pursuit of truth may be in tension with keeping a good name (witness Oedipus, Socrates, Galileo, Spinoza, Solzhenitsyn). For most of human history, the pursuit of truth (including "science") was not a reputable activity among the many, and was, in fact, highly suspect. Even today, it is doubtful whether more than a few appreciate knowledge as an end in itself. Science has acquired a "good name" in recent times largely because of its technological fruit; it is therefore to be expected that a disenchantment with technology will reflect badly upon science. Second, my own attack has not been directed against science, but against the use of *some* technologies and, even more, against the unexamined belief—indeed, I would say, superstition—that all biomedical technology is an unmixed blessing. I share the questioner's belief that the pursuit of truth is a highly moral activity. In fact, I am inviting him and others to join in a pursuit of the truth about whether all these new technologies are really good for us. This is a question that merits and is susceptible of serious intellectual inquiry. Finally, we must ask whether what we call "science" has a monopoly on the pursuit of truth. What is "truth"? What is knowable, and what does it mean to know? Surely, these are also questions that can be examined. Unless we do so, we shall remain ignorant about what "science" is and about what it discovers. Yet "science"—that is, modern natural science—cannot begin to answer them; they are philosophical questions, the very ones I am trying to raise at this point in the text.

The Cholinergic Synapse and the Site of Memory

J. Anthony Deutsch

That learning and memory are due to some form of change of synaptic conductance is a very old idea, having been suggested by Tanzi in 1893 (*1*). It is a simple idea and in many ways an obvious one. However, the evidence that learning is due to changes at the synapse has been meager (*2*). Although changes occur at a spinal synapse as a result of stimulation, there is no evidence that the changes are those utilized in the nervous system for information storage. To use an analogy, if we pass large amounts of current across resistors in a computer, temporary increases in temperature and perhaps even permanent increases in resistance occur. However, such an experiment shows only that the computer could store information by using "post-stimulation" alterations in its resistors, but it does not show that this is the actual way in which the computer stores information. Sharpless (*3*) has pointed out that learning is not due to simple use of stimulation of a pathway. He therefore questions whether the phenomena studied by Eccles (*2*) have anything to do with learning as observed in the intact organism. Nevertheless, this does not mean that learning is not due to synaptic changes of some sort. It means only that a different experimental test of the possibility must be devised.

In designing our experimental approach to this problem, clues from human clinical evidence were used. After an individual receives blows to the head, as might be sustained in accidents, he cannot recall events that occurred closest in time prior to the accident (retrograde amnesia). Such patches of amnesia may cover days or even weeks. The lost memories tend to return, with those most distant in time from the accident becoming available first (*4*). In the Korsakoff syndrome (*5*), retrograde amnesia may gradually increase until it covers a span of many years. An elderly patient may end up remembering only his youth, whereas there is no useful memory of the more recent intervening years. From such evidence concerning human retrograde amnesia we may conclude that the changes in the substrate of memory take a relatively long time and are measurable in hours, days, and even months. If we suppose from this that the substrate of memory is synaptic and that it is slowly changing, then it may be possible to follow such synaptic changes by pharmacological methods. If the same dose of a synaptically acting drug has different effects on remembering that depend on the age of the memory (and this can be shown for a number of synaptically acting drugs), then we may assume that there has been a synaptic alteration as a function of time after learning, and we may infer that such a synaptic change underlies memory.

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Pharmacological Tools to Investigate Hypothesis

Pharmacological agents are available that can either increase or decrease the effectiveness of neural transmitters (*6*). For instance, anticholinesterase and anticholinergic drugs affect transmission at synapses which utilize acetylcholine as the transmitter. During normal transmission, acetylcholine is rapidly destroyed by the enzyme cholinesterase. Anticholinesterase drugs, such as physostigmine and diisopropyl fluorophosphate (DFP), inactivate cholinesterase. Therefore they indirectly prevent the destruction of acetylcholine. Because submaximum doses of these drugs inactivate not all but only a part of the cholinesterase present, they slow down but do not stop the destruction of acetylcholine. The overall effect at such submaximum levels of anticholinesterase is to increase by some constant the lifetime of any acetylcholine emitted into the synapse, which increases the concentrations of acetylcholine in the synapse which result from a given rate of emission. Within certain limits the greater this concentration the greater is the efficiency of transmission, that is, the conduction across the synapse. Above that limit, which is set by the sensitivity of the postsynaptic membrane, any further increase in acetylcholine concentration produces a synaptic block (*6, 7*). Thus, the application of a given dosage of anticholinesterase will (by protecting acetylcholine from destruction) have different effects on the efficiency of synaptic conduction that depend on the rate of acetylcholine emission during transmission and on the sensitivity of the postsynaptic membrane. When emission of acetylcholine is small, or when the sensitivity of the postsynaptic membrane is low, an application of anticholinesterase will render transmission more efficient, a property used to good effect in the treatment of myasthenia gravis. In the treatment of this disorder, anticholinesterase is

used to raise the effective concentration of acetylcholine at the neuromuscular junction, which reduces apparent muscular weakness. On the other hand, the same dose of anticholinesterase that caused muscular contraction in the myasthenic patient produces paralysis in a man with normal function of the neuromuscular junction.

