dopa in patients with Parkinson's disease. The present study points to the value of investigating cholinomimetics in patients with Alzheimer's disease.

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References and Notes

1. J. N. Hingten and M. H. Aprison, in *Biology of Cholinergic Function* (Raven, New York, 1976), p. 515.
2. Cholinesterase inhibitors can facilitate the retrieval of previously learned habits in rats, but these effects depend on the intervals between learning, drug administration, and retrieval [see Deutsch (*3*)].
3. J. A. Deutsch, *Science* **174**, 788 (1971).
4. D. A. Drachman and J. Leavitt, *Arch. Neurol.* **30**, 113 (1974). Most studies with anticholinergics have shown that these drugs impair performance on tasks involving learning but have smaller effects on tasks involving only retrieval: D. J. Safer and R. P. Allen, *Biol. Psychiatry* **3**, 347 (1971); A. M. Ostfeld, X. Machne, K. R. Unna, *J. Pharmacol. Exp. Ther.* **128**, 265 (1960); A. M. Ostfeld and A. Araguate, *ibid.* **137**, 133 (1962); B. Soukapova, M. Vojtechovsky, V. Safratova, *Act. Nerv. Super.* **12**, 91 (1970); J. W. Dundee and S. K. Pandit, *Br. J. Pharmacol.* **44**, 140 (1972); J. Hrbeck, S. Komenda, A. Siroka, *Act. Nerv. Super.* **13**, 200 (1971); J. Hrbeck, S. Komenda, J. Macakova, *ibid.* **16**, 213 (1974); T. J. Crow and I. G. Grove-White, *Br. J. Pharmacol.* **43**, 464 (1971); *ibid.* **49**, 322 (1973).
5. A woman with a profound memory deficit secondary to an attack of herpes simplex encephalitis showed a marked improvement in both storage and retrieval of information after she received intravenously 0.8 mg of physostigmine in a double-blind procedure [B. H. Peters and H. S. Levin, *Arch. Neurol.* **34**, 215 (1977)].
6. B. H. Peters and H. S. Levin (*5*) reported that doses both higher and lower than 0.8 mg did not improve memory in their amnesic patient. Davis *et al.* (*7*) found that 2 mg or 3 mg of physostigmine administered intravenously impaired all aspects of memory functioning.
7. K. L. Davis, L. E. Hollister, J. Overall, A. Johnson, K. Train, *Psychopharmacology* **51**, 23 (1976).
8. To ensure that it was possible to detect a statistically significant physostigmine-induced improvement on the learning task, we included subjects only if they recalled more than three words but less than 15 words after a single presentation of a list of 20 words. Approximately 20 potential subjects were excluded because they recalled more than 14 words; no subject recalled less than three words.
9. The task we used was almost identical to the varied set procedure described by S. Sternberg [*Science* **153**, 652 (1966); *Am. Sci.* **57**, 421 (1969)]. On each of a series of trials, subjects were given a memory set of one to four digits and were then to decide, as rapidly as possible, whether a test digit was in the memory set. On both days response time increased linearly with memory set size and was greater when the test digit was not in memory than when it was a member of the memory set. Similar results were obtained by Sternberg.
10. The distinction between STM and LTM and the role of these stores in various memory tasks is discussed by R. C. Atkinson and R. M. Shiffrin [*Sci. Am.* **224**, 82 (August 1971); in *The Psychology of Learning and Motivation* (Academic Press, New York, 1968), vol. 2, p. 89] and by M. Glanzer [in *The Psychology of Learning and Motivation* (Academic Press, New York, 1972), vol. 5, p. 29]. The total doses received by subjects at the time of these tests are indicated in Table 1. Because of the short half-life of physostigmine, the actual anticholinesterase effect is less than the total dose. The concentration of physostigmine can be calculated from the equation: $Y = (rt/\ell n2)\ 1 - 2^{-t/T}$, where Y is the plasma concentration of physostigmine; r is the rate of infusion (1 mg/hour); T is the half-life of physostigmine (approximately one-half hour); t is the time elapsed since the beginning of the infusion; and $\ell n2$ is 0.69. The precise half-life of physostigmine in human plasma is unknown. From our previous clinical studies, in which physostigmine was administered intravenously, the biological half-life of the drug in humans was estimated to be approximately 30 minutes [K. L. Davis and P. A. Berger, *Biol. Psychiatry* **13** (No. 1), 23 (1978); Davis *et al.* (*7*); K. L. Davis, L. E. Hollister, J. D. Barchas, P. A. Berger, *Life Sci.* **19**, 1507 (1976); K. L. Davis, L. E. Hollister, F. K. Goodwin, E. K. Gordon, *ibid.* **21**, 933 (1977)]. From this equation, if one assumes a 30-minute half-life for physostigmine, one finds that the amount of physostigmine that had been administered at the time that the storage of information into LTM was significantly improved was 0.36 mg, and at the time when retrieval of information was significantly improved 20 minutes after the infusion was concluded (+80), this amount was also 0.36 mg. At the conclusion of the infusion (+60 minutes) the amount of physostigmine administered was 0.54 mg. The relation between the amount of physostigmine administered and the duration of the behavioral manifestations of the drug's central acetylcholinesterase blockade is not known.
11. An analysis of variance was performed separately for recall scores obtained at +18 minutes and at +80 minutes. At +18 minutes recall scores did not differ for physostigmine and saline administration ($F = 1.88$, $P > .10$) and did not differ on the two recall trials ($F = 0.45$). A similar analysis at +80 minutes also showed no significant difference between trials ($F = 3.91$, $P > .05$).
12. The variable effects of physostigmine at +18 minutes (0.30 mg of physostigmine) and at +80 minutes (1.0 mg of physostigmine +20 minutes) may be due to differential responses to increasing doses of physostigmine. However, see Deutsch (*3*).
13. For the procedures for this task see H. Buschke, *J. Verb. Learn. Verb. Behav.* **12**, 543 (1973); _____ and P. A. Fuld, *Neurology* **24**, 1019 (1974).
14. D. A. Drachman and J. Leavitt, *J. Exp. Psychol.* **93**, 302 (1972); V. A. Kral, *Dis. Nerv. Sys.* **27**, 51 (1966); J. G. Gilbert and R. F. Levee, *J. Gerontol.* **26**, 70 (1971).
15. E. McGeer and P. L. McGeer, in *Neurobiology of Aging* (Raven, New York, 1976), p. 389; D. M. Bowen, C. B. Smith, P. White, A. N. Davison, *Brain* **99**, 459 (1976); E. K. Perry, R. H. Perry, G. Blessed, B. E. Tomlinson, *Lancet* **1977-I**, 189 (1977); P. Davies and A. J. F. Maloney, *ibid.* **1976-II**, 1403 (1976); P. White, C. R. Hiley, M. J. Goodhardt, L. H. Carrasco, J. P. Keek, I. E. I. Williams, D. M. Bowen, *ibid.* **1977-I**, 668 (1977).
16. M. J. Kuhar, in *Biology of Cholinergic Function* (Raven, New York, 1976), p. 3.
17. This research was supported in part by the Medical Research Service of the Veterans Administration, NIDA grant DA-00854; and by NIMH Specialized Research Center grant MH-30854.

