system connections. The PGO burst cells form the final link in the chain, acting as output generators for the PGO waves by integrating information from other pontine systems and transmitting it to forebrain.

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10. We emphasize the critical dependence of the coherence value on the quality of the PGO and unit recording; since the PGO burst cells have low voltages (75 to 300 μV) it is quite possible to lose resolution of the unit and have an apparently low coherence. Conversely, any failure to detect PGO waves or any noise in the unit recording will elevate the percentage of PGO burst cell firings apparently not associated with PGO waves.
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Physostigmine: Improvement of Long-Term Memory Processes in Normal Humans

Abstract. *Nineteen normal male subjects received 1.0 milligram of physostigmine or 1.0 milligram of saline by a slow intravenous infusion on two nonconsecutive days. Physostigmine significantly enhanced storage of information into long-term memory. Retrieval of information from long-term memory was also improved. Short-term memory processes were not significantly altered by physostigmine.*

Studies in humans and animals have implicated cholinergic processes in memory functioning. Investigations with both anticholinergics and cholinomimetics indicate that fluctuations in cholinergic activity can profoundly affect storage and retrieval of information in memory. Small doses of cholinesterase inhibitors have been reported to facilitate maze learning in rats (*1–3*). In humans the anticholinergic agent scopolamine produces learning impairments similar to those found in people with senile dementia (*4*). The acetylcholinesterase inhibitor physostigmine enhanced both storage and retrieval of information in a patient with impaired cognitive function (*5*). However, any enhancement of human memory is apparently limited to a narrow dose range of physostigmine (*6, 7*).

In this study normal subjects received low doses of either physostigmine or saline placebo. When the subjects received physostigmine they showed a significant improvement in storage of information compared to their performance when they received the placebo. These results have implications for the treatment of people with a variety of memory disorders.

The subjects were 19 normal male volunteers (18 to 35 years) who gave their informed consent to participate in this study. They were chosen according to their performance on a verbal learning task identical to the test used to measure the effect of physostigmine. Subjects were excluded if their performance on the screening task indicated there was no opportunity for improvement (*8*).

The subjects received 1.0 mg of physostigmine or 1.0 mg of saline on two nonconsecutive days. The order of infusions was randomized. Physostigmine or the saline placebo was administered by a constant infusion over 60 minutes. Approximately 20 minutes prior (−20) to the start of either infusion the subjects received 0.5 mg of methscopolamine bromide subcutaneously in order to block the peripheral effects of physostigmine. When the pulse rate reached 100 beats per minute the infusion was begun.

The experiment was designed (Table 1) so that we could measure short-term (STM) and long-term (LTM) memory functions. Two tests of STM were used: the digit span and memory scanning task of Sternberg (*9*). The digit span task determines the capacity of STM by measuring the maximum number of digits that a subject can recall correctly after a single presentation (*10*). Digit span measured 9 minutes after (+9) the start of infusion with physostigmine (when the subjects had received 0.15 mg) was 6.8 digits, and with saline was 6.9 digits. The memory scanning task measures the rate of processing in STM. Subjects make decisions about the contents of STM and register those decisions allowing measurement of the response speed. Two components of response time can be distinguished: one is a function of STM processing speed and one is a function of stimulus encoding and motor response processes. A dose of 0.75 to 1.0 mg of physostigmine had no significant effect on either component (*8*). Thus, physostigmine, compared to saline, had no quantifiable effect on any aspect of STM functioning.

We assessed LTM functioning by means of two verbal learning tasks (*10*). The first tested the hypothesis that physostigmine would enhance the ability to retrieve information from LTM. Thirty minutes prior to either infusion the subjects were given two learning trials on a list of 15 concrete nouns. The 15 nouns were presented verbally to the subject at the rate of one word per 2 seconds. The subject tried to recall the 15 words at the

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end of the presentation. Before the second trial subjects were reminded of all words they failed to recall on the first trial. They then tried to recall all 15 words again. At +18 minutes and again at +80 minutes subjects attempted to recall all 15 words twice without additional reminders of missed words between trials. Figure 1A shows the total number of words recalled when physostigmine and saline were administered. Recall scores on the two learning trials prior to infusion were not, by analysis of variance, significantly different for the two infusions, that is, prior to an infusion the subjects did not differ in the number of words retrieved from LTM. Statistical analysis demonstrated that after the start of infusion, at +18 minutes (when the subjects had received 0.30 mg of physostigmine) there was a nonstatistically significant trend for subjects to retrieve more words from LTM on days they received physostigmine than on days they received saline. At +80 minutes (when all of the 1.0 mg of physostigmine had been infused) the subjects retrieved significantly more words from LTM on the day they received physostigmine (1.0 mg) than the day they received saline ($F = 5.77$, $P < .03$) (*11, 12*).

