 2, since each of the 12 pictures had a 1/6 chance of being randomly guessed correctly.

In the next session (1 day later for the picture–photographer pairs presented on sessions S1–S4, approximately 3 days later for session S0, and 4 h later for session S5), the subject was presented with another disjoint selection of 12 test images (2 pictures per photographer, randomly ordered, comprising test set 2 for the original session) and asked to identify which photographer took each picture. The number of correct choices of 12 possible was used as an estimate of the amount of the original data set of 36 picture–photographer pairs that was successfully retained over the delay interval. The final selection of 12 test images (2 pictures per photographer, randomly ordered, comprising test set 3 for the original session) was presented on a later session in order to get estimates of 3-day retention from the picture–photographer pairs presented on sessions S1 and S2, and estimates of approximately week-long retention (7–9 days) for the picture–photographer pairs presented on sessions S0, S3, S4, and S5.

Raven Puzzle Task

This problem-solving task is based upon the Standard Progressive Matrices of Raven (15), which is composed of a set of 60 progressively more difficult puzzles. In a preliminary study using a similar subject population ($n = 9$, data not shown), the puzzles were ranked in difficulty by the ratio of correct responses and the time to completion of a correct response. The easiest 10 with respect to this ranking criteria were used for demonstrating the task to the subject prior to testing on session S1. The next group of 10 easiest were used for the test set of session S1. The remaining 40 were arranged into four test sets for use in sessions S2–S5, balancing each set for equivalent difficulty using the above criteria. The order of presentation of these four equivalent test sets of 10 puzzles used for sessions S2–S5 was counterbalanced between test groups A and B.

Digit Canceling Task

This psychomotor task is based upon one described by Lezak (11) and is used to assess attention, vigilance, and sedation. The subject is presented with a page containing 36 rows by 20 columns of randomly generated, single-digit numbers printed in 4 mm height on a white sheet of paper. The subject is given a pencil and told to mark over ("cancel") all occurrences of three designated target digits, as accurately and as rapidly as possible in a 2-min period. The target sets and digit pages used for each session were counterbalanced between test groups A and B.

RESULTS

Odor Recognition Task

The experimental group had significantly poorer performance in odor recall for the 4-h delay on drug

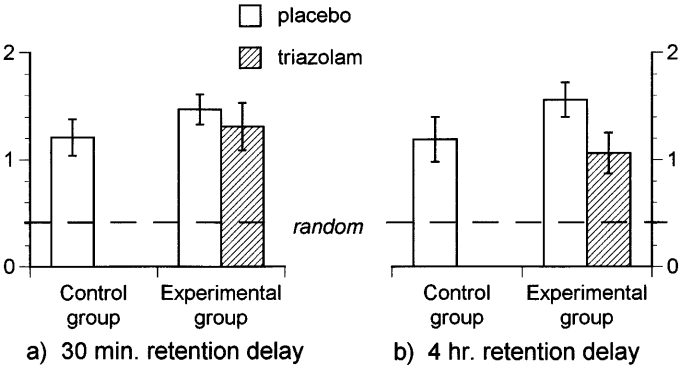

FIG. 1. Odor recognition task results by retention delay interval. One hour after either drug or placebo administration, the subjects sampled odors from two unlabeled flasks for 20 s each. After a delay of either 30 min or 4 h, the subjects were asked to identify the two odors, in the reverse order, from a randomly ordered lineup of five unmarked odor flasks. Shown are the number of correct responses of two possible (mean ± SE) for the control group (placebo only) and the experimental group (either placebo or 0.125 mg triazolam), along with the random expectation (dashed lines). (a) The triazolam treatment slightly reduced the scores after 30 min delay for the experimental group, but they were not significantly different from either their own placebo scores of from those of the control group. (b) After the 4-h delay, the experimental group triazolam treatment scores were significantly worse that their own respective placebo treatment scores ($P < 0.036$; t test).

versus placebo treatment sessions (Fig. 1b; S3 vs S4, within subject; $P < 0.036$; t test). No significant difference was observed between drug and placebo treatment for the experimental group on sessions with the 30-min delay (Fig. 1a). Note that the control group tended to perform worse than the experimental group placebo scores for both delay intervals.

