*RESTORATION OF EXPRESSION OF MEMORY LOST AFTER TREATMENT WITH PUROMYCIN**

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We have reported that memory of maze learning in mice disappears approximately 10–20 hours after intracerebral injections of puromycin, to remain absent for at least three months thereafter, the longest interval over which we have made retention tests.[1] We began the present experiments to determine how long puromycin must be present in the brain to cause loss of memory. Using intracerebral injections of saline, it was planned to dilute out puromycin at various times up to about 20 hours after its administration and then to test memory after the mice had recovered. These experiments led to others which, surprisingly, showed that injection of saline, for at least two months after treatment with puromycin, can restore memory.

Materials and Methods.—The behavioral procedures have been described.[2] White mice weighing 28–32 gm were trained in a Y maze to a criterion of nine out of ten correct responses. Shock was given for failure to move from the stem of the Y within five seconds and for errors of left-right discrimination. The same procedure was used in tests for retention of memory of the training experience. These retention tests were usually given five days after the last intracerebral treatment but if necessary were delayed until the cage behavior of a mouse appeared normal. A final test of retention of relearning was given one to two weeks after the first retention test.

The injection technique has also been described.[2] All injections were bilateral and each had a volume of 12 μl. Each bitemporal injection, used to produce loss of recent (one-day-old) memory, contained 90 μg of puromycin; each of the combined temporal plus ventricular plus frontal injections, used to produce loss of longer-term (greater than five-day-old) memory, contained 30 μg. As is routine, puromycin dihydrochloride was neutralized with NaOH. Isotonic NaCl or NaCl isosmolar with that of the neutralized puromycin solutions was used in the saline injections; they were equally effective. Bitemporal injections of saline were made in mice which had received bitemporal injections of puromycin. Bilateral temporal plus ventricular plus frontal injections of saline were made in most of the mice which received corresponding injections of puromycin. In some experiments, however, mice treated with puromycin to produce loss of longer-term memory subsequently received only two bilateral injections of saline, i.e., either frontal or temporal or ventricular. These last experiments were made to test the possibility that longer-term memory might be restored by exposure of a more limited portion of the brain to saline. Finally, a series of mice treated with puromycin to produce loss of memory were subsequently injected with puromycin instead of saline. These second injections contained 90 μg each of puromycin when bitemporal injections were used, 30 μg each, with the six combined injections.

We have taken the following precaution in the design of these experiments.

We have found in large numbers of mice of appropriate size[1] that bitemporal injections of 90 μg of puromycin and the six combined injections of 30 μg consistently caused, respectively, loss of short-term and longer-term memory.[1, 2] Nevertheless, in the present series of experiments, the effectiveness of our puromycin in obscuring memory was checked throughout the experiments in control mice; some of these mice were tested for memory as long as 78 days after treatment.

Results.—The experiments of Table 1 were made to check the effectiveness of the puromycin which was used for the present studies. It produced sustained loss of memory consistent with results previously published.[1, 2] There was no evidence for loss of the potency of the antibiotic or of the development of resistance to it in our mice.

Table 2 shows the striking improvement of memory which followed intracerebral injections of saline in mice previously treated with effective doses of puromycin. There was a relatively large proportion of miceinjected with saline at 12–20 hours after puromycin that failed to show improvement of memory. At that time cerebral edema was at its height and it was consequently often difficult to inject saline effectively into the brain. At all other times the number of mice with lost memory after saline injections was surprisingly small and the number with memory retained at a high level, surprisingly large. The group of six mice at 30–34 days showed least retention. Even in this instance, however, the means ± standard error (SE) for percentage savings of trials and errors (57.2 ± 13.2 and 67.5 ± 11.0) for the group were significantly different (t test, $P < 0.01$) from those of the control group (1.9 ± 2.0 and 3.0 ± 2.3).

One group of experiments in addition to those shown in Table 2 was made with saline following puromycin to test the possibility that expression of longer-term memory might be restored by exposure of a limited portion of the brain to saline. Ten to 14 days after training, seven mice received bilateral temporal plus ventricular plus frontal injections of puromycin. In five of these, each injection contained 30 μg of puromycin; in two, 90 μg. Six to eight days later, the mice received only two injections of saline. These were all bilateral and either frontal or ventricular or temporal injections. Tests for memory showed retention in six mice and impairment in one which had received injections of 30 μg of puromycin. The means ± standard deviation (SD) for percentage of savings of trials and errors for the mice with retained memory were, respectively, 77 ± 13 and 90 ± 3; the corresponding savings for the mouse with impaired memory, 40 and 80.

Finally, experiments were performed where initial treatment with puromycin was followed by second injections of puromycin (Table 2). The means ± SE for

TABLE 1

CONTROL EXPERIMENTS

Puromycin Injections				Number Mice with Memory:		
Days after learning	Site	μG per injection	Retention test (days after puro)	Lost	Impaired	Retained
1	T	90	6–13	5	1	0
1	T	90	33–78	5	0	0
13	T + V + F	30	13–15	4	0	0
7–14	T + V + F	30	33–50	5	0	0

Control experiments show effectiveness of bitemporal (T) injections of puromycin in producing loss of memory when given 1 day after learning and of bilateral temporal plus ventricular plus frontal ($T + V + F$) injections in causing loss of longer-term memory. The long duration of loss of memory is also shown. For the mice with loss of memory, the means ± SD for percentage of savings of trials and errors were, respectively, 0 ± 0 and 1 ± 3; for the mouse with impaired memory, 44 and 54.

