Amnesia or Reversal of Forgetting by Anticholinesterase, Depending Simply on Time of Injection

Abstract. *The effect of intracerebral injections of the anticholinesterase drug diisopropyl fluorophosphate in rats was to produce good recall of an otherwise almost forgotten habit learned 28 days before. The same injections produced temporary amnesia for the same habit, otherwise well remembered, learned 14 days before. The injections had no effect on the memory of the same habit when it was only partly learned 14 days before. The results support the hypothesis that the physiological basis of memory lies in an increase, and forgetting in a decrease, in synaptic conductance.*

In a previous report (*1*) the effects of intracerebral injections of the anticholinesterase drug diisopropyl fluorophosphate (DFP) on the memory of otherwise well-remembered habits was described. Amnesia was produced by such injections for a habit 14 days old but not for the same habit only 3 days old. Here we report the effect of the same injections on a habit acquired 28 days before, where forgetting has already set in. Gleitman *et al.* (*2*) report forgetting in rats 25 days after training.

As in the previous study (*1*), male Sprague-Dawley rats (350 g) were trained to a criterion of ten successive correct choices to escape electric shock to their feet by running to the lit arm of a Y-maze. After learning, the rats were divided into five groups by matching initial learning scores. The object was to compare the effects of DFP on 14-day-old and 28-day-old habits. The first two groups were injected 14 days after training, the first with DFP and the second with the vehicle, peanut oil. The second two groups were injected 28 days after training. Thus the third group was injected with DFP and the fourth with peanut oil 28 days after training. The fifth group was used in a different part of the experiment. The rats in all the first four groups were retested 24 hours after operation. The operation was performed under nembutal anesthesia in a stereotaxic instrument, and injections were made through a hypodermic needle bilaterally in two hippocampal loci. The placement of the bilateral injection was anterior 3, lateral 3, vertical +2 and anterior 3, lateral 4.75, vertical −2, according to the atlas of DeGroot (*3*). Each injection contained 0.1 ml of anhydrous peanut oil containing (in the case of groups 1 and 3) 1 mg of DFP per milliliter (Floropryl isoflurophate, U.S.P.). The dose of drug and placement of injection was chosen to be the same as in the previous report (*1*) to make the results comparable. A sample of ten brains was histologically examined and confirmed the correctness of placement of injection tracks.

Substantial forgetting or memory loss took place in the first and fourth groups (Table 1). The first group had been injected with DFP 14 days after initial learning and the fourth with peanut oil 28 days after initial learning. The results of the first group corroborate the results already reported (*1*), while the results of the fourth group show that after 28 days the habit had undergone a process of forgetting. (However, the poor performance of the fourth group may be due to an interaction of some forgetting with the aftereffects of the operation 24 hours before.) On the other hand, excellent or good recall of the habit is shown by groups 2 and 3. Group 2 was injected with peanut oil 14 days after training, whereas group 3 was injected with DFP 28 days after initial training. Taken as a whole, the results show that an otherwise almost forgotten habit becomes well remembered through an injection of DFP. This contrasts with the amnesia induced by DFP for an otherwise well-remembered habit.

It has been suggested (*4*) that, as a result of learning, an initially nonfunctional synapse is modified to eject transmitter and so is rendered functional. As this increase in the capacity to eject transmitter takes some time before it levels off, synapses modified recently should be less affected by anticholinesterase than those modified a longer time before. A reduction in cholinesterase should produce a swifter accumulation of acetylcholine (leading to synaptic block) at synapses where the amount of acetylcholine during transmission is high. This would explain why 14-day-old habits are vulnerable to a certain dose of DFP while 3-day-old habits are immune. On the other hand, where amounts of transmitter are marginal, anticholinesterase should improve transmission by allowing otherwise ineffective amounts of acetylcholine to accumulate, a fact used in the treatment of myasthenia gravis. If forgetting is due to a decline of transmitter at the synapses which store the habit, we can explain why almost forgotten habits become available again when an anticholinesterase is injected, as has been shown in this report.

