EXPERIMENTAL NEUROLOGY **146,** 553–559 (1997)
ARTICLE NO. EN976581

Enhancement by an Ampakine of Memory Encoding in Humans

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A centrally active drug that enhances AMPA receptor-mediated currents was tested for its effects on memory in humans. Evidence for a positive influence on encoding was obtained in four tests: (i) visual associations, (ii) recognition of odors, (iii) acquisition of a visuospatial maze, and (iv) location and identity of playing cards. The drug did not improve scores in a task requiring cued recall of verbal information. The selectivity of drug effects on memory was confirmed using tests of visual recognition, motor performance, and general intellectual functioning. These results suggest that positive modulators of AMPA receptors selectively improve at least some aspects of memory.

INTRODUCTION

Facilitation of glutamatergic transmission promotes the formation of long-term potentiation (1), a type of synaptic plasticity hypothesized to be involved in the encoding of memory (5). Recently, a novel class of drugs ("ampakines") has been developed which augments currents gated by AMPA-type glutamate receptors in excised patches (3) and, as expected from this, increases the size of excitatory synaptic responses in brain slices (13, 25). These compounds rapidly cross the blood–brain barrier and enhance field EPSPs as well as long-term potentiation in freely moving rats (25, 26). Behavioral studies indicate that ampakines improve the encoding of various forms of memory in rats in tasks involving spatial cues (13, 25), odor discriminations (17), eyeblink conditioning (21), and cue matching (26). The drugs are sufficiently potent to reverse the memory deficits that occur in middle-aged rats (14). The consistency of effects noted across such a variety of paradigms (7) suggests that ampakines have a generalized effect on plasticity mechanisms and thereby enhance learning and memory.

The present study tested if, as predicted from the above-cited results, ampakines have positive effects on human memory. The drug selected for the experiment, 1-(quinoxalin-6-ylcarbonyl)piperidine (CX516; previously BDP-12) is known to have the physiological profile described above (2, 7) and has been widely used in animal studies. CX516 was recently tested for safety in humans at dosages up to 1200 mg and, as part of this effort, evidence was obtained suggesting that it has a positive effect on the recall of nonsense syllables (19).

METHODS

Subjects

Twenty-four subjects were included in the experiments reported here. Prospective subjects were solicited from the population of medical students resident at the Karolinska Hospital. Assignment to groups was random, and all testing was double blind. Inclusion criteria were healthy males 20 to 35 years of age (25.4 ± 1.2 for experimental group, $24.7 \pm .92$ for control group, $x \pm$ SE) with (i) willingness to participate and adhere to the experimental schedule; (ii) no known history or sign of head trauma, birth complication, or substance abuse, (iii) absence of major kidney, liver, or heart disease and of epilepsy disorder including next of kin; (iv) no concomitant medication; and, to reduce variability in the odor, maze, and biography tests, to be (v) nonsmokers, right handed, and native speakers of Swedish. A physical examination including blood pressure, blood tests, and a clinical EEG predated participation in the study. Of all those initially screened, two were excluded due to ongoing medication, five due to inability to conform to the planned test schedule, and one delayed exclusion after 1 day of testing due to a late report of abnormal s-ASAT and s-ALAT blood tests. All other subjects who entered the study concluded the program.

General Procedures

Following initial interviewing and acquaintance with the testing procedures, each subject visited the laboratory on 5 consecutive days plus one follow-up visit 6 or 7 days later. Several assays of memory as well as of simple visual recognition, motor performance and coordination, and general intellectual performance were included in the daily test battery, which was administered during a 45-min period commencing 20 min after

0014-4886/97 $25.00

oral ingestion of a drug or placebo capsule. All subjects received capsules on each of the 5 testing days; all of the capsules given to control subjects ($n = 12$) were placebo capsules; experimental subjects ($n = 12$) were given placebo capsules on Days 1, 4, and 5 and identical CX516 capsules (300 mg) on Days 2 and 3. This design allowed for within-subject (drug vs no drug days) as well as between-group comparisons. All aspects of the study were conducted in a double blind fashion, and scores were not unblinded until all subjects had completed the entire study. The testing regimen was drawn from that used in a previous study in which the effects of oral triazolam on memory encoding and retention were assessed (8).