TABLE 2

MEMORY OF MICE TREATED WITH PUROMYCIN (P) AND SUBSEQUENTLY WITH SALINE (S) OR P

Time after Initial Puromycin of: Saline injections	Second P injections	Number Mice with Memory: Lost	Impaired	Retained
4–10 hr	—	0	3	17
12–20 hr	—	11	9	12
30 hr	—	0	1	7
2–12 days	—	1	9	13
3–18 days*	—	0	1	4
30–34 days	—	1	3	2
60 days	—	0	2	3
—	1–9 days	8	3	0
—	2–12 days*	4	3	0
—	30–60 days	2	3	1

* In these experiments, bilateral temporal plus ventricular plus frontal injections of 30 μg P were made 13–15 days after learning; corresponding injections of S or of 30 μg P were made at indicated times after these initial injections. In all other experiments, bitemporal injections of 90 μg P were made 1 day after learning; bitemporal injections of S or 90 μg P were made subsequently at indicate times. For the mice with loss of memory, the means ± SD for percentages of savings of trials and errors were, respectively, 1 ± 2 and 9 ± 5; for those with impaired memory, 43 ± 20 and 60 ± 18; for those with retention of memory, 88 ± 12 and 91 ± 10.

percentage savings of trials and errors for this group were, respectively, 21.1 ± 6.3 and 25.6 ± 6.8 and were significantly different ($P < 0.01$) from the control group of Table 1.

The mice of all experiments were given a final test for retention of relearning one to two weeks after the first retention test. All had memory of this relearning at a high level.

Discussion.—Up to the time of this study we had thought that puromycin permanently destroys memory in mice perhaps as a consequence of destruction of essential mRNA.[3] This interpretation of the results is not tenable in view of the restoration with saline of expression of memory lost, for long periods of time, after treatment with puromycin. It now seems clear that puromycin blocks expression of memory in mice without substantially altering the process which maintains the basic memory trace. As will be discussed, we can now only speculate about the mode of action of saline.

Two of the experimental observations need further comment. The first concerns the return of the expression of memory which followed after two injections of saline (bilateral temporal or ventricular or frontal) in mice previously treated with six injections of puromycin (bilateral temporal plus ventricular plus frontal) to produce loss of longer-term memory. It has been shown that omission of any group of two of the combined injections of puromycin fails to produce loss of longer-term memory.[1, 2] This result was interpreted to mean that large areas of the brain participate in longer-term memory but that a relatively small area is sufficient to sustain it. The results with two injections of saline support this finding and interpretation; it seems only necessary to free a relatively small area of the brain from the block of expression of memory produced by puromycin for longer-term memory to reappear.

The second comment concerns the status of memory in mice which followed second injections of puromycin. We have found that resistance frequently develops to puromycin after a first treatment. Mice which had lost their memory after puromycin were retrained. In most instances the standard treatment with puromycin then failed to destroy memory. As shown with tritiated puromycin and by the reduced level of inhibition of protein synthesis, the antibiotic is more

rapidly lost from the brain after the second injections, probably because of vascular changes which persist after the first injections.[1] As stated above, a saline solution isosmolar with that of the neutralized puromycin dihydrochloride was just as effective in restoring memory as isotonic saline. If saline completely removed the block to expression of memory caused by first injections of puromycin, it would be anticipated that in most instances memory would be improved by a second injection of a solution of puromycin with its contained saline. The finding that most mice with second injections of puromycin did not recover memory (Table 2) suggests that the contained saline in these mice only partially removed the blocking process which was then restored to an effective level by the puromycin present. In those mice with improved memory, it is supposed that saline was most effective and/or puromycin least effective.

The most attractive possibility which presently comes to mind to explain puromycin's effect on memory is that abnormal peptides formed in its presence alter in a reversible way the characteristics of neuronal synapses. This possibility is supported by several observations: (1) It has been shown by Murphy and Miller[4] and by Bohus and de Wied[5] that different peptides can delay or accelerate the rate of extinction of a conditioned avoidance response. (2) After treatment with puromycin, established memory disappears only after 10–20 hours,[1] possibly because some substance must accumulate with time to sufficient concentration to produce its effect. (3) Puromycin does not affect memory in the presence of heximide,[3] which inhibits the formation of peptides.[6] The present difficulties with this view are that (1) so far, our efforts to find abnormal peptides linked to puromycin have been unsuccessful,[1] although other methods might be more effective; and (2) it is difficult to understand the long survival time of the postulated abnormal peptide(s), expression of memory having been demonstrated to disappear reversibly for at least two months. So little is known, however, of the interaction of peptides with membranes that this difficulty may simply be an expression of ignorance. It can be imagined, for example, that the abnormal peptide(s) in combination with some element of the membrane is in such conformation that it is protected against dissociation from the membrane and that treatment with saline results in a conformational change through which this protection is lost.

We are now attempting, with injection of saline, to unmask whatever memory may be established when puromycin is injected immediately after training[7] and when training is conducted in the presence of puromycin.[8] We are also studying the effects of ionic strength and species of ions on the restoration of memory.

Summary.—Expression of memory of maze learning in mice, lost after treatment with puromycin, can be restored by intracerebral injections of saline for at least two months after the treatment with puromycin.

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