Over a period of time if there are changes after learning in the amounts of acetylcholine emitted at the modified synapse, then such a synapse should show either facilitation or block, depending on just when, after learning takes place, we inject the same dose of anticholinesterase. A similar argument with regard to the action of anticholinesterase can be applied if we assume that, instead of a presynapatic increment in transmitter, it is the postsynaptic membrane that becomes more sensitive to transmitter as a function of time after learning. But the use of an anticholinesterase does not allow us to decide which of these alternative arguments actually holds for the learning situation. I describe below how the use of other types of drugs, such as the cholinomimetics, allows us to surmise that postsynaptic sensitization is the more likely mechanism.

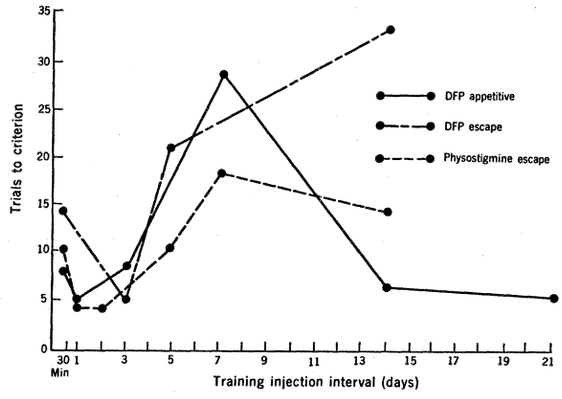


Fig. 1. The effect of anticholinesterase injection on memories of different ages [from (*8, 14, 16*)]. Trials to criterion during retest are plotted against the time that elapsed between retest and original learning; the larger the number of trials to criterion, the greater the amnesia. The time between injection and retest was constant. The differences past the 7-day point probably represent differing rates of forgetting in the three situations.

Memory Block and Facilitation with Anticholinesterase

The first two experiments (*8, 9*) show that facilitation or block of a memory can be obtained with the same dose of anticholinesterase simply as a function of time of injection after original learning, as might be expected if synaptic change formed the substrate of memory. In the first experiment, rats were trained on a simple task (*10*). Then anticholinesterase was injected intracerebrally at intervals after initial training, the time being varied from one group of subjects to another. After they were injected, all rats, irrespective of the group to which they were assigned, were tested again 24 hours after injection. Thus, the time between training and injection was varied, whereas the time between injection and retest was kept constant. Any difference between groups was therefore due to the time between initial training and injection.

Rats were placed on an electrified grid in a Y-maze. One arm of the Y was illuminated but not electrified, and its position was changed at random from trial to trial. The rats learned to run into the illuminated arm. The criterion of learning was met when they had chosen this arm ten trials in succession, whereupon training was concluded.

Then, at various times after training, the rats were injected intracerebrally with DFP dissolved in peanut oil (*11*). This dose did not increase the number of trials to criterion in a naive group of rats, and the result showed that learning capacity during training was not affected by the drug in the amounts used. At 24 hours after injection, the rats were retrained to the same criterion of ten correct trials in succession. The number of trials to criterion in this retraining session represented the measure of retention.

The first group was injected 30 minutes after training. Its retention was significantly worse than that of a control group injected only with peanut oil (*12*). By contrast, a group injected with DFP 3 days after training showed the same amount of retention as did the control group. Thus, up to this point it seems that the longer the item is stored, the less susceptible is memory to DFP. In fact, a subsidiary experiment (*13*) has established that injections of DFP on habits 1 and 2 days old have no effect, which shows that the initial stage of vulnerability lasts less than 1 day. Beyond 3 days, however, the situation seems to reverse itself; the memory is more susceptible to DFP the older it is because a group injected with DFP 5 days after training showed only slight recollection at retest, and a further group injected 14 days after training showed complete amnesia. The score of the group trained 14 days before injection was the same as the score of the previously mentioned naive group that had not been trained before but had simply been injected with DFP 24 hours prior to testing. The amnesia of the DFP group trained 14 days before injection was not due to normal forgetting, because other controls showed almost perfect retention over a 15-day span. Using the same escape habit, Hamburg (*14*) obtained similar results with intraperitoneal injections of the anticholinesterase physostigmine.

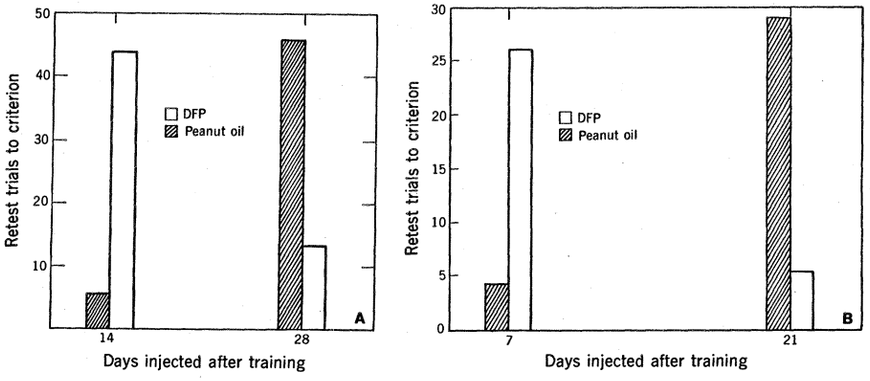


Fig. 2 (A and B). The effect of injection of the anticholinesterase DFP and peanut oil on habits that were well retained or almost forgotten. Trials to criterion are plotted against time between retest and original training. When controls remember well, DFP-injected animals forget. When controls forget, DFP-injected animals remember well [after (*9, 16*)].