For all comparisons, statistical tests were run to ascertain that performance was correlated within subject between conditions; if not, then statistical significance was not imputed. For instances in which scores were found to be correlated, statistical tests were run. All changes seen were in the predicted direction (improvement of performance with ampakine), justifying one-tailed statistical tests; nonetheless, two-tailed tests of statistical significance are reported on all tasks except one.

Verbal and Visual Cues

Subjects were read a ~250-word biographical sketch containing 12 items of information (e.g., name, age, place of birth, etc.) for each of six "photographers" on the initial interview day and on each of the subsequent five sessions. Following the biography, they were presented with 36 digitized color photographs (of diverse subjects taken from popular magazines, e.g., National Geographic) on a computer screen, for 5 s each, alongside a portrait of one of the six "photographers" and were asked to remember which "photographer" had taken each picture.

All stimuli were presented randomly and controlled for order of presentation. The material was tested in three parts, each with four biographical questions and 12 pictures, the first to be tested at once (approximately 10 min after presentation), the second the next day, and the last one after either 3 or 7–8 days. Material was therefore never tested more than once, minimizing any effects of rehearsal. This testing schedule was designed to enable within-subject comparisons at multiple latencies as well as between-subject comparisons.

After the appropriate delay, subjects (Ss) were asked to recall the answer to four biographical questions (a point for each correct answer with partial credit for partial answers) and were then asked to select, from a screen containing all six "photographers," the one who took each of 12 individually presented pictures. One point was scored for each correct identification.

Olfactory Cues

Five scents designated A through E, which had been shown in previous tests to be sufficiently distinct to enable recognition and yet lacked obvious verbal descriptions (8), were dissolved in glycerine to achieve similar moderate intensities and placed in salt-shaker-size opaque flasks. All five scents were presented to the Ss on the initial interview day. On each test session, Ss were asked to sample two designated odors for 20 s apiece in immediate succession and after the appropriate delay (45 min on Days 1, 2, and 5; 220 min on Days 3 and 4) were allowed to sample all five odors freely and asked to identify, in order, those presented earlier. The subject was never permitted to touch the flasks. Each identification of a correct odor in the right sequence was scored one point, such that the maximum possible score was two. No points were given for odors identified correctly but in the wrong order. All Ss were given odor pair CB on Day 1. The sequence for Days 2, 3, 4, and 5 was BD, DE, AC, EA for half of the controls and half of the experimental subjects; the reverse sequence (EA, AC, DE, BD) was followed for the remaining Ss.

Spatial Mazes

Ss were seated at a computer on which was displayed a maze pattern, through which they could maneuver by tapping keys corresponding to four directions (left, right, up, down). Ss could back up (undo a previous movement through the maze) by tapping an "erase" key. A different maze was employed in each session. An overall time limit of 2 min for any given maze was imposed; after 1 min and 45 s a warning message asked the subject to complete the best pathway within 15 s. After completion, the subject could still make changes and was required to press a "completion" key to end a maze run. The composite score was composed of (i) median number of correctly traversed maze segments per second; (ii) median time in seconds between completion and acknowledging the solution by pressing the completion key; (iii) 10th percentile of responses faster than 800 ms; (iv) number of erasures (see ref. 11).

Types and Positions of Playing Cards

In each session, a different selection of 8 cards from a 4×4 array of 16 face-down playing cards was turned over and shown in place to Ss for 4 s apiece, in succession. After the appropriate delay (1 min on Days 1, 2, and 5; 220 min on Days 3 and 4) the subjects were asked to do the following: (i) name the cards that had been shown, in the order shown; (ii) indicate (yes or no), for each of six locations in the card array, whether that card had been shown (three had) and, following a positive answer, name the face-down card at that location; (iii) for 6 named cards (e.g., red queen, black eight), to indicate (yes or no) whether that number–

color combination had been shown (three had) and, following a positive answer, to point to the location of that card in the array; (iv) point to the locations of the 8 cards in the array that had not been shown. Each correct answer was scored 1 point, and they were added to yield the composite score, except for task (i), which was scored 1 point for each card named in the right order, plus 1 point for each card that had been shown irrespective of order. The maximum score was thus 42.