Misplaced Objects Task

The experimental group made significantly more collision errors in the initial room choice encoding on the drug versus placebo treatment days (Fig. 2a; $P < 0.0158$; t test), indicating an impairment in immediate memory induced by the drug. The experimental group placebo collision error score was not significantly different from the control group score. During the delayed free-recall of object names, subjects in the experimental group identified fewer correct names after drug treatment for both delay intervals (Fig. 2b; $P < 0.0202$ for 20 min delay, $P < 0.0033$ for 4 h delay; t test) and exhibited a tendency to include falsely extra incorrect names at the longer delay interval (Fig. 2c; $P < 0.0602$ for 4 hr delay; t test). The experimental group also had a tendency for poorer object placement recall performance after the drug treatment, although this tendency only approached statistical significance for the shorter delay interval (Fig. 2d; $P < 0.0693$ for 20 min delay, $P < 0.0919$ for 4 h delay; t test).

Card Layout Task

The experimental group actually performed better on the drug treatment session than on the placebo treatment session for the short delay interval (Fig. 3a; $P < 0.0432$ for 1 min delay; t test), indicating a short-term facilitation effect of the drug on the card layout task. However, this may be primarily due to the rela-

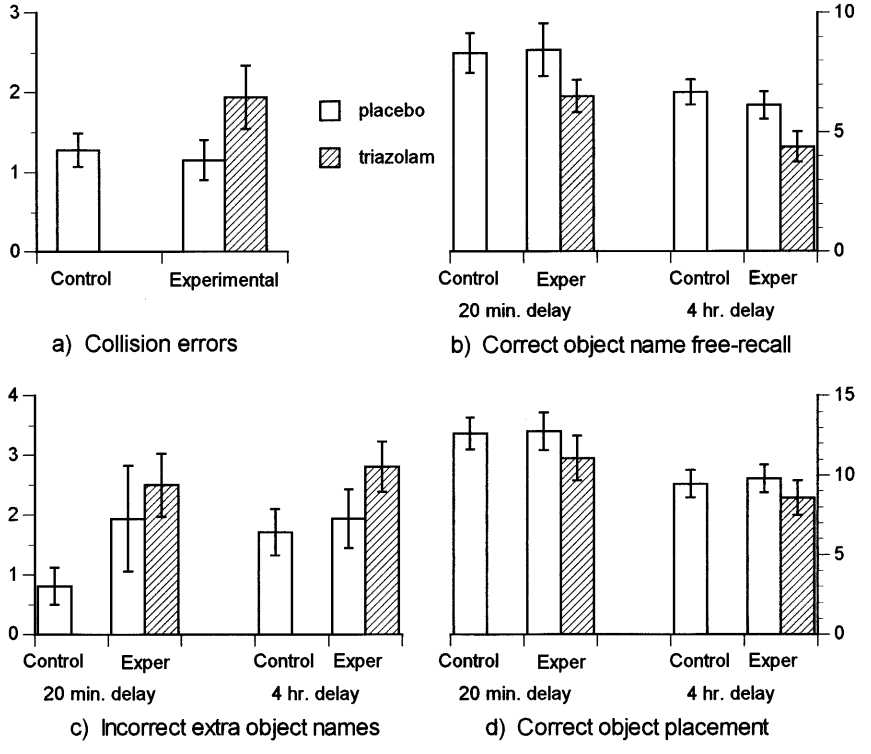

FIG. 2. Misplaced objects task results by retention delay interval. The subjects sequentially placed each of 20 cards representing common household objects into one of 12 "rooms" in a diagram of a house and were asked to remember in which room they had placed each object, with at most 2 objects in each room. After the subject had placed an object in a room, the tester removed the object from view. (a) Each attempt to place an object into a room in which 2 objects had already been placed was recorded as a "collision error" and the subject was asked to pick another room for that object. Shown are the number of collisions for each group (mean ± SE). On days after triazolam administration, the experimental subjects committed significantly more collision errors compared to days after placebo administration ($P < 0.0158$; t test). (b) After either a 20-min or a 4-h delay, each subject was asked to recall the names of the 20 objects shown that day in a free-recall format. Shown are the number of correctly recalled object names for each group after each delay amount. Experimental group subjects recalled significantly fewer names correctly after the triazolam treatment than subjects after the placebo treatment for both delay amounts ($P < 0.0202$ and $P < 0.0033$ for 20-min and 4-h delay, respectively; t test). (c) The number of incorrect extra names in the free recall was slightly greater for the experimental group with triazolam treatment, but this only approached statistical significance with the 4-h delay ($P < 0.0602$; t test). (d) After either a 20-min or a 4-h delay, each subject was shown each of the 20 objects (in a randomized order) and asked to point to the room that each object had been previously placed in. Shown are the total number of correct responses for each group. While the experimental group scores after triazolam tended to be worse than their respective placebo scores on each delay amount, the differences did not reach statistical significance ($P < 0.0693$ and $P < 0.0919$ for 20 min and 4 hr delay, respectively; t test).