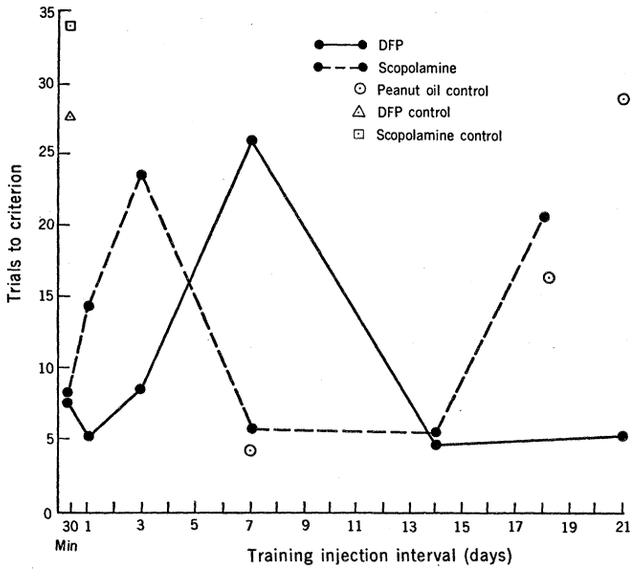


Fig. 3. The effects of the injection of the anticholinergic scopolamine compared with that of the anticholinesterase DFP and the control of peanut oil on the retention of an appetitive task at various times after original learning. The time between injection and retest was constant. Also indicated is the number of trials to criterion when rats were injected with scopolamine (scopolamine control) or DFP (DFP control) before original learning to give an estimate of actual amount of amnesia produced [from (*16*)].

Biederman (*15*) confirmed the shape of the amnesic function with physostigmine in an operant situation. He used a latency measure of forgetting and a bar-press response.

To make sure that we were not observing some periodicity due to fear or emotionality interacting with the drug, we conducted an experiment with an appetitive rather than an escape task. The rats were taught to run to a reward of sugar water, the position of which always coincided with the illuminated arm of a Y-maze (*16*). These results and the results from the preceding experiments show a similar pattern of amnesia as a function of time of learning before injection (Fig. 1). It is, therefore, most likely that we are in fact studying memory. The divergences in the curves after 7 days are probably due to differences in rates of forgetting among the three groups.

In this first set of experiments that dealt with the effects of the anticholinesterases DFP and physostigmine on habits that are normally well retained, the effects of these drugs were to decrease the retention of a habit depending on its age. Thus, one of the predicted effects of an anticholinesterase was verified. However, the other predicted effect, facilitation, was not shown. The reason for this is that the habit that was acquired was so well retained without treatment over 14 days that one could not, on methodological grounds, show any improvement of retention subsequent to injection of the drug. It may be the case that habits that were trained 1, 2, and 3 days prior to injection and retest were facilitated instead of merely being unaffected, but the design of the experiment would not allow us to detect this because there is an effective ceiling on performance. Therefore, an attempt was made to obtain facilitation where it was methodologically possible to detect it, namely, where retention of the habit by a control group was imperfect. For example, it was found that 29 days after learning, the escape habit described above was almost forgotten by a group of animals injected with peanut oil only, 24 hours before. On the basis of this observation, we devised a second kind of experiment.

Rats were divided into four groups. The first two groups were trained 14 days before injection, the second two groups, 28 days before injection. One 28-day group and one 14-day group were injected with the same dose of DFP, and the other 28-day group and the other 14-day group were injected with the same volume of pure peanut oil. The experimental procedure and dosage were exactly the same as previously described.

On retest, poor retention was exhibited by the 14-day group injected with DFP and by the 28-day group injected with peanut oil. By contrast, the 28-day group injected with DFP and the 14-day group injected with peanut oil exhibited good retention. The results of anticholinesterase injection show a large and clear facilitation of an otherwise almost-forgotten habit that was 28 days old, whereas they confirm the obliteration of an otherwise well-remembered habit that was 14 days old, as already demonstrated in the previous experiments (Fig. 2A). The same facilitation of a forgotten habit was shown by Wiener and Deutsch (*16*) using an appetitive habit and by Squire (*17*) using mice injected with physostigmine. Biederman (*18*) showed an improvement in memory in pigeons when physostigmine is injected 28 days after a line-tilt discrimination was partly learned. A well-learned color discrimination acquired by the same subjects showed no such improvement under the same conditions. Thus, these results also lend strong support to the notion that forgetting is due to a reversal of the change in synaptic conductance that underlies learning (Fig. 2B). It must be emphasized, however, that both the block and facilitation of a memory are temporary and wear off as the injected drug wears off.