14 October 1977; revised 28 March 1978

Human Serial Learning: Enhancement with Arecholine and Choline and Impairment with Scopolamine

Abstract. *Arecholine (4 milligrams), a cholinergic agonist, and choline (10 grams), a precursor of acetylcholine, significantly enhanced serial learning in normal human subjects. The subjects received methscopolamine prior to both arecholine and placebo injections. Conversely, scopolamine (0.5 milligram), a cholinergic antagonist, impaired learning and this impairment was reversed by a subsequent injection of arecholine. The degree of enhancement produced by arecholine and choline and the impairment after scopolamine were inversely proportional to the subject's performance on placebo; that is, "poor" performers were more vulnerable to both the enhancing effect of cholinergic agonist and precursor and the impairment after cholinergic antagonist than "good" performers.*

Evidence from studies with rodents (*1*) suggests that acetylcholine may be involved in learning and memory mechanisms. In humans the evidence is less clear. Although scopolamine, an anticholinergic agent, produced impairment of learning and recall (*2, 3*), there are no controlled studies in normal humans showing enhancement of memory after the administration of cholinergic agonists (*4*). We have studied the effects of arecholine, which in low doses is reported to be a specific central muscarinic cholinergic agonist (*5*), and of scopolamine, an antimuscarinic agent (*6*), in normal human volunteers. To further confirm our finding with arecholine, we administered choline, a normal dietary constituent which recently has been shown to increase whole brain and hippocampal acetylcholine in rats (*7*), to normal subjects in a follow-up study.