To test this interpretation, two other groups of rats were used. Group 5 was given only 30 trials during initial training (instead of being given training to criterion) so that the amount of relearning and, by inference, the amount of modification at the relevant synapses was much smaller. This group was then injected with DFP in the same manner and dose as above, and retested

Table 1. Effect, on rats trained to criterion, of injection of DFP (groups 1 and 3). Control groups (2 and 4) were injected with peanut oil (PO).

Group	No. in group	Injection: days after training	Substance injected	Initial learning scores		Retest scores	
				Means	Medians	Means	Medians*
1	10	14	DFP	44.5	46	44.2	47
2	7	14	PO	45	49	6.0	1
3	8	28	DFP	47.4	50	14.5	12
4	9	28	PO	51	56	46	46

* Probabilities of differences between medians of various groups, computed by the Mann-Whitney U-test, are: groups 1 and 3, $p < .01$; groups 3 and 4, $p = .001$; groups 1 and 2, $p < .001$; groups 2 and 4, $p < .001$.

Table 2. Effect, on partially trained rats (group 5), of injection of DFP (all three groups) compared to effect of injection in a group trained to criterion (group 1), and effect on fully trained rats (group 6) retested 5 days after injection.

Group	No. in group	Injection: days after training	Retested: days after injection	Initial learning scores		Retest scores	
				Means	Medians	Means	Medians*
5	9	14	1			17	8
1	10	14	1	44.5	46	44.2	47
6	9	14	5	44.2	49	17.8	13

* Probabilities of differences between medians of various groups, computed by the Mann-Whitney U-test, are: groups 5 and 1, $p < .005$; groups 1 and 6, $p < .01$.

24 hours later. It should, according to the hypothesis, resemble a group that has partially forgotten. There should therefore be a smaller amnesic effect than in the 14-day group trained to criterion (group 1), or even an enhancement of memory, as in the 28-day group (group 3). The results (Table 2) show that on retest this group required a mean of 17 trials to criterion, making the total number of trials to criterion 47, while the average of initial training for the other five groups is 46.4. The result, showing no evidence of amnesia in undertrained rats, is in good agreement with the hypothesis.

A further prediction from the hypothesis is that the amnesia caused by DFP should not be permanent, but that memory should return. Some evidence in favor of this has already been reported (*1*). Group 6, trained and treated in the same way as group 1, was retested 5 days later, instead of 1 day later. Return of memory was almost complete (Table 2). Besides supporting the view that the substrate of memory is a change in synaptic conductance, mediated by increasing amounts of transmitter, the present report suggests that forgetting lies in a reversal of this change.

Whether the observed effects on memory are related to the hippocampal locus of injection is at present undetermined. That there is considerable spread of the drug can be seen because the intrahippocampal injection of the drug in the experimental animals causes pupillary constriction. It seems likely that the effects on memory are due to anticholinesterase action of DFP. Injections of the anticholinergic scopolamine (*5*) produce the greatest amnesia 1 to 3 days after initial learning and a minimum of effect on memory at 14 days, the reverse of the case with DFP (*1*). This corroborates the notion that cholinergic synapses are being affected and that the quantity of transmitter emitted at these synapses soon after learning is low and gradually increases with time after learning. From the results reported here it seems the amount of transmitter at synapses modified by learning finally declines, rendering transmission across such synapses progressively less efficient and so producing behavioral forgetting.