Digit Cancellation Task

Ss were given 2 min in each session to cross out, as rapidly and accurately as possible, all instances of three named digits from a sheet of paper containing 36 rows and 20 columns of randomly generated digits. The test is based on a design described in ref. 18 which is reported to be sensitive to alterations in vigilance and attention.

Finger Tapping Test

This motor performance test consisted of five phases, each performed for 15 s: (i) tapping with right index finger on a keyboard key, (ii) tapping with left index finger, (iii) alternating right index and middle fingers, (iv) alternating left index and middle fingers, (v) alternating right and left index fingers. Each part of the motor sequence was timed. The score combined number of taps per second, maximum time (ms) of key up, maximum time of key down, and means and SDs of key up and key down.

RESULTS

Associative Memory for Complex Visual Cues

On the picture:photographer association portion of the test, Ss in both groups had very high scores (10–11 correct of a possible 12), with a 10-min delay, but showed significantly less retention 24 h later. Importantly, within-subject correlations across days of testing were evident under both 10-min and 1-day delay conditions, indicating that the test reliably sampled individual differences in the encoding/retention of visual associations across these latencies. Figure 1 summarizes the group data for these two conditions. Ss in the experimental group exhibited significantly better delayed retention for material learned on drug days (2 and 3) than they did for material learned on placebo days (1 and 4) ($P = .028$, paired two-tailed t test). Placebo subjects had virtually identical delayed retention scores for associations acquired on Days 1 and 4 vs those acquired on Days 2 and 3.

There were no meaningful within-subject correlations across days for tests carried out with longer delays nor did the scores correlate with those at shorter delays. The biography test also failed to produce reliable within-subject

