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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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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Table 1. Tyrosine hydroxylase activity and catecholamine concentrations in four brain regions in controls, in rats killed 2 to 5 days after amphetamine pellet implantation (during pellet), and in rats killed 115 days after pellet implantation and 108 days after pellet removal (after pellet).

Brain region	Tyrosine hydroxylase			Dopamine			Norepinephrine		
	Controls [pmole (mg protein)$^{-1}$ min^{-1}]	Pellet (% control)		Control (ng/g)	Pellet (% control)		Control (ng/g)	Pellet (% control)	
		During	After		During	After		During	After
Cortex	0.15 ± 0.02	86 ± 8	121 ± 5*	450 ± 43	82 ± 8	87 ± 10	305 ± 24	79 ± 4*	115 ± 9
Caudate	2.91 ± 0.33	50 ± 5†	74 ± 7*	3221 ± 68	63 ± 10*	87 ± 13	500 ± 42	84 ± 7	92 ± 11
Hypothalamus	0.38 ± 0.04	123 ± 11	115 ± 18	831 ± 69	98 ± 13	78 ± 18	1412 ± 53	68 ± 4†	103 ± 5
Brainstem	0.22 ± 0.02	102 ± 9	122 ± 20	119 ± 23	179 ± 19*	117 ± 14	641 ± 29	59 ± 3†	93 ± 5

*Means $P < .05$. †Means $P < .01$, Dunnett's tests.

ceive low doses of amphetamines every several hours for a number of days (*1*). Many studies have shown that cumulative behavioral and biochemical effects occur in animals given daily injections of amphetamines for long periods (*2*), but the continuous amphetamine intoxication regime used in the human studies is laborious to reproduce in animals by means of repeated intraperitoneal injections. It can be simulated more easily by using subcutaneous implants of silicone pellets containing *d*-amphetamine base (*3*); such pellets release amphetamine continuously for at least 10 days. Rats with these implants go through a series of behavioral stages, culminating 5 days after implantation in a late stage of socially disruptive behaviors and heightened startle responses (*4*).

Many of the actions of amphetamine are mediated by the catecholamines dopamine and noradrenaline (*5*), and we therefore studied the effects of amphetamine pellet implantation on brain catecholamines using fluorescence microscopy. We used groups of male hooded rats weighing 360 to 420 g. In each rat we implanted subcutaneously in the back area silicone pellets containing either polyethylene glycol vehicle (controls, $N = 14$) or 49 mg of *d*-amphetamine base. The experimental animals were killed either 2 days ($N = 6$) or 5 to 6 days ($N = 12$) after pellet implantation, or from 5 to 33 days after pellet removal 7 days following pellet implantation ($N = 11$). The control animals were killed at similar time intervals. All animals were perfused with 2 percent glyoxylic acid and the brains were rapidly removed and sectioned on a vibratome. The sections were dipped in glyoxylic acid, air dried, and baked for 45 minutes in formalin vapors at 100°C (*6*).

At 2 days after amphetamine pellet implantation we observed many of the alterations in catecholamine fluorescence reported by others to occur in animals injected with amphetamine (*7*): reduced background fluorescence throughout the forebrain and diffusion around cortical axons. At 5 days after amphetamine pellet implantation, cortical axons were more difficult to study because of their faint, pale appearance, but axons with normal-appearing varicosities were still present in several cortical regions receiving norepinephrine innervations. However, highly distinctive alterations had appeared in the caudate nucleus. In the normal caudate nucleus there were many small terminals and few fluorescent axons, resulting in a diffuse and cloudy appearance (Fig. 1A), but in animals that had received amphetamine pellet implants 5 days previously there was decreased background in most areas of the caudate and many long, bright fluorescent axons with large varicosities (Fig. 1B). These swollen axons were particularly prevalent in the more ventral regions of the caudate, in the region where dopamine fibers enter the nucleus. When the pellet was removed 7 days after implantation and a recovery period given, there was a gradual and partial reappearance of background fluorescence and finer axons in most of the caudate, but bright and extremely swollen axons were still present in some regions (Fig. 1C). Similar swollen and enlarged fluorescent axons in the ventrolateral caudate have been observed after lesions of the caudate nucleus have been made by partial aspiration (*8*), and these enlargements are presumed to reflect an accumulation of amines in the remaining axons. Our observations therefore imply that damage to dopamine terminals in the caudate can be produced by the continuous amphetamine administration achieved through pellet implantation.

