Adrenalectomy and the Suppression of Memory by Puromycin*

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Abstract. It has previously been shown that expression of memory of maze-learning in mice is blocked by puromycin injected intracerebrally one or more days after the training experience. Bilateral adrenalectomy before training has now been found to protect memory against the effects of puromycin. This protection is absent when adrenalectomy follows training. In view of control experiments, we conclude that adrenalectomy before training modifies factors necessary for the expression of memory and that this alteration makes puromycin ineffective in blocking memory.

Introduction. The expression of the memory of maze-learning by mice is blocked for long periods of time by the intracerebral injection of puromycin dihydrochloride (neutralized with NaOH) one or more days after the training experience.1, 2 The experiments reported here were designed to test the possibility that hormonal imbalance might modify this suppressive action of puromycin and so indicate a change from the normal properties of one or another element essential for the maintenance of memory. We have used adrenalectomized mice since it has been shown that the extinction of a conditioned avoidance response is markedly decreased by ACTH.3, 4

Materials and Methods. The mice used were male and female Swiss-Webster, 5–10 months old, from our closed colony. They were trained in a Y-maze with a grid floor through which an electric shock, usually of 40 v, could be applied intermittently. For training, a mouse was placed in the stem of the Y. To avoid shock, it had to move into the correct arm within 5 sec. If a mouse entered the incorrect arm, intermittent shock was given until it moved to the correct arm. Training was conducted in one session of 10 to 20 min to a criterion of nine out of ten correct responses. Total errors were the sum of incorrect choices and of latencies greater than 5 sec—i.e., all mistakes were added until, in ten consecutive runs in the maze, the mouse had performed correctly in nine of them. The same procedure was used in tests for the retention of memory of the training experience. These retention tests were given 1–2 weeks after learning. A final test of retention of relearning was given 1–2 weeks after the first retention test.

The injection technique has been fully described.5 Puromycin·2HCl (Nutritional Biochemicals Corp.) was neutralized to pH 6 with 1 eq of NaOH. All injections were bilateral and each had a volume of 12 μl. All were made one day after training and were either bitemporal or combined bitemporal + biventricular + bifrontal. The bitemporal injections contained 90 μg of puromycin per injection in mice weighing 29–32 g and 120 μg in those weighing 36–42 g. Mice treated with the six combined injections received 30 μg of puromycin per injection site regardless of their weight. Both adrenals were removed
under epival anesthesia using a dorsal approach and with the aid of a binocular magnifier having a magnification of 2.3x. The adrenalectomized mice were maintained in our routine way, with free access to Purina laboratory chow and tap water. After completion of the behavioral studies all adrenalectomized mice were examined for completeness of removal of the adrenals.

**Experimental plan and its rationale:** After finding that mice adrenalectomized before our training experience have normal retention of memory, we designed three sets of experiments. In the first set, mice were adrenalectomized, trained at a later time, and one day after training injected intracerebrally with puromycin. Adrenalectomy was performed 1 day (4 mice), 5 days (7 mice), 7 days (18 mice), or at 9 to 13 days (8 mice) before training. The failure of puromycin to block memory in these mice was initially observed when adrenalectomy had been performed 7 days before training; the shorter intervals between adrenalectomy and training were used to determine the approximate time of onset of protection against puromycin and the longer intervals, to test for its persistence. As controls for this and the following two sets of mice, the effectiveness of our puromycin solutions in blocking the memory of normal mice was checked throughout the experiments. For this purpose, normal mice were trained and 1 day later injected with puromycin (bitemporal or combined bitemporal + biventricular + bifrontal injections were used); then retention was tested. Most of these mice also served as controls for other experiments which were performed concurrently with those reported here. In addition, we have tested the possibility that the effective locus of the memory trace might develop beyond the hippocampal area to include large areas of the neocortex more rapidly in the adrenalectomized than in the normal mice. In a normal mouse, bitemporal injections of puromycin are effective for 3 days after training, whereas combined bitemporal + biventricular + bifrontal injections are necessary to suppress memory at later times. Accordingly, nine of the mice that had been adrenalectomized 5–13 days before training were treated 1 day after training with the six combined injections, the remaining mice of this series having received bitemporal injections of puromycin.