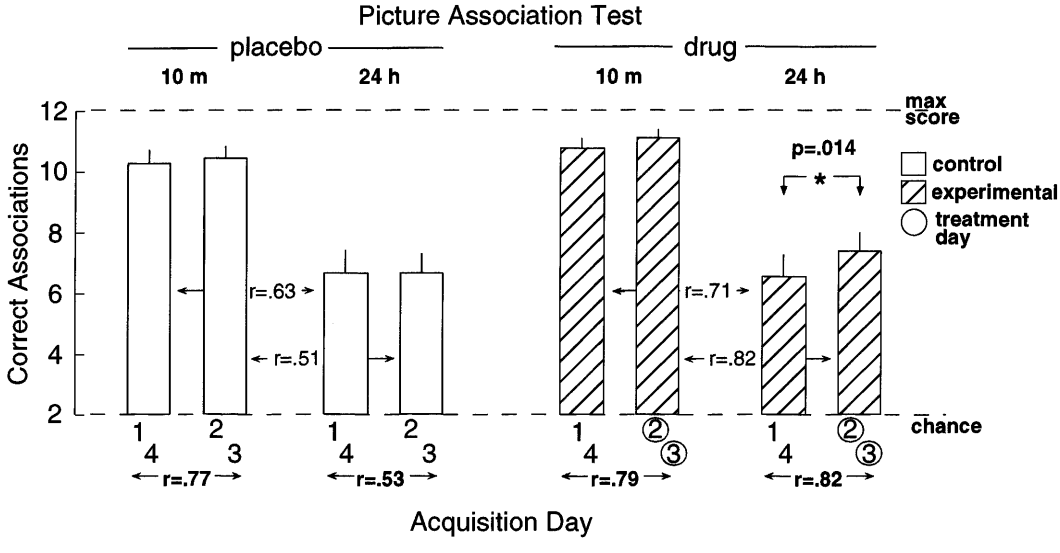

FIG. 1. Retention scores ($x \pm$ SE) in an associative memory task. Ss were presented with novel picture:photographer combinations on Days 1, 2, 3, and 4 and then tested for retention 10 min (10 m) and 24 h later. CX516 was administered to subjects in the experimental group prior to acquisition on Days 2 and 3. Each subject's retention scores for associations learned on Placebo Days 1 and 4 were compared with those for material acquired on Drug Days 2 and 3. (Left side) Results for subjects ($n = 12$) given placebo (open bars) on all days. Immediate retention was excellent (10 of a maximum possible score of 12, where 2 is chance), and within-subject scores for Days 1 and 4 vs 2 and 3 were correlated (horizontal bars below). Delayed (24 h) retention was poorer and not different for Days 1 and 4 vs Days 2 and 3. (Right side) Results for subjects ($n = 12$) given CX516 (striped bars) on Days 2 and 3 and placebo on all other testing days. Within-subject retention scores on the delayed test were higher for the picture associations acquired on Drug Days 2 and 3 than for those presented on Nondrug Days 1 and 4 ($P = 0.028$, paired t test, two-tailed).

results across days. CX516 did not measurably affect encoding under these uncorrelated conditions.

Recognition Memory for Odors

Comparisons for drug vs placebo groups are shown in Fig. 2. As expected from past studies (8), some odor pairs were more easily discriminated and/or remembered than others. The left panel of the figure summarizes the results for the subgroups administered the "easy" pairs on Test Days 2 and 3; as indicated, drug (striped bar) and placebo (open bars) Ss approached the ceiling for these pairs. The right panel of Fig. 2 illustrates the data for the subgroups who received the "difficult" pairs on Test Days 2 and 3; performance by the CX516 group was superior to that of control subjects ($P < 0.035$ vs controls tested during the same sessions; $P < 0.03$ for all controls, Mann–Whitney U tests, one-tailed).

Learning of a Visuospatial Maze

Ss were tested each day on a computerized maze test described in prior publications. Learning in this paradigm is assessed as improvement over days rather than as recognition or recall of specific material (11). Day 2 scores for placebo Ss were well correlated with those for subsequent days and were used as a baseline measure. Thus the experimental question was whether Ss given CX516 on Days 2 and 3 would show greater improvement as measured on Day 4 (the first postdrug day) than those receiving placebos on Days 2 and 3. The results are summarized in Fig. 3. As shown, the two groups had the same scores on Day 2 but within-subject improvement (right panel) was substantially greater for the CX516 group ($P = .04$; two-tailed t test). Nearly 30% of the variance in the experimental group was due to a single outlier; excluding this individual from the analysis substantially increased the magnitude and statistical significance of the differences ($P = .012$; two-tailed t test).

Recall of Visual and Spatial Information

Scores for individuals in the experimental group varied markedly across days and immediate recall was not predictive of delayed recall. Comparisons between groups revealed a tendency for superior performance in immediate recall by subjects who received CX516 vs those in the control group ($P = .063$; unpaired two-tailed t test); delayed recall scores were highly variable and not significantly different between groups (see Fig. 4).

None of the Ss attributed any subjective experience to the ingestion of drug, and daily guesses of the content of the capsules resulted in chance scores. A limited inventory for mood changes did not reveal any effects. Finally, CX516 had no detectable influence on tests in which Ss were required to (i) cross out as many