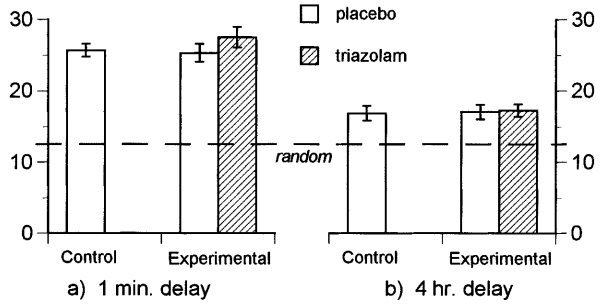

FIG. 3. Card layout task total correct by retention delay interval. The subjects were sequentially shown the faces of 8 cards from a box containing 16 cards in a 4 × 4 face-down arrangement. After a delay interval of either 1 min or 4 h, the subjects were asked a series of questions: to point to the locations of the 8 cards in the same order as they had been shown; to recall whether any of 6 verbally specified cards had been shown, and if so, to indicate its location in the box; to recall whether any of 6 cards pointed to by the tester had been shown, and if so, to indicate its face value; and to point out the 8 cards that were not shown. These questions had a total of 42 possible correct answers, with a random expectation of 12.7 (dashed lines). (a) The total scores after 1 min delay for the experimental group (mean ± SE) show significantly higher scores after triazolam treatment compared with placebo treatment ($P < 0.0432$; t test), indicating a possible short-term facilitation effect of the drug in this task. (b) After the 4-h delay, there was no difference between any group scores, although they had all dropped much closer to random values (dashed line).

tively poor performance of all subjects on the initial session S1, which was the session used for comparison on the 1-min delay in which all groups received placebo treatment. There was no discernible difference between the groups at the longer 4-h delay interval (Fig. 3b).

Photographer Biography Task

The control group did not show consistent acquisition scores in the photographer biography task for each session (Fig. 4a), as measured with the first question set approximately 10 min after the biography was read to the subjects. The initial session scores tended to be the lowest, but there was not steady improvement in later sessions. This brings into question the within-subject reliability of this task. However, the retention scores for question sets with the same delay amounts were not significantly different for each session, indicating that perhaps the retention scores are more reliable than the acquisition scores. As might be expected, the retention scores for 1-day delay were significantly below the acquisition scores (Fig. 4b; $P < 0.002$; t test). Although the retention score at 1-week delay was actually larger than the retention score at 3 days, the difference did not approach statistical significance, with both being significantly below the 1-day retention score ($P < 0.001$ for each compared with 1-day retention score; t test) and approaching zero, indicating that the control subjects did not recall the details of the biographical information correctly after more than a 1-day delay.

The experimental group showed more consistent acquisition scores for each session, as compared with the control group, even including the drug treatment sessions S2 and S3 (Fig. 4c). Although the S2 and S3 acquisition scores were slightly lower than the placebo session scores, these differences did not reach statistical significance. The 1-day and 1-week retention scores for question sets from biographies read after drug treatment on sessions S2 and S3 were significantly lower than the placebo session scores (Fig. 4d; $P < 0.001$ for 1-day; $P < 0.01$ for 1-week; t test). There was no significant difference between 3-day retention scores for triazolam and placebo treatments of the drug group. Although the experimental group placebo acquisition scores were slightly below the control group acquisition scores ($P < 0.085$; Mann–Whitney U test), there was no significant difference between the group retention scores for the control group and the placebo treatment scores for the experimental group with any other retention delay amount (Fig. 4e).