Memory Block with Anticholinergics

We have shown that the anticholinesterases DFP and physostigmine have effects on memories that differ with the age of the memories. Although their actions on memory are consistent with, and plausibly interpreted by their anticholinesterase action, some other property besides their indirect action on acetylcholine could in some unknown manner produce the same results. Therefore, we conducted an independent check on the hypothesis that the effects observed might be due to an effect on acetylcholine by using an anticholinergic drug. An anticholinergic such as atropine or scopolamine, reduces the effective action of a given concentration of acetylcholine at the synapse without

actually changing the concentration itself. It does this apparently by occupying some of the receptor sites on the postsynaptic membrane without producing depolarization. It thus prevents acetylcholine from reaching such receptor sites, which attenuates the effectiveness of this transmitter. We would therefore expect an anticholinergic to block conduction at a synapse where the postsynaptic membrane is relatively insensitive, whereas it would simply diminish conduction at synapses where the postsynaptic membrane is highly sensitive. If the interpretation of the effects of DFP is correct, we would then expect the reverse effect with the administration of an anticholinergic drug. That is, we would expect the greatest amnesia with anticholinergics precisely where the effect of anticholinesterase was the least; and we would predict the least effect where the effect of anticholinesterase was the largest. It will be recalled that the least effect of anticholinesterase was on habits 1 to 3 days old.

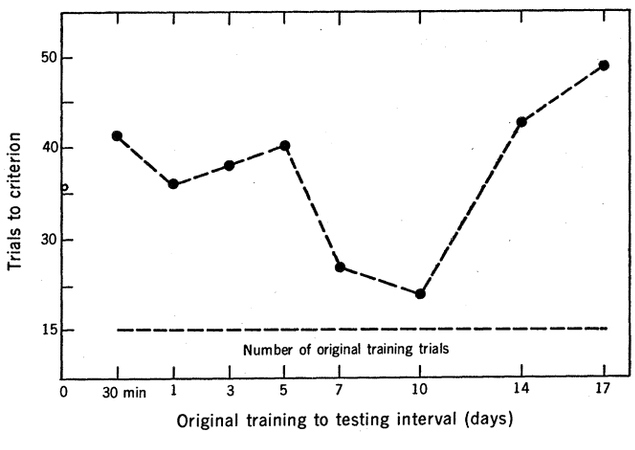
In a third set of experiments (*16*, *19*), the anticholinergic agent injected was scopolamine. The experimental procedure and the amount of oil and the location for the injection were the same as in the experiments with DFP (*20*). A group injected 30 minutes after training showed little if any effect of scopolamine. However, a group injected 1 and 3 days after training showed a considerable degree of block. Groups injected 7 and 14 days after training showed little if any effect. The results from the appetitive and escape situations were very similar.

As far as the experimental methodology allows us to discern, the anticholinergic effect is the mirror image of the anticholinesterase effect (Fig. 3); there is an increase of sensitivity between 30 minutes and 1 to 3 days which is followed by a decrease of sensitivity. This observation further confirms the notion that there are two phases present in memory storage. Finally, it is of interest that amnesia can result in man from anticholinergic therapy (*21*).

Memory Fluctuation without Drugs

The above experiments support the idea that at the time of learning some unknown event stimulates a particular group of synapses to alter their state and to increase their conductivity. At this point two questions may be asked. Why does such an increase in synaptic conductivity not manifest itself with the passage of time when no drugs are injected, and why has it not been noted that habits are better remembered a week after initial learning than, say, 3 days after such learning? There are various possible answers. One is that the phenomena we have described are some artifact of drug injection. Another is that animal training has, in general, stretched over days in other studies and has blurred in time the initiation of a memory. In addition, and partly as a consequence of the foregoing, it is difficult to find studies on retention where the age of the habit, measured in days, has been used as an independent variable.

The question then arises as to whether or not we should have seen such an improvement in recall in our control groups. This would have been unlikely because our animals were trained to the very high criterion of ten out of ten trials correct. Given a score that was initially almost perfect, it was nearly impossible to observe any subsequent improvement in retention that might in fact actually exist. To rid ourselves of this limitation, we devised a study in which no drugs were used and in which rats were initially undertrained to escape from shock. The rats were given 15 trials and then were tested on some subsequent day to see how many trials it would take for them to reach our strict criterion (*22*). The