The subjects were paid normal volunteers, who were either college students or recently graduated from college, and were free of significant physical or psychiatric difficulty (*8*).

In experiment 1 we examined the effects of arecholine and scopolamine on categorized serial learning. Before receiving a subcutaneous injection of arecholine, the subjects were treated with either methscopolamine or scopolamine (injected intramuscularly), both of which block the peripheral cholinergic side effects. Scopolamine, unlike methscopolamine, crosses the blood-brain barrier and has central antimuscarinic effects (*6*). Methscopolamine itself has been reported not to affect human memory (*2, 3*). The drugs were administered on four nonconsecutive days (A, B, C, and D) (Table 1). Categorized serial learning consists of learning a fixed sequence of ten words belonging to a familiar category (for example, vegetables, cities, or fruits). The words were presented at a rate of one every 2 seconds. Each list was repeated until the subject recalled all ten words in correct se-

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Table 1. The results of experiment 1, comparing the effect of three drugs on categorized serial learning. On each of four nonconsecutive randomly ordered days (A, B, C, and D), 14 subjects (11 male and 3 female; mean age, 22.4 years) were studied under two treatments, for a total of eight treatment conditions (1 through 8). Each day the subjects received an intramuscular injection (first treatment) of either methscopolamine (days A and B) or scopolamine (days C and D); 45 and 105 minutes later they received a subcutaneous injection (second treatment) of either placebo (normal saline) or arecholine (in various doses). On days A and B, the order of treatments (placebo and arecholine) was randomized; on days C and D, placebo preceded arecholine. Categorized serial learning was tested 10 minutes after each subcutaneous injection. Experimental conditions 1 to 8 were analyzed by means of a one-way analysis of variance with repeated measures [$N = 14$ subjects (*14*), F (7, 91) = 8.33, $P < .001$]. Two subsequent tests were used to compare different experimental conditions. (i) The contrast of means technique (*15*, p. 170) was used because the two placebo conditions (1 and 3, on days A and B) did not differ from each other and were therefore both compared to the other drug conditions. (ii) The Newman-Keuls test (*15*, p. 191) was used with pairwise comparison of placebo conditions (average of conditions 1 and 3, which is 5.18 ± 0.53 trials) and other conditions. Only comparisons that reached significance on both the above tests are listed in Table 1.

Schedule	First injection	Second injection and conditions	Trials to criterion (mean ± S.E.M.)
Baseline 1	None	None	5.0 ± 0.68*
Day A	Methscopolamine (0.3 mg)	1. Placebo	5.16 ± 0.56
Day A	Methscopolamine (0.3 mg)	2. Arecholine (4 mg)	3.75 ± 0.26†
Day B	Methscopolamine (0.3 mg)	3. Placebo	5.20 ± 0.45
Day B	Methscopolamine (0.3 mg)	4. Arecholine (2 mg)	4.86 ± 0.45
Day C	Scopolamine (0.25 mg)	5. Placebo	5.25 ± 0.83
Day C	Scopolamine (0.25 mg)	6. Arecholine (4 mg)	4.50 ± 0.35
Day D	Scopolamine (0.5 mg)	7. Placebo	6.35 ± 0.82†
Day D	Scopolamine (0.5 mg)	8. Arecholine (6 mg)	4.92 ± 0.51‡
Baseline 2	None	None	5.31 ± 0.80§

*Not statistically significant when compared with conditions 1 and 3. Two-tailed paired t-tests were used to compare baseline 1 with placebo ($N = 14$, d.f. = 13) and baseline 2 with baseline 1 ($N = 9$, d.f. = 8). †$P < .01$ when compared with conditions 1 and 3. ‡$P < .01$ when compared with condition 7. §Not statistically significant when compared with baseline 1.

quence on two consecutive trials. Previous studies show that categorized serial learning is sensitive to psychoactive drugs (*9*). Prior to formal testing, the procedure was explained and the subject learned one list to criterion (baseline 1). After the formal study, nine subjects were retested with one list to criterion (baseline 2) so that we could determine whether practice per se had any effects on learning. During each of the two experimental conditions on days A, B, C, and D, the subjects learned two lists to criterion; the results for each subject were averaged for that experimental condition.