Picture–Photographer Association Task

The control group showed consistent acquisition scores in the picture–photographer association task on each session (Fig. 5a), as measured in the first test set given immediately after the picture presentations (approximately 10 min mean delay time per picture), so approximately the same proportion of pictures were initially encoded on each session. The retention scores on the 1-day delayed recall test sets were slightly lower for the pictures shown on session S1, indicating a small practice effect in the task, but were not significantly different from the scores for the other sessions. The retention scores on the 3-day delayed recall test sets tended to be lower for the pictures shown on session S1, but not significantly so. The retention scores on the 1-week delayed recall test sets from sessions S3, S4, and S5 were not significantly different from each other, but were still greater than the random expected score of 2.0 (Fig. 5a), indicating that the subjects still retained some information even after 1 week. It is also interesting that the score from the 4-h delayed recall test from the S5 pictures (although given to only four of the eight control subjects) is significantly lower than the acquisition score (Fig. 5b; $P < 0.002$; t test) and only marginally larger than the 1-day retention score ($P < 0.0964$; t test), indicating that the majority of the decay in retention measured after 1 day has likely already occurred within the first 4 h after presentation. This suggests that the retention in this task is subject to a fast initial decay on the order of a few hours, followed by a slower, long-term decay over a period of at least 1 week.

The experimental group showed consistent acquisition scores on each session, with slightly (but not significantly) lower scores on session S1, similar to the