In the second set of experiments, mice were trained, one day later adrenalectomized, and subsequently injected intracerebrally with puromycin. The initial purpose of these experiments was to test the possibility that puromycin might also be ineffective in blocking memory when adrenalectomy occurred after training, rather than before. The first three mice of this group that were injected bitemporally with puromycin 1 day after adrenalectomy had, however, total loss of memory. The remaining nine mice of the series were treated with the six combined injections of puromycin 8 days after adrenalectomy, to match the most frequently used interval between adrenalectomy and injection of puromycin in the group where adrenalectomy preceded training.

In the third set of experiments, mice were trained, injected bitemporally with puromycin on the next day, and adrenalectomized 1 day (5 mice) or 6 days (3 mice) later. These mice, like those of the second set, had loss of memory. As additional controls for these last two sets, mice were trained and then adrenalectomized 1 day (8 mice) or 6 days (3 mice) later. They served to test the possibility that adrenalectomy after training might in itself interfere with memory.

**Results.** Mice recovered quickly from the effects of adrenalectomy and within a day showed essentially normal cage behavior. Treatment with puromycin gave the same symptoms as in normal mice—about two days of lethargy, reduced intake of food and water, and occasional convulsions. Small amounts of adrenal tissue were found in nine instances, on post-mortem examination. In six of these, adrenalectomy-precceeded training; in three, it followed training. The status of memory of these mice was indistinguishable from that of the remaining members of their respective groups in which adrenalectomy was complete.

Adrenalectomy caused little change in maze performance. For the 43 mice trained after adrenalectomy, the number of trials and total errors to criterion
were, respectively, 13.1 ± 0.6 (S.E.) and 13.6 ± 0.6; for the 71 normal mice (those trained before any treatment was applied), 10.8 ± 0.6 and 12.2 ± 0.8. The difference between the two sets of means is slight; the difference for errors is not significant (t test, P > 0.1); that for trials, however, is significant (P < 0.01).

The effects on memory of the experimental and control procedures are given in Table 1. Adrenalectomy before training had no effect on retention of memory

Table 1. Effect of change in sequence of adrenalectomy, training, and injection of puromycin on retention of memory.

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedure</th>
<th>No. of Mice with Memory Retained</th>
<th>Lost</th>
<th>Impaired</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>Adrenalectomize 5 days → Train</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>1b</td>
<td>Adrenalectomize 1-13 days → 1 day Train → Puro (T)</td>
<td>2</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>1c</td>
<td>Adrenalectomize 5-13 days → 1 day Train → Puro (T + V + F)</td>
<td>0</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>1 day Train → Puro (T; T + V + F)</td>
<td>39</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1 day Train → 1 or 8 days Adrenalectomize → Puro (T; T + V + F)</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1 day Train → Puro (T) → 1 or 6 days Adrenalectomize</td>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>1 day or 6 days Train → Puro (T; T + V + F) → Adrenalectomize</td>
<td>0</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>

Number of days over arrows indicates intervals between procedures. Group 1a served as control for groups 1b and c; group 2, for groups 1, 3, and 4; group 5, for groups 3 and 4. Puro = puromycin · 2HCl neutralized with NaOH. T = bitemporal and T + V + F = bitemporal + biventricular + bifrontal injections. For the mice with retained memory, the means ± S.D. for percentage of savings of trials and errors were, respectively, 90 ± 12 and 93 ± 7; for those with impaired memory, 35 ± 23 and 63 ± 13; and for those with lost memory, 0 ± 2 and 4 ± 7.

of the training experience (group 1a). Injection of puromycin into such mice was largely ineffective in suppressing memory; only 5% had complete loss of memory, whereas 70% retained memory at a high level (groups 1b and 1c). There was no significant difference in the mean savings whether the mice were treated with the bitemporal or the six combined injections. The percentage savings of trials and errors in the bitemporally injected groups were, respectively, 73.7 ± 6.4 (S.E.) and 81.4 ± 5.3; in the group injected at six sites, 68.8 ± 8.8 and 77.2 ± 6.2. The difference in retention of memory between these mice and the controls treated with puromycin (group 2) was striking.