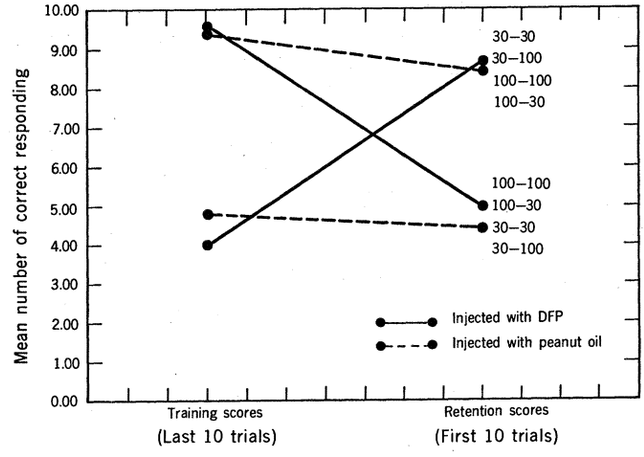


Fig. 4 (left). The effects of delay between original partial training (15 trials) and subsequent training to criterion. Plotted are trials to criterion in subsequent training against time since original partial training. Control (○) indicates the number of trials to criterion taken by a group that received its training all in one session. Fig. 5 (right). The effects of injection of DFP on the retention of well-learned and poorly learned habits. The mean number of correct responses of the last 10 of 30 trials for two groups is shown on the left. One group had to learn to run to the alley illuminated by a normal 120-volt bulb with 30 volts across it to make it look dim; the other had to learn the same task except that the 120-volt bulb had 100 volts across it to make it look much brighter. As can be seen from the last ten trials, the dim light offered to the 30-volt group posed a difficult task that produced little learning by the end of the 30 trials. The group learning by the brighter cue (100 volt) displayed excellent acquisition. Because of the different rates of acquisition of the 100-volt and 30-volt habits, half of each group was shifted to retest on the other brightness and half was retrained on the same brightness (30-30, 100-100 retested on the same brightness; 30-100 trained on 30, retested on 100; 100-30 trained on 100, retested on 30). The scores of animals trained on the same brightness are combined. Half of the animals were injected with DFP, the other half with peanut oil. There is little change in the scores of the peanut oil–treated animals. However, there is a complete reversal of the animals injected with the drug, showing block of the well-learned habit and facilitation of the poorly learned habit.

rats took only about half the number of trials to reach the criterion when tested after 7 or 10 days than after 1 or 3 days (Fig. 4). Using an appetitive task Huppert (*23*) has now shown an analogous improvement. Finally, McGaugh has pointed out that there are old studies on animals that purport to find similar effects (*24*). This shows that our conclusions about the varying substrate of memory were not due to some pharmacological artifact.

Gradation of Memory Change

We may now ask whether the inferred modification of a synapse represents an all-or-none process or a graded process. In other words, can a synapse be modified only once during learning or does a repetition of the same learning task after some learning has already occurred further increase conductance at a single synapse? If we postulate an all-or-none process, then how according to such a model can we explain empirical increases in "habit strength" with increased training? Possibly they are due to a progressive involvement of fresh synapses and a spread involving more parallel connections in the nervous system. In support of a graded process, we may hypothesize that successive learning trials modify the same synapses in a cumulative way by producing an increase either in the rate at which conductance increases or in the upper limit of such conductance, or both.

There are tests of these two alternatives. If, with increased training a synapse becomes more conductive, then a habit should become increasingly more vulnerable to anticholinesterase with increased training. Furthermore, the memory of the same habit should be facilitated when its level of training is very low. In other words, we should be able to perform the same manipulations of memory by varying the level of training as we were already able to perform when we varied the time after training.

If, on the other hand, increases in training simply involve a larger number of synapses but no increase in transmission at any one synapse, then increases in training should not lead to an increased vulnerability of a habit to anticholinesterase. Rather, the opposite should be the case. As the number of synapses recruited is increased, some of the additional synapses will, by chance variation, be less sensitive to a given level of anticholinesterase. Thus, a larger number of synapses should be left functional after anticholinesterase injection when we test an overtrained habit. Three experiments (*25–27*) show a large and unequivocal effect. Poorly learned habits are enormously facilitated, and well-learned habits are blocked (Fig. 5). This supports the hypothesis that a set of synapses underlying a single habit remains restricted, and each synapse within such a set simply increases in conductance as learning proceeds.

Table 1. The effect of carbachol injection on recall of habits that were 3 and 7 days old. Criterion was seven correct trials in succession. Numbers in parentheses indicate number of rats tested.

Treatment	Median number of trials to criterion	
	3 days	7 days
Carbachol	6.0 (15)	20 (15)*
Saline	4.0 (8)	0 (7)

* $P < .01$ compared with saline, Mann-Whitney U test.

Interval during Retest and Memory Block

The results presented so far have been interpreted in terms of the action of drugs on synapses that alter their conductance as a function of the time after training and of the amount of training. We can use our model to generate a somewhat different kind of prediction. An anticholinesterase in submaximum concentrations simply slows down the rate of destruction of acetylcholine. Because we have hypothesized that amnesia is due to a block resulting from an acetylcholine excess, we should predict no amnesia if we spaced our trials so that all or most of the acetylcholine emitted on the previous trial is destroyed by the time the next trial comes along. Bacq and Brown (*28*) showed that (with an intermediate dose of anticholinesterase) block at a synapse occurred only when the intervals between successive stimuli were shortened. Accordingly, an experiment was performed where we varied the interval during retest between 25 and 50 seconds (*29*). Using a counterbalanced design, we found that rats tested under physostigmine at 25-second intervals showed amnesia for the original habit, whereas those tested at 50-second intervals showed no amnesia.