To test the hypothesis that physostigmine enhances storage of information in LTM, we gave the subjects a 20-word verbal learning task at +30 minutes (0.5 mg of physostigmine) (*13*). After the entire list of 20 nouns was presented verbally at a rate of one word per 2 seconds, the subjects tried to recall the entire list. Prior to each of five subsequent recalls the subjects were reminded of all words that they failed to recall on the previous trial. Figure 1B shows the number of words recalled on the six learning trials when either physostigmine or saline was administered. An analysis of variance showed that subjects recalled more words on later trials than earlier trials [F (5, 85) = 81.7, $P < .001$] and recalled more words on days when physostigmine was administered than on days when saline was administered [F (1, 17) = 9.98, $P < .006$]. Thus the subjects stored more information during the physostigmine infusion than during the saline infusion. This improvement may be mediated in part by an effect of physostigmine on retrieval, attentional, and motivational mechanisms. These results suggest that cholinomimetics might improve LTM in certain situations.

There was considerable variability between subjects in the augmentation of LTM by physostigmine. Although no subject had a performance decrement, some subjects were essentially unchanged. Factors that are predictive of a physostigmine-induced improvement in LTM are not readily apparent. However, it is tempting to speculate that improvement may be correlated with a subject's baseline level of central cholinergic activity. Thus the dose of physostigmine that facilitates a maximum improvement in LTM could be quite different in each subject.

The subjects in this study were selected to maximize the likelihood of exhibiting an improvement in memory function. Since they were presented with lists of 20 nouns to store, those subjects with baseline recall scores above 15 had less opportunity to demonstrate a statistically significant improvement than subjects with lower baseline scores. A revised task, allowing subjects with better baseline performance the opportunity to recall 25 nouns, might be an effective strategy to determine whether LTM functioning can be improved in this group. However, subjects with a high baseline level of cognitive functioning may already have an optimal level of cholinergic activity; therefore, any physostigmine-enhanced cholinergic transmission might produce no improvement or even a decrement.

Storage of information into LTM is affected by aging and by senile dementia. Storage into LTM was significantly improved in this study (*14*). Neurochemical studies indicate that patients with Alzheimer's disease have diminished activities of choline acetyltransferase (*15*); this diminished activity may indicate a relative depletion of central cholinergic neurons (*16*). If cholinergic receptor binding was relatively normal in patients with Alzheimer's disease cholinomimetics might improve memory functioning in a manner analogous to the efficacy of levo-

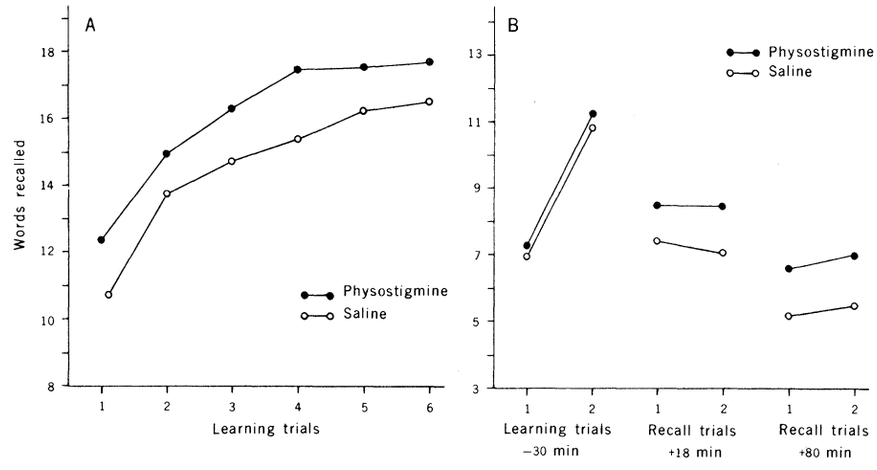

Fig. 1. (A) The effect of physostigmine on retrieval. The data show the total number of words recalled from a list of 15 words. Two learning trials occurred 30 minutes prior to the start of the infusion. Two recall trials occurred 18 minutes into the infusion. Two recall trials occurred 80 minutes after the start of the infusion. (B) The effect of physostigmine on storage. The data show the total number of words recalled from a list of 20 words on each of six learning trials.

Table 1. Schedule of procedures for a single session.

Total dose of physostigmine received (mg)	Time (minutes)	Procedure
	−30*	Two learning trials on list of 15 concrete nouns†
	−20	Methscopolamine (0.5 mg, injected subcutaneously)
	0	Start of infusion
0.15	+ 9	Digit span
0.30	+18	Two recall trials on list of 15 concrete nouns presented at −30 minutes‡
0.50	+30	Six learning trials on list of 20 categorized nouns†
0.70	+42	Short-term memory scanning task
1.00	+60	End infusion
1.00	+80	Two recall trials on list of 15 concrete nouns presented at −30 minutes‡

*Thirty minutes before start of infusion. †At the start of the first learning trial the entire list of words was presented; on subsequent learning trials only words not recalled on the previous trial were presented. ‡No words were presented on recall trials.

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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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.
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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*).
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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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