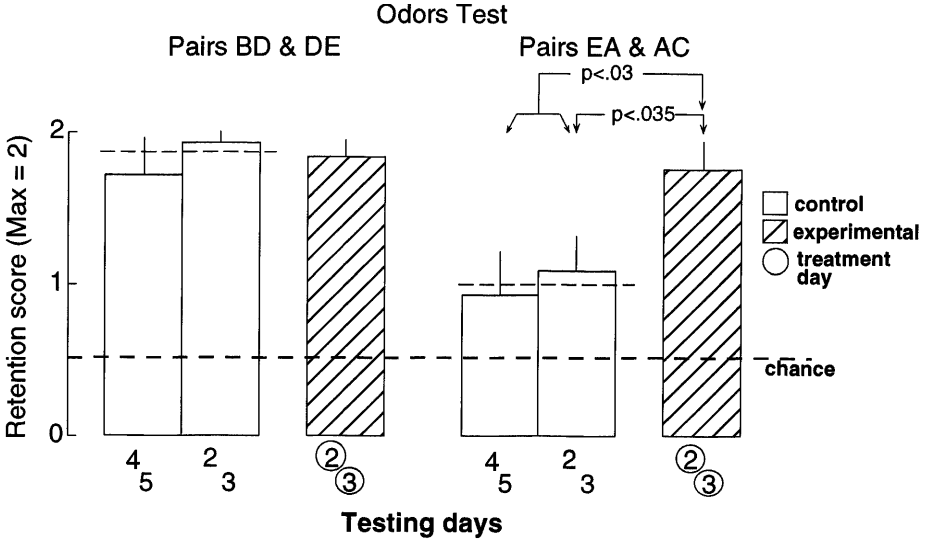

FIG. 2. Retention scores ($x \pm$ SE) in an olfactory recognition task. Ss were presented with two scents in succession. After a delay of 45 (Days 1, 2, and 5) or 220 (Days 3 and 4) min, Ss were asked to select the two previous odors from the group of five and to specify the order in which they had been sampled. (Left side) Results ($x \pm$ SE) obtained with the odor pair BD vs DE. Subjects administered drug (striped bar) and their controls on the same days (open bars, 2 and 3) and on different days (open bars, 4 and 5) had near ceiling retention scores for these odors. The dotted line denotes the average for all controls ($n = 12$). (Right side) Results obtained with odor pairs EA vs AC. Control subjects had much poorer retention for these odors than for pairs BD and DE; subjects administered CX516 had substantially better retention than placebo subjects tested during the same sessions (open bars, 2 and 3) and those tested during different sessions (open bars, 4 and 5); again, the dotted line denotes the average for all controls ($n = 12$). The difference between drug and placebo subjects is statistically significant ($P = .035$ vs controls tested during the same sessions; $P = .03$ vs all controls, Mann–Whitney U test, one-tailed; $P = .043$ vs all controls, unpaired t test, one-tailed).

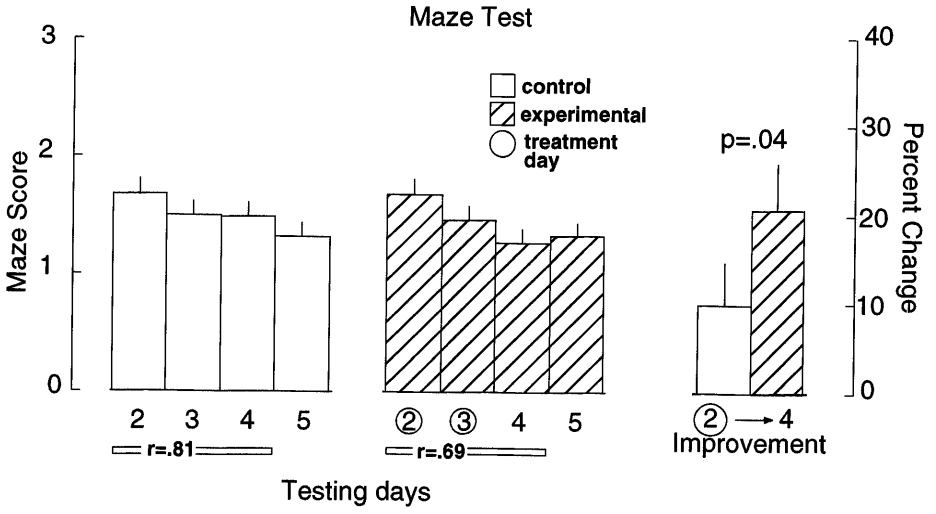

FIG. 3. Shown are composite performance scores (see ref. 10) for placebo (open) and drug groups (striped) across Testing Days 2–5 ($x \pm$ SE) for a visuospatial maze task. Initial (Day 2) scores were identical for both groups; as subjects acquired skill in the task, scores improved (became lower) while remaining highly correlated within subjects (horizontal bars). After 2 consecutive days of receiving CX516 (Days 2 and 3), within-subject scores in the drug group had improved approximately twice as much as those of the placebo group (right panel; $P = .04$; two-tailed t test).

as possible of three indicated digits from a sheet filled with single digits (18) or (ii) repeatedly tap left or right fingers alone or in combination (see Fig. 5).