By contrast, when mice were trained and then adrenalectomized either before or after injection of puromycin, suppression of memory occurred with regularity (groups 3 and 4), the loss of memory in these two groups being indistinguishable from the memory loss in puromycin-treated controls (group 2). As has been stated, nine of the mice of group 3 were injected with puromycin 8 days after adrenalectomy, which was the interval between adrenalectomy and treatment with puromycin most frequently used in groups 1b and 1c. Adrenalectomy alone after training had no effect on memory (group 5).

Only three of all the mice used in these experiments had impaired memory on their final retention tests; the remainder showed retention of relearning at a high level.
Discussion. The protection of memory against the effects of puromycin when adrenalectomy precedes training and the absence of this protection when adrenalectomy follows training is of major interest to us. We have considered several explanations for the protective effects of adrenalectomy before training, in part related to two previous experimental situations. Thus, we have reported that puromycin fails to block memory in mice which are overtrained, on the average to 60 trails beyond criterion.\(^1\) The mean number of trials and errors to criterion in adrenalectomized mice is so close to that of normal mice that this possibility clearly appears to be ruled out; similar results with adrenalectomized rats have been obtained by others in a different training situation.\(^6\)\(^,\)\(^7\) We have also reported that when puromycin is neutralized with KOH instead of NaOH, it fails to affect memory.\(^3\) This observation raises the possibility that the hyperkalemia associated with adrenal insufficiency might account for protection of memory against puromycin. There are numerous other possibilities, among them an increased rate of escape of puromycin from the brain, a decreased rate of penetration into neurones, and an alteration in the types of puromycin-peptides\(^8\) which are released from ribosomes. All appear untenable in view of the drastic loss of memory caused by puromycin when injected into mice which have been adrenalectomized after training (group 3), adrenalectomy itself having been shown to be without effect on memory (group 5). This conclusion is reinforced by the following observations: (a) mice adrenalectomized before training and then treated with puromycin show the usual toxic reactions of the central nervous system to the antibiotic and (b) two adrenalectomized mice injected with tritiated puromycin and killed 7 hr later had normal amounts of free puromycin and of peptidyl puromycin in their brains. Finally, we must reject the possibility that, in the adrenalectomized mouse, an effective memory trace develops more rapidly than normally in wide areas of the neocortex\(^3\) since treatment 1 day after training with the six combined injections of puromycin was just as ineffective as the bitemporal injections. We conclude from these considerations that adrenalectomy before training modifies factors responsible for the expression of memory and that this alteration makes puromycin ineffective in blocking memory.

Although it is premature to attempt to identify the agent responsible for this change, two possibilities may be briefly discussed. The first concerns ACTH. Its concentration in plasma has been found to be 4–6 times normal three days after adrenalectomy (the earliest time examined\(^9\)) and to remain at a high level for at least several weeks.\(^10\) It consequently appears reasonable to suppose that plasma ACTH is elevated throughout the period between adrenalectomy and training. The observations that ACTH delays extinction of a conditioned avoidance response\(^3\)\(^,\)\(^4\)\(^,\)\(^7\) and that removal of the posterior and intermediate lobes of the pituitary facilitates extinction\(^11\) suggest that ACTH may modify factors responsible for the expression of memory.

The second possibility concerns norepinephrine. Its rate of turnover after adrenalectomy has been found to be increased in both heart\(^12\) and brain.\(^13\) Preliminary experiments made with R. B. Roberts indicate that this possibility may be an important one.
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