In a second experiment, the rats had to learn an escape habit during the retest that was the reverse of the one they had learned during training. To escape shock they had to learn not only to run to the dark alley but also to inhibit the original learning of running to the illuminated alley. Thus, provided that the original habit was remembered at the time the reversal was being learned, the time to learn the reversal should take longer than the time to learn the orig-

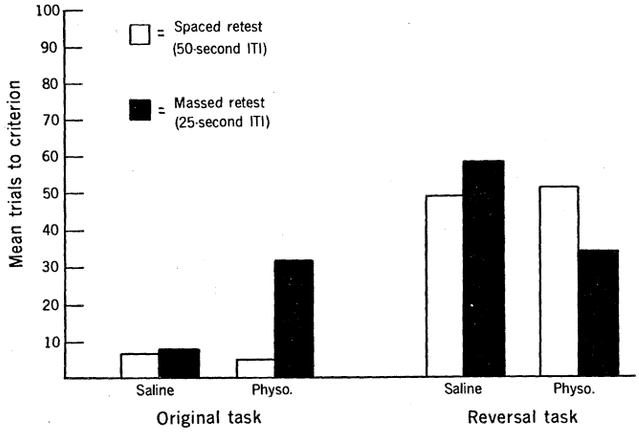


Fig. 6. The effect of massing and spacing trials during retest on amnesia induced by anticholinesterase. On the left, retest consisted of relearning original habit (run to light, avoid dark). On the right, retest consisted of unlearning original habit. On retest, the animal had to learn to run to dark and avoid light (reversal). *ITI*, intertrial interval; *Physo.*, physostigmine.

inal habit. But if the original habit was not remembered there should be no difference in trials to criterion between original learning and retest. The results showed that, at 50 seconds between trials, animals in both the physostigmine-treated and the saline-control groups took almost twice as long to reverse as it took them to learn the original habit, indicating in fact that they remembered the original habit (Fig. 6). At 25 seconds between trials, the animals treated with physostigmine learned the reversal as quickly as the original habit, whereas again the control animals took much longer. This second experiment shows that the amnesia of the 25-second group injected with physostigmine in the first experiment is not due to either disorientation or incapacity to perform or learn, but to an amnesia. We might explain the high relearning scores of the same habit of the rats at 25-second intervals under physostigmine by saying that the rats were somehow incapacitated by the physostigmine if they had to run at 25-second intervals. However, it is difficult to see how such incapacitation could produce abnormally low learning scores of the reversal habit. This dependence of the amnesia on the precise interval between trials during retest should of course not be seen with anticholinergics or cholinomimetics but only with anticholinesterases. This further prediction from the hypothesis should be tested.

Postsynaptic Change More Likely

So far, then, it seems that the drugs we are using to block or facilitate memory have their effect on synaptic conductance. However, what is it that changes when synaptic conductance alters? As was mentioned previously, the two main hypotheses are (i) that the amount of transmitter emitted at the presynaptic ending increases or (ii) that the postsynaptic ending increases in its sensitivity to transmitter. To test this idea, carbachol (carbamoylcholine chloride) was injected before retest. This drug is a cholinomimetic. It acts on the postsynaptic membrane much like acetylcholine. However, it is not susceptible to destruction by the enzyme acetylcholinesterase. Therefore, by injecting this drug, we can test the sensitivity of the postsynaptic membrane. It seems that habits learned 7 days before injection and retest are blocked by a dose of this cholinomimetic that leaves a

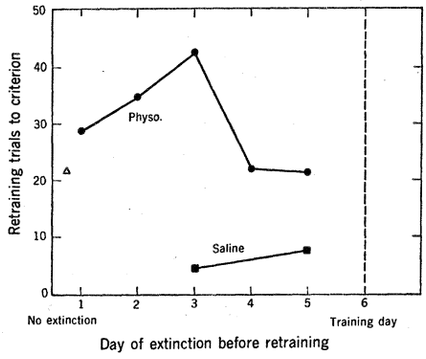


Fig. 7. The effect of physostigmine on retraining after extinction. The time between original learning and retraining is the same for all groups. When time of extinction is close to original learning, there is amnesia but no difference from the group receiving no extinction. At extinction 3 days before learning, the number of trials to relearn is almost double. *Saline*, scores of controls injected with saline; *Physo.*, scores of animals injected with physostigmine.

habit learned 3 days before unaffected (Table 1). This would indicate that it is probably the postsynaptic membrane that has increased its sensitivity and so increased synaptic conductance.

One of the questions that often arises is why it is that we do not block all cholinergic synaptic activity with the drugs we use. As was seen above, rats learn appetitive tasks at a normal rate under doses of drug that under some circumstances produce complete amnesia. There is very little in the overt behavior of the rat to indicate that it has been drugged. The doses of drugs used produce no apparent malaise or incoordination. The dose we use only seems to affect what one might call the "memory" synapses. Therefore, it would seem that these are more sensitive to our drugs. Such an abnormal sensitivity may be more apparent than real. We know that there are some levels of training and times after training where a habit is unaffected by the dosage of drug we use, and this shows that memory synapses are not always affected. It seems that the memory synapses have a much larger range of postsynaptic sensitivity, whereas normal synapses are in the middle of the sensitivity range of the memory synapse. In other words, sensitivity of the memory synapse must range from extreme insensitivity to transmitter to extreme sensitivity in order to manifest those changes in conductance that we have demonstrated. It will therefore be much more susceptible to anticholinergic agents when conductance is low and to anticholinesterases and cholinomimetics when conductance is high. In the middle of the range, sensitivity to all agents will resemble that of a normal synapse, and only grossly toxic doses will affect memory. This, of course, will have to be further tested. So far, the experiments implicate the cholinergic system in memory. It is, of course, possible that other systems, such as the adrenergic, may have a similar function, and this, too, we hope to test.