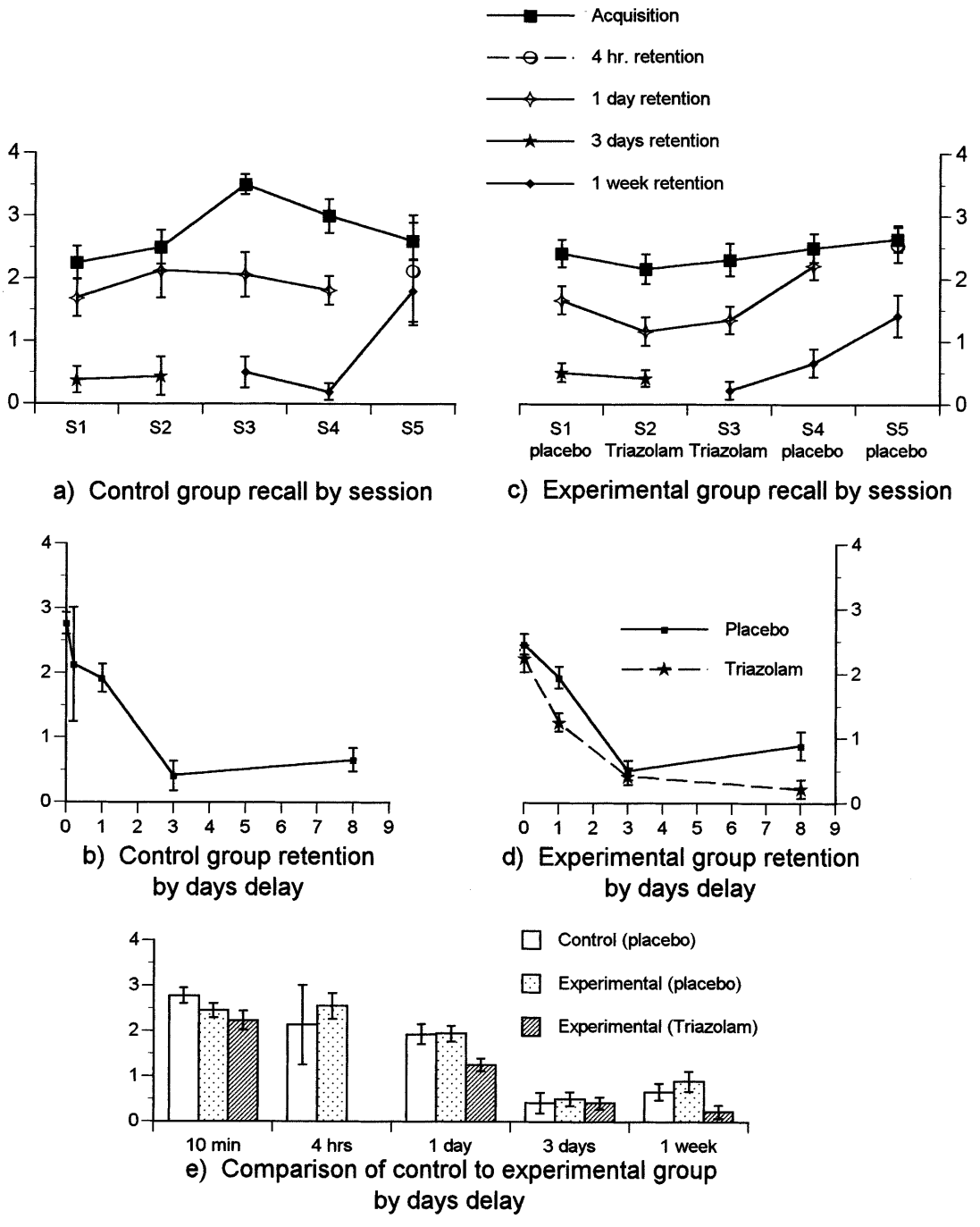

FIG. 4. Photographer biography task results. The subjects were shown portraits of six faces representing photographers for the picture–photographer association task (see text and Fig. 5). On each session, the subjects were read a short (approximately 100 word) biographical story for one of the photographers and asked to remember the details. After various delay intervals (7 min, 4 h, 1 day, 3 days, 1 week), subjects were asked a set of four verbal questions about the photographer (each photographer had three such sets of questions, each asked only once). (a) Shown are the scores for the control group (mean ± SE) for the particular biographies presented on each session, grouped by delay interval. The acquisition scores (7 min delay) were not consistent across sessions, although performance clearly deteriorated with increasing delay intervals. (b) Combining scores from the same delay intervals indicates a significant reduction in performance after 1 day delay ($P < 0.002$; t test), with further performance reduction at 3 days and 1 week delays ($P < 0.001$ for each compared with 1 day delay; t test). (c) Experimental group scores for each session grouped by delay intervals show more consistent acquisition performance than those for the control group, with a drop in performance for biographies given on the sessions with triazolam treatment (S2, S3). (d) Combined scores by delay interval and treatment condition indicate that both 1-day retention and 1-week retention were significantly worse for biographies presented on sessions after triazolam treatment than for those presented on sessions after placebo treatment ($P < 0.001$ and $P < 0.01$ for 1 day and 1 week, respectively; t test). (e) The experimental group placebo acquisition scores were slightly, but not significantly, below the control group acquisition scores ($P < 0.085$; Mann–Whitney U test), although there was no other significant non-drug-related group difference.