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References and Notes

1. J. A. Deutsch, M. D. Hamburg, H. Dahl, *Science* **151**, 221 (1966).
2. H. Gleitman, F. Steinman, J. W. Bernheim, *J. Comp. Physiol.* **59**, 461 (1965).
3. DeGroot, *Verhandel. Konink. Ned. Akad. Wetenschap. Afdel. Natuurk. Sect. II*, **52**, 1 (1957).
4. J. A. Deutsch, *Diseases Nervous System*, in press.
5. J. A. Deutsch and K. W. Rocklin, in preparation.
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6-Hydroxylation: Effect on the Psychotropic Potency of Tryptamines

Abstract. *6-Hydroxy-5-methoxy*-N,N-*dimethyltryptamine and 5-methoxy*-N,N-*dimethyltryptamine were synthesized and their psychotropic effects compared on trained rats in a Skinner box. The nonhydroxylated form was the more potent. The metabolism of 5-methoxytryptophol acetate ester was also studied to determine whether hydroxylation might occur in other than the six position with exogenous indoles. One metabolite was formed, with properties of a hydroxy-5-methoxyindole-3-acetic acid, which proved on chromatography not to be the 6-hydroxy structural isomer. Pharmacologic and metabolic studies suggest that psychotropic activity of tryptamines may result from metabolites other than the 6-hydroxylated forms.*

It has been generally held (*1*) that in mammals, when most exogenous indoles that are substituted in the three position are metabolized by hydroxylation, this occurs at the six position. Szara and Hearst (*2*) postulated that the behavioral effects of *N,N*-dimethyltryptamine in rats are due to a metabolite because a substance having high potency was isolated from urine of rats receiving this compound. They suggested, on the basis of chromatography, that the 6-hydroxyl metabolite was the active form (*3, 4*). This is an important premise since it suggests that other indoles might also have their biological actions enhanced by 6-hydroxylation.

We have synthesized by an unequivocal chemical route the 6-hydroxylated analog of 5-methoxy-*N,N*-dimethyltryptamine, a potent psychotropic agent (*5*). It was prepared from 6-benzyloxy-5-methoxyindole by treatment with oxalyl chloride and dimethylamine to give 6-benzyloxy-*N,N*-dimethyl-5-methoxyindole-3-glyoxylamide. The amide was reduced with lithium aluminum hydride and the benzyl group was removed from the resulting amine by hydrogenation (*6*). The structure of the parent compound (I), synthesized according to an established procedure (*7*), and that of the hydroxylated form (II) are shown in Fig. 1.

The two compounds were compared by measuring their interference with standardized behavior in rats trained to press a bar for food reward in a Skinner box (*8*). White male rats (average age 14 weeks), weighing between 320 and 395 g, were used. They were fed a diet of 12 to 16 g of Rockland rat and mouse chow each evening and trained 1 hour daily for a minimum of 8 weeks in a Skinner box on a positive-reinforcement, variable-interval schedule. Animals were not fed each day prior to their stay in the chamber. Intervals between opportunities for reward were variably spaced so that the rats were unable to remember their duration. For the rats to obtain maximum profit from the situation they continued to press the bar at a steady rate for the hour in the chamber. Their efforts expressed as bar presses per hour were automatically recorded. Each animal had an individual mean work rate ranging from 26 to 68 bar presses per minute. Normal performance of each animal served as its own control against work rates when under test.

Standard deviation based on data for 12 typical control days ranged from ± 7.9 percent to ± 21.4 percent. Dosages required for known psychotomimetic agents to alter the work rates of rats were higher than those which produced mental changes in human beings. Psilocybin produced marked reductions of work rates at 3 mg/kg, and 150 μg/kg of LSD-25 were required in these studies (intraperitoneal injections).

The animals were divided into four groups according to the compound and dose they received (see Table 1). At doses of 6-hydroxy-5-methoxy-*N,N*-dimethyltryptamine ranging from 6.7 to 7.5 mg/kg (15.9 to 17.7 μmole/kg) (group 1) no significant changes in rates occurred in any of the six experiments (Table 1). However, in the same dose range, the nonhydroxylated analog (group 2) caused significant changes of performance in all six experiments, with values approaching complete extinction of work rates. Three animals (group 3) that received lower doses (5.6 to 6.2 mg/kg) of the nonhydroxylated tryptamine all exhibited signifi-