Analysis of Extinction through Selective Amnesia

When an animal is rewarded for performing a habit, such a habit will be learned or acquired. However, when the habit is no longer rewarded, the

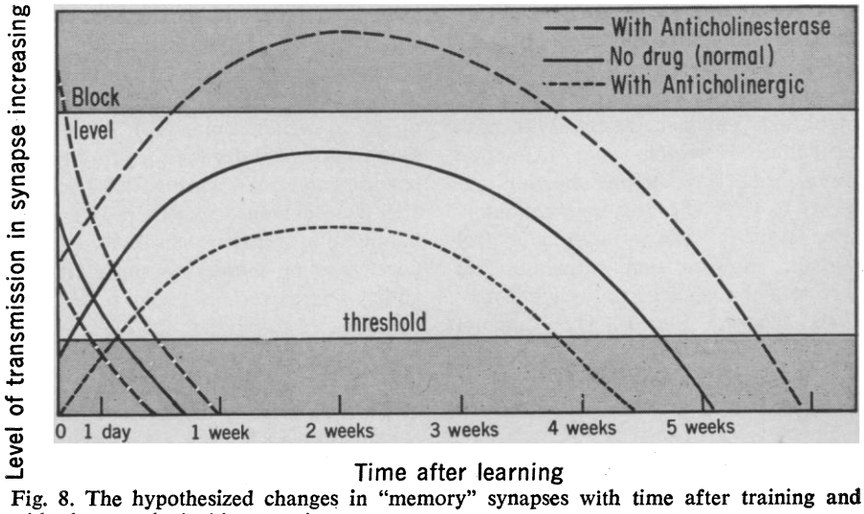


Fig. 8. The hypothesized changes in "memory" synapses with time after training and with pharmacological intervention.

animal will cease to perform the habit. Another kind of learning takes place, and this is called extinction. If initial learning consists of the formation of some synaptic (or other) connection, does extinction consist of the weakening or uncoupling of this connection? Or is it the formation of some other connection that then works to oppose the effects of the first (learning) connection? If extinction consists of weakening the connection set up in original learning, then an extinguished habit should be similar to a forgotten habit pharmacologically. We have already shown that a habit that is almost forgotten is facilitated by anticholinesterase. We would, then, on the "weakening" hypothesis of extinction, expect an injection of an anticholinesterase to produce less amnesia of an extinguished habit than of the same unextinguished habit.

If, on the other hand, during extinction there is another habit acquired that inhibits the expression of the original habit, another pattern of results should be discernible after injection with an anticholinesterase. If original learning occurs 7 days before anticholinesterase injection and retest, there should be amnesia for the original habit. If extinction of the habit is given close in time to its acquisition, there should be amnesia for both the original learning and extinction. If, on the other hand, original learning is 7 days before injection and retest, and the extinction is 3 days before injection and retest, then the original habit should be lost but the extinction habit should be retained. (As we noted above, 3-day habits are unaffected by our dose of anticholinesterase.) When extinction was given to rats close in time to the original training, both the original training and extinction were blocked by physostigmine (*30*). These rats took the same number of trials to relearn as control animals, which were trained, not extinguished, and then injected with physostigmine. However, when extinction was placed 3 days before injection and retest, it took the rats approximately twice as many trials to learn as control animals, showing that extinction has been retained whereas the original habit was blocked (Fig. 7). This supports the idea that extinction is the learning of a separate habit that opposes the performance of the initially rewarded habit.

It has also been suggested (*31*) that different systems, such as excitatory or inhibitory systems, are subserved by different transmitters. Habits acquired during extinction have been viewed as inhibitory. However, the last experiment we have outlined also shows that extinction placed close to original learning is equally as vulnerable to anticholinesterase as original learning. Habits can probably not be classified into synaptically inhibitory and excitatory on the basis of behavioral excitation or inhibition. However, as all habits compete for behavioral expression, there must be excitation and reciprocal inhibition connected with all habits.

Conclusions

A simple hypothesis can explain the results obtained to date if we disregard those results when we wait 30 minutes after original learning to inject. The hypothesis is that, as a result of learning, the postsynaptic endings at a specific set of synapses become more sensitive to transmitter. This sensitivity increases with time after initial learning and then declines. The rate at which such sensitivity increases depends on the amount of initial learning. If the curve of transmission plotted against time is displaced upward with anticholinesterases then the very low portions will show facilitation, and the high portions will cause block (Fig. 8). The middle portions will appear unaffected (unless special experimental tests are made). If the curve of transmission is displaced down with anticholinergics, then the middle portion will appear unaffected and only the very early or late components will show block.

The results are evidence that synaptic conductance is altered as a result of learning. So far it seems (i) that cholinergic synapses are modified as a result of learning and that it probably is the postsynaptic membrane that becomes increasingly more sensitive to acetylcholine with time after learning, up to a certain point. (ii) After this point, sensitivity declines, leading to the phenomenon of forgetting. (iii) There is also good evidence that there is an initial phase of declining sensitivity to cholinesterase or increasing sensitivity to anticholinergics. This could reflect the existence of a parallel set of synapses with fast decay that serve as a short-term store. (iv) Increasing the amount of learning leads to an increase in conductance in each of a set of synapses without an increase in their number. (v) Both original learning and extinction are subserved by cholinergic synapses.