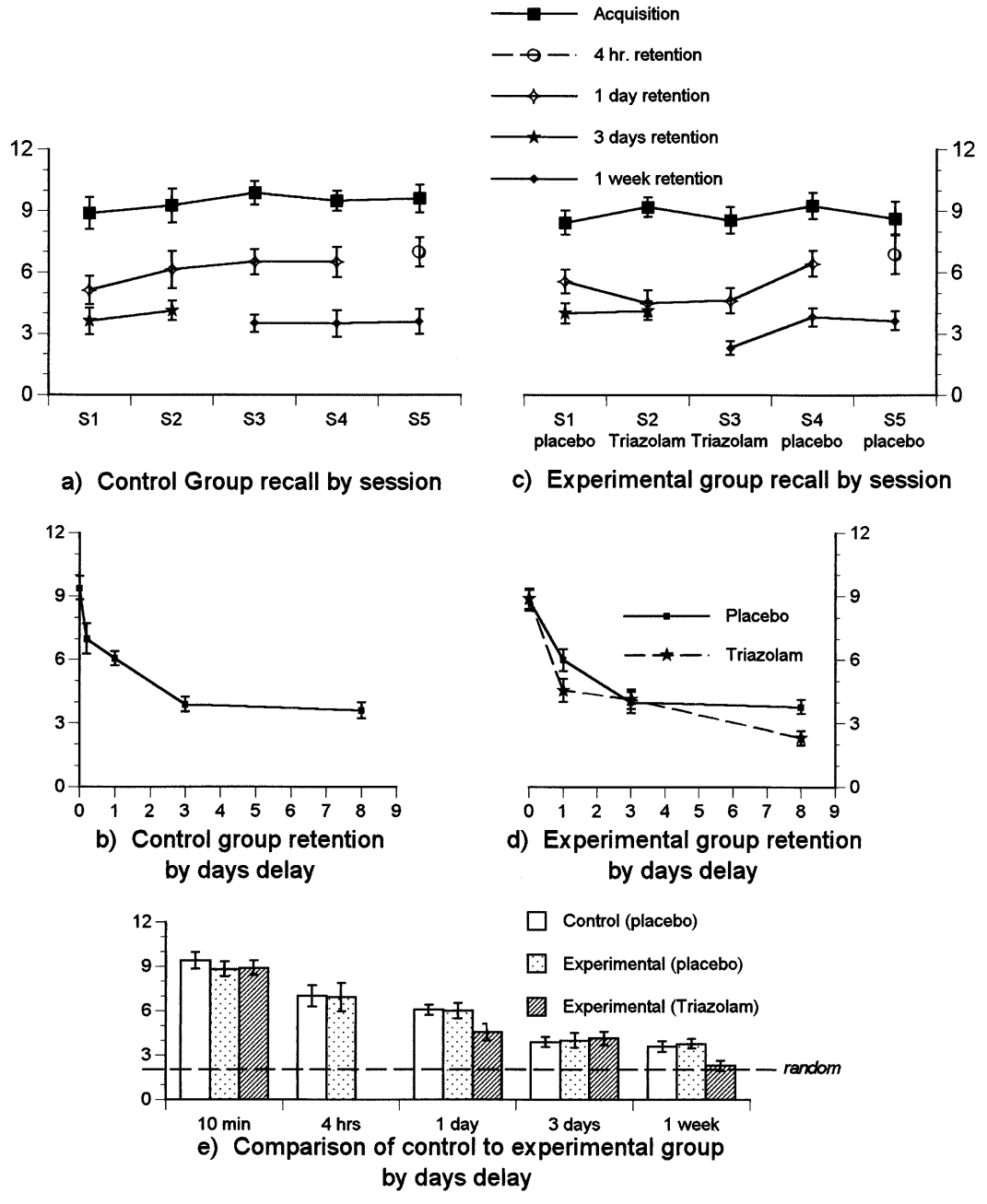

FIG. 5. Picture–photographer association task results. The subjects were shown 36 new pictures per session, each shown for 5 s simultaneously with a portrait of one of six faces (from the photographer biography task in Fig. 4) representing the photographer who "took" that picture. After various delay intervals (10 min, 4 h, 1 day, 3 days, 1 week), subjects were shown a randomly ordered subset of 12 of the pictures along with all 6 photographer portraits and asked to point to the correct photographer associated with each picture (each photograph was tested only once). (a) Shown are the scores for the control group (mean ± SE) for the photographs presented on each session, grouped by delay interval. All scores were consistent across sessions, although performance for pictures shown on the initial session were slightly lower. (b) Combining scores from the same delay intervals indicates a significant reduction in performance after 1 day delay ($P < 0.0001$; t test), with further performance reduction at 3 days and 1 week delays ($P < 0.0006$ for each compared with 1 day delay; t test). (c) Experimental group scores for each session grouped by delay intervals also show consistent performance, with a drop in performance for pictures shown on the sessions with triazolam treatment (S2, S3). (d) Combined scores by delay interval and treatment condition indicate that both 1-day retention and 1-week retention were significantly worse for pictures shown on sessions after triazolam treatment compared to sessions after placebo treatment ($P < 0.017$ and $P < 0.005$ respectively; t test). (e) The experimental group placebo scores were no different the control group scores. Note that the triazolam 1-week retention was not significantly above the random expectation score of 2.0 (dashed line).

control group (Fig. 5c). This indicates that the drug had no significant immediate effect on the encoding of picture–photographer associations (triazolam sessions S2 and S3 compared to placebo sessions S1, S4 and S5). The 1-day and 1-week retention scores for pictures presented after drug treatment on sessions S2 and S3 were significantly lower than placebo scores (Fig. 5d; $P < 0.017$ for 1 day, $P < 0.005$ for 1 week; t test). In fact, the 1-week retention score for drug treatment was not significantly above chance expectation (2.31 ± 0.33, mean $\pm$ SE; random expectation 2.0). There was no significant difference between 3-day retention scores for triazolam and placebo treatments for the experimental group. Furthermore, there was no significant difference between the group scores for the control group and the placebo treatment scores for the experimental group on any delay amount (Fig. 5e).