DISCUSSION

Safety trials with CX516 have used doses up to 1200 mg but did not include the successive daily administrations required by the design of the present study. Accordingly, a relatively low dose of 300 mg was employed. This approximately 4 mg/kg level is below the threshold doses (12.5 mg/kg) found to reliably enhance memory in animal studies (13), but it is not unusual for effective human dosages to be significantly lower than those for rats. For instance, diazepam produces psychoactive effects in humans at ⅓ to ⅒ the threshold concentrations needed to alter exploratory activity by rats (6, 16).

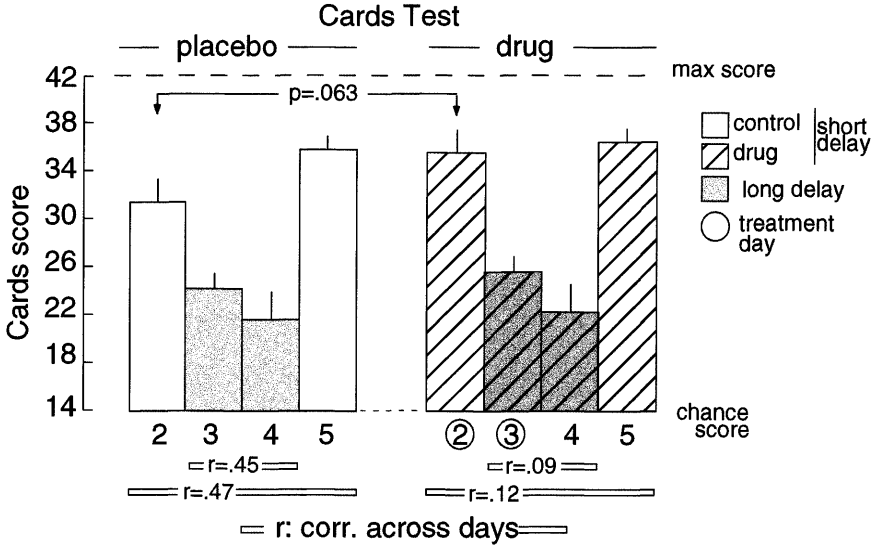

FIG. 4. Composite retention scores are shown for placebo (open) and drug groups (striped) on Testing Days 2–5, at delays of 1 min (Days 2 and 5) or 4 h (Days 3 and 4; shaded) on a card placement task. No changes were evident between groups on those days when the drug group received placebo (Days 1 and 4). Within-subject variance was high and scores on this task across days were uncorrelated (horizontal bars). On Day 2, drug subjects (striped) receiving CX516 exhibited somewhat higher scores than placebo subjects in the immediate (1-min) retention test ($P = .063$; two-tailed t test).

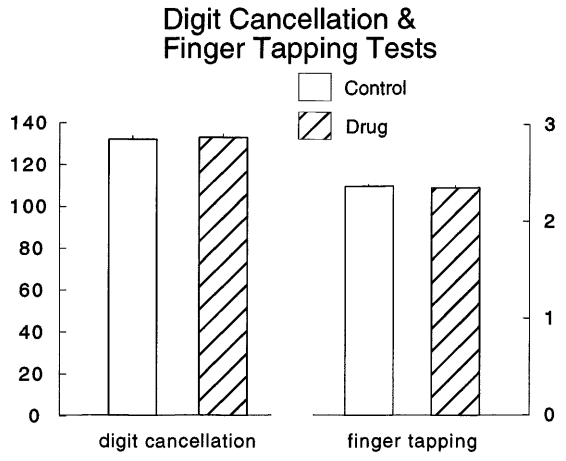

FIG. 5. Composite scores ($x \pm$ SE) are shown for two tests, digit cancellation and finger tapping, for Days 2–5 of the task. No differences among groups were evident, and no effect of CX516 was seen.