References and Notes

1. E. Tanzi, *Riv. Sper. Freniat.* **19**, 149 (1893).
2. Eccles has attempted to supply such evidence by studying the effect of stimulation on transmission across a synapse at the spinal level. After repetitive stimulation, a stimulus produces a larger effect at the synapse than before stimulation. J. C. Eccles, in *Brain Mechanisms and Learning* (Thomas, Springfield, Ill., 1961), pp. 335–352; *The Physiology of Synapses* (Academic Press, New York, 1964). W. A. Spencer and R. Wigdor [*Physiologist* **8**, 278 (1965)] and F. B. Beswick and R. T. W. L. Conroy [*J. Physiol. London* **180**, 134 (1965)] have also shown such an effect. On the other hand, J. C. Fentress and R. Doty [*Fed. Proc.* **25**, 573 (1965)] have reported a depression of responsiveness at a synapse after stimulation.
3. S. K. Sharpless, *Annu. Rev. Physiol.* **26**, 357 (1964).
4. W. R. Russell and P. W. Nathan, *Brain* **69**, 280 (1946).
5. G. A. Talland, *Deranged Memory* (Academic Press, New York, 1965).
6. L. S. Goodman and A. Gilman, *The Pharmacological Basis of Therapeutics* (Macmillan, New York, 1965).
7. W. Feldberg and A. Vartiainen, *J. Physiol. London* **83**, 103 (1934); R. L. Volle and G. B. Koelle, *J. Pharmacol. Exp. Ther.* **133**, 223 (1961).
8. J. A. Deutsch, M. D. Hamburg, H. Dahl, *Science* **151**, 221 (1966).
9. J. A. Deutsch and S. F. Leibowitz, *ibid.* **153**, 1917 (1966).
10. The rats were Sprague-Dawley males approximately 350 grams at the start of the experiment.
11. The animals were placed in a stereotaxic instrument under Nembutal anesthesia. They were intracerebrally injected in two bilateral loci that were symmetrically placed. The placements were: anterior 3, lateral 3, vertical +2 and anterior 3, lateral 4.75, vertical −2, according to the atlas of DeGroot [J. DeGroot, *Verh. Kon. Ned. Akad. Wettensch. Afdel-Natuurk.* Sect. II, 52 (1957)]. Peanut oil (0.01 milliliter) containing 0.1 percent of DFP was injected in each locus.
12. Except as otherwise stated, the results quoted are significant beyond the 1 percent level. The tests used were the *t*-test, Mann-Whitney U test, and analysis of variance.
13. J. A. Deutsch and J. Stone, in preparation.
14. M. D. Hamburg, *Science* **156**, 973 (1967).
15. G. B. Biederman, *Quart. J. Exp. Psychol.* **22**, 384 (1970).
16. N. I. Wiener and J. A. Deutsch, *J. Comp. Physiol. Psychol.* **66**, 613 (1968).
17. L. R. Squire, *Psychon. Sci.* **19** (1), 49 (1970).
18. G. B. Biederman, *ibid.*, in press.
19. J. A. Deutsch and K. Rocklin, *Nature* **216**, 89 (1967). See also A. Pazzagli and G. Pepeu, *Int. J. Neuropharmacol.* **4**, 291 (1964).
20. Deutsch and Rocklin (*19*) used an injection of scopolamine at the same loci as in (*11*). Peanut oil (0.01 milliliter) containing 0.58 percent of scopolamine was injected in each placement. Wiener and Deutsch (*16*) used only the first locus, but doubled the amount injected at that site (both of scopolamine and DFP).
21. W. C. Cutting, *Handbook of Pharmacology; The Actions and Uses of Drugs* (Appleton-Century-Crofts, New York, 1964).
22. F. A. Huppert and J. A. Deutsch, *Quart. J. Exp. Psychol.* **21**, 267 (1969).
23. F. A. Huppert, personal communication.
24. A. C. Anderson, *J. Comp. Psychol.* **30**, 399 (1940); M. E. Bunch and W. K. Magdsick, *ibid.* **16**, 385 (1933); M. E. Bunch and E. S. Lang, *ibid.* **27**, 449 (1939); H. B. Hubert, *Behavior Monogr.* **2**, No. 6 (1915).
25. J. A. Deutsch and S. F. Leibowitz, *Science* **153**, 1017 (1966).
26. J. A. Deutsch and H. Lutzky, *Nature* **213**, 742 (1967).
27. S. F. Leibowitz, J. A. Deutsch, E. E. Coons, in preparation.
28. Z. M. Bacq and G. C. Brown, *J. Physiol. London* **89**, 45 (1937).
29. J. A. Deutsch and K. Rocklin, in preparation.
30. J. A. Deutsch and N. I. Wiener, *J. Comp. Physiol. Psychol.* **69**, 179 (1969).
31. P. L. Carlton, in *Reinforcement and Behavior*, J. T. Tapp, Ed. (Academic Press, New York, 1969), pp. 286–327.