Raven Puzzle Task

The control group showed progressive improvement in the Raven puzzle task between each session (Fig. 6). One possible explanation is that the subjects were able to transfer some newly acquired procedural knowledge of the techniques needed to solve the puzzles from one session to the next, using that knowledge to improve their future scores. By contrast, the experimental group showed no apparent improvement until the last session of the week (S5). The drug apparently impaired the ability of the subjects to transfer the new procedural knowledge gained on a triazolam treatment day to the next session, thus preventing improvement. However, once the experimental subjects were allowed to perform the task without drug on session S4, they were apparently able to use the knowledge gained during this session to improve their scores significantly on the final placebo session S5 ($P < 0.01$ for S5 compared to S4; t test).

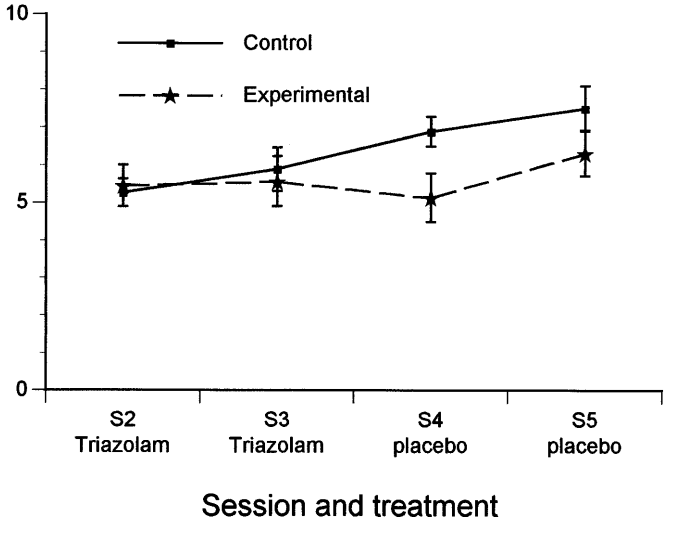

FIG. 6. Total correct for Raven matrix task. The subjects were shown 60 puzzle-solving tasks from the Standard Progressive Matrices of Raven (15), arranged in sets of 10 each, including a demonstration set, and easy set (session S1) and 4 sets previously ranked to have approximately equal difficulty (sessions S2–S5). Shown are the scores (mean $\pm$ SE) for each group. The control group score improved each session in a linear fashion. The experimental group showed no improvement until a session occurred without drug treatment ($P < 0.01$ for session S5 compared to S4).

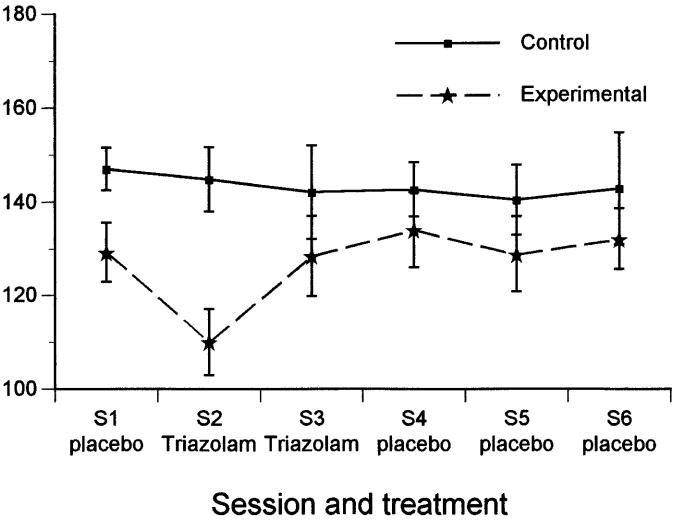

FIG. 7. Number of target digits correctly marked in 2 min for digit canceling task. The subjects were given a page containing 36 rows by 20 columns of randomly generated digits and asked to mark over ("cancel") with a pencil all occurrences of three designated target digits within 2 min. Shown are the numbers of digits correctly marked (mean $\pm$ SE) for each group. The control group scores were essentially constant for each session. The experimental group scores were significantly lower on session S2 compared with all other sessions ($P < 0.002$; t test), which was 1.5 h after triazolam administration. Their scores on session S3 (4.5 h after triazolam administration) were no different from their scores on placebo sessions (S1, S4, S5) or on the follow-up (no medication) session (S6).