As shown in Table 1, no practice effect was noted (that is, baselines 1 and 2 did not differ significantly). Methscopolamine alone had no effect (that is, conditions 1 and 3 were similar to baseline 1). Similarly, there were no significantly observable effects caused by the order of administration of drugs, the word list used, or the order of presentation of lists.

The mean values (number of trials to criterion) for the eight experimental conditions differed significantly ($P < .001, F$ (7, 91) = 8.33, analysis of variance). The mean values for placebo treatments after methscopolamine administration (conditions 1 and 3) did not differ. These two groups (conditions 1 and 3) are together referred to as "placebo" condition because the subjects did not receive any centrally active agents and their performance during the two conditions was comparable. Compared to "placebo" (conditions 1 and 3), 4 mg of arecholine (condition 2) enhanced ($P < .01$) and 0.5 mg of scopolamine (condition 7) impaired ($P < .01$) learning significantly. Arecholine (6 mg; condition 8) significantly reversed ($P < .01$) the impairment produced by 0.5 mg of scopolamine (condition 7).

In experiment 2, we examined the effect of choline chloride on uncategorized serial learning in ten subjects (seven males, three females; mean age, 24.3 years). A list of ten unrelated (uncategorized) words was repeated until the subject recalled the ten words in correct or-

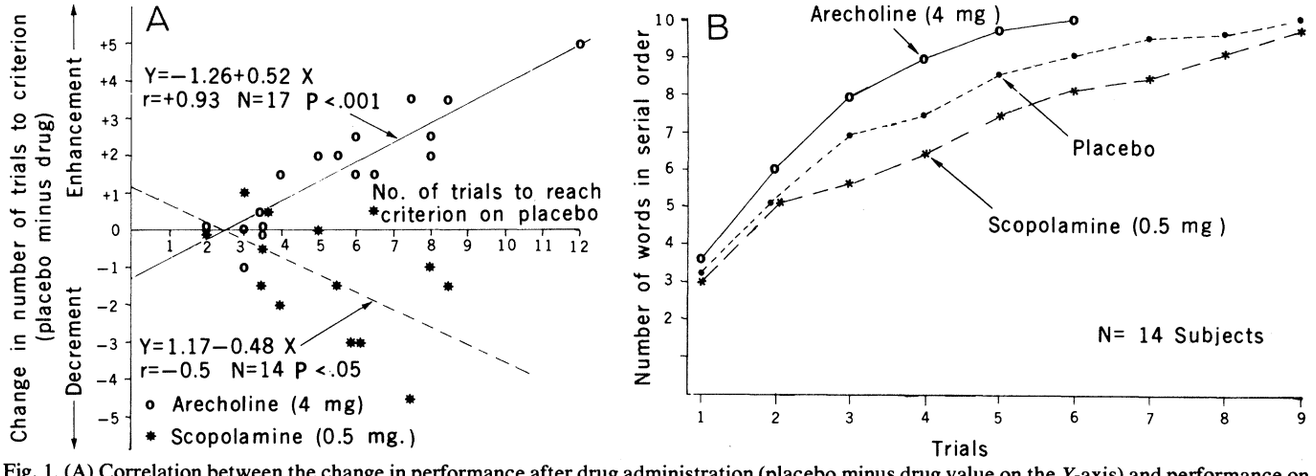

Fig. 1. (A) Correlation between the change in performance after drug administration (placebo minus drug value on the Y-axis) and performance on placebo (average of conditions 1 and 3 on the X-axis). All 17 subjects who received placebo and 4 mg of arecholine were used in the analysis. The correlation would still be highly significant if only the 14 subjects who underwent all conditions were included ($r = 8.89$, $Y = 1.7 + 0.61 X$, $N = 14$, $P < .01$). Product moment correlation between change after arecholine administration [that is, placebo minus arecholine (4 mg)] and change after scopolamine was also significant ($r = -0.64$, $Y = -0.56 + 0.77 X$, $N = 14$, $P < .05$) indicating that "slow" learners were more vulnerable to change in performance after both arecholine and scopolamine administration than "fast" learners. (B) The learning curve for subjects receiving arecholine (4 mg) ends at trial 6, since all 14 subjects had reached criterion by then. Analysis consisted of one-way analysis of variance between the three treatment conditions (placebo, arecholine, and scopolamine) for trial 1, and a two-way analysis of variance with one repeated measure across trials 2 through 6 [see (*10*)].

der on two consecutive trials. The subjects were tested on two separate days and were required to reach criterion on one list beginning 90 minutes after the oral administration in random order of an elixir containing choline chloride (10 g) or a placebo matched for taste, color, and consistency.