It is noteworthy that all changes observed in the study were in the direction predicted, i.e., subjects receiving ampakine exhibited better performance on drug days than either their own performance on nondrug days or than the performance of the control group.

The paradigms in which CX516 had positive effects (pictures, maze, odors, and perhaps cards) are likely to sample different memory systems. One-trial encoding of visual associations involves episodic or data memory and is generally thought to be dependent upon temporal lobe structures including hippocampus. In contrast, the maze test exploits incremental learning of a complex skill, something which presumably incorporates procedural memory. PET studies utilizing this particular task indicate that the primary areas of activation during the task include the parietal lobes, posteriorlateral cortex, and premotor areas; decreased activity is seen in the medial temporal cortex including hippocampus and in medial prefrontal cortex (12).

The olfactory test differs from the other paradigms in its dependency on primary paleocortical regions (e.g., piriform cortex) as opposed to sensory neocortex. Work with animals indicates that acquisition of odor discriminations in the absence of overtraining is dependent upon the retrohippocampal cortex (10, 23) and there is evidence implicating the dorsomedial nucleus–frontal cortex system as well (9, 22, 24). Improvement with ampakine administration on similar odor recognition tasks has been found in animals (17, 26).

In all, then, it appears that the effects of the ampakine CX516 are not restricted to particular cortical systems or to narrow categories of cortically dependent memory. At the same time, they are not attributed to general arousal or to an influence on supervisory attentional systems; the digit cancellation and finger tapping tests are both sensitive to these variables (20) and neither was affected by the drug.

The present results provide evidence that facilitation of AMPA receptors constitutes a new route for enhancing at least some aspects of memory. It will be of considerable interest to test higher doses of the drug used in the present study and to explore the far more potent ampakines that have recently become available (4, 7).

ACKNOWLEDGMENTS

This work was supported in part by the Air Force Office of Scientific Research, the Swedish Medical Research Council, The Swedish Bank Tercentenary Foundation, The Knut and Alice Wallenberg Foundation, and Cortex Pharmaceuticals, Inc. We sincerely thank Ellenor Andersson, Predrag Petrov, and Rasha Zabaneh for assistance in running human trials and Jim Whitson for his assistance in assembling the picture association test.

REFERENCES

1. Arai, A., and G. Lynch. 1992. Factors regulating the magnitude of LTP induced by theta pattern stimulation. *Brain Res.* **598:** 173–184.
2. Arai, A., M. Kessler, G. Rogers, and G. Lynch. 1996. Effects of a memory enhancing drug on AMPA receptor currents and synaptic transmission in hippocampus. *J. Pharm. Exp. Ther.* **278:** 627–638.
3. Arai, A., M. Kessler, P. Xaio, J. Ambros-Ingerson, G. Rogers, and G. Lynch. 1994. A centrally active drug that modulates AMPA receptor gated currents. *Brain Res.* **638:** 343–346.
4. Arai, A., and G. Lynch. 1996. Response to repetitive stimulation of AMPA receptors in patches excised from fields CA1 and CA3 of the hippocampus. *Brain Res.* **716:** 202–206.
5. Bliss, T. V. P., and T. Lømo. 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol.* **232:** 331–356.
6. Clarke, P. R. F., P. S. Eccersley, J. P. Frisby, and J. A. Thornton. 1970. The amnesic effect of diazepam (Valium). *Br. J. Anaesthesiol.* **42:** 690–697.
7. Davis, C. M., B. Moskovitz, M. A. Nguyen, A. Arai, G. Lynch, and R. Granger. 1997. A profile of the behavioral changes produced by facilitation of AMPA-type glutamate receptors. *Psychopharmacology.* [In press]
8. Davis, C. M., J. Ambros-Ingerson, R. Granger, J. Wu, R. Zabaneh, M. Abdelnaby, and G. Lynch. 1997. Triazolam impairs delayed recall but not acquisition of various everyday memory tasks in humans. *Exp. Neurol.* [In press]
9. Eichenbaum, H., K. Shedlack, and K. Eckmann. 1980. Thalamacortical mechanism in odor guided behavior. I. Effects of lesions of the mediodorsal thalamic nucleus and frontal cortex on olfactory discrimination in the rat. *Brain Behav. Evol.* **17:** 255–275.
10. Eichenbaum, H., A. Fagan, and N. J. Cohen. 1986. Normal olfactory discrimination learning set and facilitation of reversal learning after medial-temporal damage in rats: implications for an account of preserved learning abilities in amnesia. *J. Neurosci.* **6:** 1876–1884.
11. Elithorn, A., S. Mornington, and A. Stavrou. 1982. Automated psychological testing: Some principles and practice. *Int. J. Man–Machine Stud.* **17:** 249–263.
12. Ghatan, P., J. Hsieh, A. Wirsenmeurling, R. Wredling, and others. 1995. Brain activation induced by the perceptual maze