Digit Canceling Task

The number of target digits correctly marked over for each control subject was essentially constant across sessions, with no significant differences between any of the sessions (Fig. 7). For the experimental group, the only session with a significantly different score was the first drug treatment session (S2), in which the task was administered 1.5 h postmedication. For this session, the scores were significantly lower than on all other sessions (Fig. 7; $P < 0.002$; t test), which is consistent with the sedative effects of triazolam. For the other drug treatment session (S3), in which the digit canceling task was administered in the afternoon segment (5.5 h postmedication), the scores were not significantly different from those on the placebo sessions (S1, S4, S5) or on the follow-up session (S6). This is consistent with triazolam's reported active half-life of 4.5 h (14) and the relatively low medication dose of 0.125 mg used in this

study. The mean number of nontarget digits incorrectly marked over was never significantly greater than zero for either the experimental or the control groups on any session, indicating that accuracy in this task was unaffected by drug treatment.

DISCUSSION

The dose of 0.125 mg triazolam used in this study was enough to have a measurable impairment of psychomotor performance, as measured in the digit canceling task, at 1.5 h postmedication. However, the drug did not impair recall of previously learned information, nor did it impair immediate recall in most tasks, indicating that the effect is not a general impairment of recall, nor a general impairment of encoding. In fact, the drug possibly enhanced the immediate recall of the location and identity of playing cards, although this observed effect may be an artifact of the tendency to score lower on the initial testing session. The only task to show any drug-induced impairment of immediate recall was the increase of room collision errors in the misplaced objects task. The drug had no significant immediate effect on the recall of details from a verbal biography, nor in correct identification of picture–photographer associations.

For relatively short delay amounts of 20–30 min, the drug impaired free recall of object names and slightly impaired object placement in the misplaced objects task, although there was no impairment in the odor recognition task at this delay. With a 4-h delay, the drug impaired cued odor recall, impaired free recall of object names as well as increased inclusion errors of incorrect names, and slightly impaired correct object placement in the misplaced objects task, while not affecting correct recall of the location and identity of playing cards. For the long-term delay amounts of 1 day to 1 week, the drug impaired the recall of details from a verbal biography and correct identification of picture–photographer associations.

The memory impairments with triazolam treatment described above seem dependent upon the testing delay interval, not upon either the stimulus or memory type. Similar time-dependent deficits were observed for tasks involving olfactory, verbal, spatial, and visual stimuli. Although we were unable to determine if any specific factors in the particular tasks (such as reliance on explicit vs procedural memory, loading factors, task pacing) contributed significantly to these impairments, further work should help to clarify which types of tasks are most vulnerable to triazolam impairment.

The observation that triazolam impairs memory is not a new result, and the current findings are in agreement with many previous benzodiazepine studies. What is new and interesting in the present results is the detail of the time course of the triazolam effects and the comparison with its reported effects on long-term potentiation (LTP) over these same time periods. That triazolam did not impair recall of previous information in any task is consistent with many previous reports (for reviews see 2, 5) and is also consistent with triazolam's lack of effect on monosynaptic burst responses in piriform and hippocampal slices (4), indicating that it presumably does not affect the expression of previously encoded LTP events. Similarly, triazolam's relative lack of impairment in immediate recall over delays from seconds to minutes is also consistent with the drug's lack of effect on the induction and initial expression of LTP (4). However, at delays greater than 15–30 min, recall performance began to be impaired in many of the tasks in this test battery, consistent with results demonstrating that triazolam can cause recently induced potentiation to decay back to baseline by this time (4). However, even though the time courses of these impairments are consistent with triazolam's effects on LTP, it is unknown which aspects of the tasks are directly LTP-dependent.

Recently, novel centrally active AMPA-type glutamate receptor enhancers ("Ampakines"), which enhance LTP (19), have also been shown to enhance memory retention in rats (6, 7, 10, 19). Furthermore, one of these compounds has been shown to enhance human memory performance in many of the same tasks presented in the current study (9). Taken together with the memory deficits reportedly produced by LTP-impairing agents in animals (13, 3) and humans (2, 5), these findings add significant evidence to the hypothesis that LTP underlies certain forms of mammalian memory.

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