The results of experiment 2 indicate that subjects reached criterion significantly faster after they received choline chloride (5.2 ± 0.69 trials) than after they received placebo (6.1 ± 0.87 trials) ($P < .05$, two-tailed paired t-test).

The extent of change induced by arecholine, choline chloride, and scopolamine was significantly related to the individual's performance when he or she was tested under placebo conditions (without centrally active cholinergic agents). As shown in Fig. 1A, the performance under placebo conditions (mean of conditions 1 and 3) correlated positively with the change induced by 4 mg of arecholine (that is, "placebo" minus the 4-mg arecholine value; $r = 0.93$, $P < .001$, $N = 17$) and negatively with the change induced by 0.5 mg of scopolamine ($r = -0.55$, $P < .05$, $N = 10$). The change induced by choline chloride was also significantly correlated with performance under placebo treatment ($r = 0.59$, $P < .05$, $N = 10$). Thus, poor performers showed a relatively greater improvement after they received arecholine and choline and a greater impairment after scopolamine than good performers.

As shown in Fig. 1B, the mean number of words learned per trial of the categorized serial learning task did not differ on trial 1 between the placebo condition (mean = 3.18 ± 3.2 words), 4 mg of arecholine (mean = 3.78 ± 2.4 words), or 0.5 mg of scopolamine (mean = 3.04 ± 5.4 words) (P = N.S., F (2, 39) = 1.06). On trials 2 through 6, however, 4 mg of arecholine increased the rate of learning compared with placebo (conditions 1 and 3) while scopolamine decreased it (*10*).

Our data in human subjects are consistent with evidence that acetylcholine participates in learning and memory mechanisms in other animals (*1*). The strong correlation between learning ability under placebo conditions and enhancement after the administration of arecholine and choline is consistent with a report by Stanes and Brown (*11*) that physostigmine selectively impaired and facilitated performance of naturally "fast" and "slow" learning rats, respectively. In addition, Mandel and Ebel (*12*) noted increased concentrations of choline acetyltransferase (E.C. 2.3.1.6; synthetic enzyme for acetylcholine) in the frontal and temporal cortex of an inbred strain of mice with a high capacity for maze-learning compared to mice that were poor learners.

It is of clinical interest that a specific decrease of choline acetyltransferase has been reported in the frontal cortex of patients with Alzheimer's disease and other presenile dementias (*13*). In view of our data, the possible use of arecholine or choline as a therapeutic agent in dementia needs further exploration.

Note added in proof: In a recent experiment (unpublished) intravenous infusion of 2 mg of arecholine over 30 minutes, starting immediately after learning a list of words resulted in a significantly higher percentage of words recalled after an hour compared to placebo infusion.