test—A pet study of cognitive performance. *Neuroimage* **2:** 112–124.

13. Granger, R., U. Staubli, M. Davis, Y. Perez, L. Nilsson, G. Rogers, and G. Lynch. 1993. A drug that facilitates glutamatergic transmission reduces exploratory activity and improves performance in a learning dependent task. *Synapse* **15:** 326–329.
14. Granger, R., S. Deadwyler, M. Davis, B. Moskovitz, M. Kessler, G. Rogers, and G. Lynch. 1996. Facilitation of glutamate receptors reverses an age-associated memory impairment in rats. *Synapse* **22:** 332–337.
15. Hughes, R. 1993. Effects on open-field behavior of diazepam and buspirone alone and in combination with chronic caffeine. *Life Sci.* **53:** 1217–1225.
16. Korttila, K., and M. Linnoila. 1974. Skills related to driving after intravenous diazepam, flunitrazepam or droperidol. *Br. J. Anesthesiol.* **46:** 961–969.
17. Larson, J., T. Lieu, B. LeDuc, H. Ngo, G. A. Rogers, G. Lynch. 1995. Facilitation of olfactory learning by a modulator of AMPA receptors. *J. Neurosci.* **15:** 8023–8030.
18. Lezak, M. 1983. *Neuropsychological Assessment*, 2nd ed. Oxford Univ. Press, New York.
19. Lynch, G., M. Kessler, G. Rogers, J. Ambros-Ingerson, R. Granger and R. S. Schehr. 1996. Psychological effects of a drug that facilitates brain AMPA receptors. *Int. Clin. Psychopharmacol.* **11:** 13–19.
20. Posner, M., and J. Driver. 1992. The neurobiology of selective attention. *Curr. Opin. Neurobiol.* **2:** 165–169.
21. Shors, T., R. Servatius, R. Thompson, G. Rogers, and G. Lynch. 1995. Enhanced glutamatergic neurotransmission facilitates classical conditioning in the freely-moving rat. *Neurosci. Lett.* **186:** 153–156.
22. Slotnick, B. M., and N. Kaneko. 1981. Role of dorsomedial thalamic nucleus in olfactory discrimination learning in rats. *Science* **214:** 91–92.
23. Staubli, U., G. Ivy, and G. Lynch. 1984. Hippocampal denervation causes rapid forgetting of olfactory information in rats. *Proc. Natl. Acad. Sci. USA* **81:** 5885–5887.
24. Staubli, U., F. Schottler, and D. Nejat-Bina. 1987. Role of dorsomedial thalamic nucleus and piriform cortex in processing olfactory information. *Behav. Brain Res.* **25:** 117–129.
25. Staubli, U., G. Rogers, and G. Lynch. 1994. Facilitation of glutamate receptors enhance memory. *Proc. Natl. Acad. Sci. USA* **91:** 777–781.
26. Staubli, U., Y. Perez, F. Xu, G. Rogers, M. Ingvar, S. Stone-Elander, and G. Lynch. 1994. Centrally active modulators of glutamate receptors facilitate the induction of long-term potentiation *in vivo*. *Proc. Natl. Acad. Sci. USA* **91:** 11158–11162.