This hypothesis was tested by other methods. Damage to the nigrostriatal pathway leads to long-lasting decreases in dopamine levels and tyrosine hydroxylase (TH) activity in the caudate (*9*), and so we assayed catecholamine levels and TH activity in several brain regions at various times after amphetamine pellet implantation. Groups of seven rats each were killed by decapitation 5 days after implantation of control pellets and at 2, 5, and 117 days after amphetamine pellet implantation. The pellet in the 117-day group was removed 7 days after implantation. The brains were rapidly removed, cooled in Dry Ice, and sectioned into 3-mm slabs. We dissected 50- to 80-mg pieces from the frontal poles, caudate nucleus, hypothalamus, and brainstem (combining pieces containing locus coeruleus and substantia nigra). Tyrosine hydroxylase activity was measured in homogenates of these

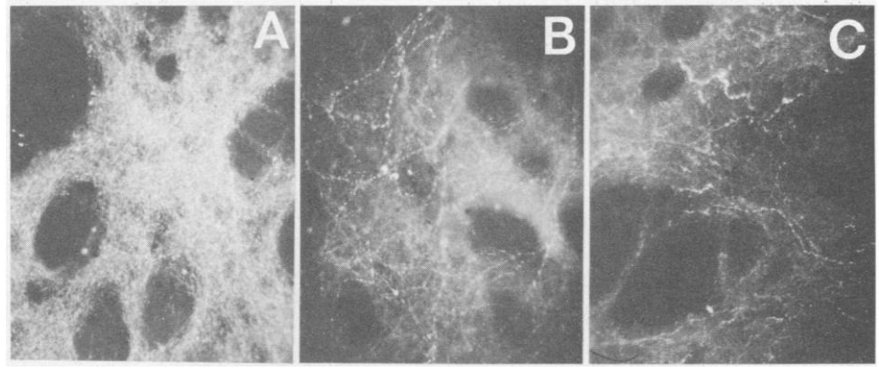

Fig. 1. Alterations in catecholamine fluorescence in the caudate nucleus produced by implantation of an amphetamine pellet. (A) Normal caudate, with numerous fine terminals. (B) Five days after pellet implantation there is decreased background and many long axons with varicosities. (C) Thirteen days after pellet implantation and 6 days after pellet removal swollen axons are still present. All photographs ×200, oil immersion, with identical exposure time.

pieces by means of the ($^{14}CO_2$) evolution method (*10*). The remainder of the cortex, caudate, and contralateral hypothalamus and brainstem (from mammillary bodies 6 mm posterior, excluding cerebellum) were assayed for catecholamine concentrations by an acetone extraction procedure (*11*).

The results from animals killed 2 and 5 days after pellet implantation were similar, and so were pooled for statistical analysis (see Table 1). The largest changes in TH activity (to 50 percent of control values) during pellet implantation were in the caudate nucleus. Dopamine content of the caudate was also significantly decreased, while there was a converse increase in dopamine concentrations in the brainstem. Norepinephrine concentrations were also decreased during pellet implantation in most brain regions. These results are similar to those reported by others (*12*) to occur after single injections of the drug, and are presumed to reflect the depletions produced by amphetamine-stimulated release of catecholamines. But while catecholamine concentrations recovered to near control levels in the animals killed 110 days after pellet removal, caudate TH activity remained significantly less than in controls, although it was elevated in the other brain regions. This long-lasting decrease in caudate TH activity again indicated selective alterations in nigrostriatal dopamine fibers produced by amphetamine pellet implantation (*13*).

Further studies were undertaken to determine whether this was due to the total amount of amphetamine released by the pellet [25 mg during 7 days (*3*)] or to the continuous nature of the drug regimen. Four groups of eight rats each were used. In one group of rats we implanted amphetamine pellets which we removed at 7 days; these rats were given a 60-day recovery period. The rats in a second group received, in seven daily intraperitoneal injections (3.7 mg per day), an amount of amphetamine equivalent to that released by the pellet. The rats in a third group also received an equivalent amount of amphetamine, but in 30 daily intraperitoneal injections of amphetamine sulfate (3.2 mg per kilogram per day). A control group received either control pellet implants or seven daily saline injections. Tyrosine hydroxylase activity in the caudate, measured 60 days after pellet removal or cessation of injections, was 4.72 ± 0.36 pmole per milligram of protein per minute (± standard error) in the controls (*14*). The animals with pellet implants showed reduced TH activity in the caudate (75.2 ± 7.6 percent of controls; $P < .05$, t-test), whereas the two groups receiving daily injections did not (91.6 ± 10.3 percent for the group receiving seven injections, and 112 ± 5.3 percent for the group receiving 30 injections, both $P > .30$).

These results indicate that amphetamine that is administered continuously can induce long-lasting structural and biochemical alterations in dopamine terminals in the caudate nucleus. Although the decreased TH activity in the animals killed 110 days after amphetamine pellet removal could be explained by a persisting increase in dopamine cell activity (which would lead to decreased caudate TH activity because of feedback inhibition), the behavior of rats during the late stage which occurs several days after amphetamine pellet implantation also implies a decrease in dopaminergic transmission. After pellet implantation, rats are initially hyperexploratory but then gradually develop motor stereotypies, that are probably mediated by striatal dopamine (*15*). These stereotypies occur continuously for 2 days, but then disappear as the animals enter a later stage characterized by heightened startle responses, increased fight and flight behaviors, and tolerance to the motor stereotypies induced by dopamine receptor stimulators (*4*).

Hyperactivity in dopamine systems has frequently been implicated in schizophrenia (*16*). It is noteworthy that the same regime of amphetamine intoxication which leads to schizophrenic-like behavior in humans selectively produces long-term alterations in the nigrostriatal dopaminergic pathways of rats. These alterations appear to be indicative of damage caused by continuous overstimulation. The further study of the mechanisms underlying these effects may thus have implications for the understanding of the pathological processes involved in schizophrenia.

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