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References and Notes

1. J. A. Deutsch, *Science* **174**, 788 (1971); J. M. Whitehouse, *Psychopharmacology* **9**, 183 (1966); J. A. Rosecrans, A. T. Dren, E. F. Domino, *Int. J. Neuropharmacol.* **7**, 127 (1968); L. O. Stratton and L. F. Petrinovich, *Psychopharmacology* **5**, 47 (1963).
2. D. A. Drachmann and J. Leavitt, *Arch. Neurol.* **30**, 113 (1974); M. M. Ghoneim and S. P. Mewaldt, *Psychopharmacology* **52**, 1 (1977); R. C. Paterson, *ibid.*, p. 283.
3. D. A. Drachman, *Neurology* **27**, 783 (1977).
4. B. H. Peters and H. S. Levin [*Arch. Neurol.* **34**, 215 (1977)] reported improvement of memory functions after intravenous administration of physostigmine, but this was in a single patient with profound dementia secondary to herpes simplex encephalitis. Physostigmine did not improve performance in normal volunteers (*3*).
5. K. I. Yamamoto and E. F. Domino, *Int. J. Neuropharmacol.* **6** (No. 5), 357 (1967); J. L. Riehl, J. Paul-David, K. R. Unna, *J. Neuropharmacol.* **1**, 393 (1962); R. W. Baker, C. H. Chothia, P. Pauling, T. J. Petcher, *Nature (London)* **230**, 439 (1971); C. C. Pfeiffer and E. H. Jenny, *Ann. N.Y. Acad. Sci.* **66**, 753 (1957). The relative potency of arecholine in reducing specific [^{3}H]quinuclidinyl benzilate binding in rat brain by 50 percent (median infective dose, ID_{50} = 2 μM) was recently calculated by H. I. Yamamura, K. J. Chang, M. J. Kuhar, and S. H. Snyder [*Fed. Proc.* **34**, 1915 (1975)] to be lower than oxotremorine (ID_{50} = 0.5 to 0.8 μM) and higher than acetylcholine itself (ID_{50} = 3 μM).
6. D. J. Safer and R. P. Allen, *Biol. Psychiatry* **3**, 347 (1971).
7. E. L. Cohen and R. J. Wurtman, *Science* **191**, 561 (1976); M. J. Hirsch, J. H. Growden, R. J. Wurtman, *Brain Res.* **125**, 383 (1977).
8. The subjects gave their informed consent to participate in this study. The use of the medications in normal volunteers and the administration of the memory tests were approved by the Human Research Review Sub Panel of the National Institute of Mental Health.
9. H. Weingartner, S. Snyder, L. A. Faillace, H. Markley, *Behav. Sci.* **15**, 297 (1970).
10. Number of words recalled under placebo conditions on trials 2 and 6 were: 5.14 ± 0.66, 6.89 ± 0.63, 7.36 ± 0.65, 8.5 ± 0.43, and 9.04 ± 0.43, respectively; after arecholine (4 mg) administration: 5.96 ± 0.38, 7.86 ± 0.52, 8.86 ± 0.40, 9.71 ± 0.19, and 10 ± 0.00; after scopolamine administration (0.5 mg): 5.07 ± 0.69, 5.57 ± 0.84, 6.39 ± 0.77, 7.36 ± 0.75, and 8.07 ± 0.66. A two-way analysis of variance with one repeated measure revealed significant interaction between treatment and trials 2 to 6 (F = 4.54, d.f. = 2, 26, $P < .05$). Newman-Keuls' pairwise comparison revealed that arecholine differed significantly ($P < .05$) from placebo on trials 4, 5, and 6. Scopolamine differed significantly ($P < .05$) on trails 3, 5, and 6 from placebo.
11. M. D. Stanes and C. P. Brown, *Psychopharmacology* **46**, 269 (1976).
12. P. Mandel and A. Ebel, in *Neurochemistry of Cholinergic Receptors*, G. E. De Robertis and J. Schacht, Eds. (Raven, New York, 1974), pp. 131–139.
13. J. A. Spillane, P. White, M. J. Goodhardt, R. H. A. Flack, D. M. Bowen, A. N. Davison, *Nature (London)* **266**, 558 (1977).
14. Three additional subjects completed testing on day A, making a total of 17 subjects. The trials to criterion for 17 subjects were 5.32 ± 0.5 on placebo and 3.83 ± 0.3 on arecholine (4 mg) ($P < .01$, two-tailed paired t-test). Since the data in Table 1 were statistically evaluated by the analysis of variance with repeated measures, these three subjects were not included in the data or analyses in Table 1.
15. B. J. Winer, *Statistical Principles in Experimental Design* (McGraw-Hill, New York, ed. 2, 1962).
16. We thank Angela Moore and the late Denise Clendaniel for technical assistance and Joan Barber for preparing the manuscript.

20 December 1977; revised 20 March 1978

Long-Term Changes in Dopaminergic Innervation of Caudate Nucleus After Continuous Amphetamine Administration

Abstract. *Silicone pellets containing* d-*amphetamine base were implanted subcutaneously in rats. These pellets release amphetamine continuously for at least 10 days. Several days after implantation, swollen dopamine axons concomitant with large decreases in tyrosine hydroxylase activity were observed in the caudate nucleus. Decreased tyrosine hydroxylase activity was still present 110 days after pellet removal in the caudate but not in several other brain regions, nor in the caudate of rats injected with an equivalent amount of amphetamine in daily injections. This implies that continuous amphetamine administration has a selective neurotoxic effect on dopamine terminals in the caudate.*

Chronic amphetamine addicts develop intake patterns during which several days of continuous amphetamine intoxication occur. A similar drug regimen is used during studies of amphetamine psychosis. A model psychosis which resembles paranoid schizophrenia in many respects develops in humans when they re-

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