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Effect of Corticosteroids in the Hippocampus on Passive Avoidance Behavior in the Rat[1]

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COTTRELL, G. A. AND S. NAKAJIMA. *Effect of corticosteroids in the hippocampus on passive avoidance behavior in the rat.* PHARMAC. BIOCHEM. BEHAV. 7(3) 277–280, 1977. – The site of action of corticosteroids in avoidance learning was investigated in 110 rats. Injection of cycloheximide, 30 min before one-trial training on a passive avoidance task suppressed corticosteroid secretion in response to footshock, and produced an avoidance deficit in a test 6 days later. However, an additional injection of hydrocortisone, either subcutaneously or intra-hippocampally within 5 min of training, restored the avoidance response in the test. Septal and hypothalamic injections of the hormone were ineffective in reversing the cycloheximide effect, whereas the effect of hormone injection into the amygdala was equivocal because of an increased level of activity. Corticosteroids secreted following an aversive experience appear to act upon the steroid-sensitive neurons in the hippocampus to influence the animal's later performance of passive avoidance response.

Amnesia Amygdala Corticosteroids Cycloheximide Hippocampus Hydrocortisone Passive avoidance

THE AMNESIC effect of cycloheximide has been attributed to the suppression of protein synthesis in the brain at the time of training [1,2], and considered as important evidence for the hypothesis that cerebral protein synthesis is required for the formation of lasting memory traces. However, Nakajima[19] found that injection of an exogenous corticosteroid counteracted the amnesic effect of cycloheximide in a passive avoidance situation. Since cycloheximide still suppressed protein synthesis in the brain after corticosteroid injection, it is unlikely that the behavioral effect of cycloheximide is related to its direct chemical effect on the brain. In view of the fact that cycloheximide also inhibits the synthesis (and therefore secretion) of corticosteroids in response to ACTH [9,10], Nakajima [19] concluded that it was the absence of stress-induced corticosteroid secretion that was responsible for the amnesic effect.

Implantation of corticosteroids into the brain results in the enhancement or suppression of stress-induced ACTH release depending on the site of implantation [3,13]. Systemic injection of corticosteroids affects the firing rate of neurons in the limbic system and hypothalamus of the rat [6, 14, 21]. Recent studies [8, 12, 15, 17] indicate that corticosterone is retained by high-affinity cytosol and nuclear receptors in the hippocampus, amygdala, septum and hypothalamus. These findings suggest that a number of structures in the brain contain hormone-sensitive neurons which are involved in detecting circulating levels of corticosteroids. Some of them may control stress secretion while others may be involved in behavioral regulation. The present experiment demonstrated that the detection mechanism for passive avoidance behavior is in the hippocampus.

METHOD

Animals

Male hooded rats of the Long-Evans strain were obtained from Canadian Breeding Farm (Montreal), and weighed between 267 and 395 g at the start of behavioral testing. They were housed individually and had free access to food and water. The animal colony was illuminated for 15 hr daily (08:00–23:00). Those animals which were to receive intracranial injections had stainless-steel cannulae (23 ga) implanted bilaterally into the brain under sodium pentobarbital anesthesia (50 mg/kg, IP). The sites of injection and their coordinates [7] were: the dorsal hippocampus (A 3.0, L 3.0, V +2.0), the amygdala (A 4.6, L 5.0, V −3.0), the septal area (A 7.8, L 0.5, V +2.0) and the hypothalamus (A 6.2, L 0.9, V −2.0). At the end of the operation, a stainless-steel stopper was inserted into each cannula, and Penicillin G (30,000 IU, IM) was injected into the hindlimbs.

Apparatus

The step-through passive avoidance apparatus was similar to the one used by Bureš and Burešová [5]. It consisted of a large gray compartment (40 × 40 cm) and a small black compartment (15 × 20 cm) connected by a doorway (10 × 10 cm). The floor of the large compartment was divided by

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white lines into nine squares of equal size. The small compartment had a grid floor that could be electrified to administer a footshock. Each compartment was covered with a clear pastic lid. The large compartment was placed under indirect illumination and the small compartment was kept relatively dark.

Behavioral Tests

All behavioral tests were conducted between 18:00 and 22:00 hr and commenced at least 7 days following surgery. For Test 1, the rat was placed in the centre square of the large compartment, facing away from the open doorway, and its behavior was observed for 600 sec. During this period, the number of lines crossed in the large compartment (activity score), the time it took the animal to enter the small compartment (latency) and the number of feces (defecation score) were recorded. The entire apparatus was thoroughly cleaned after testing every animal.

Twenty-four hours later, the animals were injected with cycloheximide and given one training trial a half hour later. They were individually placed into the small compartment, which had been separated from the large one by closing a sliding door. Thirty sec later, the grid floor was electrified to deliver 1.0 mA footshock for 0.5 sec. The animal was removed after another 30 sec and injected with hydrocortisone. Test 2 was conducted 6 days later, in the same manner as Test 1.

It should be noted here that this procedure of passive avoidance test, originally developed by Bureš and Burešová [5], enables the experimenter to record the behavioral indices in Test 1 before cycloheximide injection. The training is conducted under the influence of the drug, but the direct effect of the drug would have disappeared by the time of Test 2. Therefore, the behavioral indices are relatively free from possible perceptual, motivational, and motor effects of the drug.

Injections

Cycloheximide (Sigma Chemical Company) was dissolved in physiological saline (0.9% NaCl) at a concentration of 1.5 mg/ml, and injected 1.5 mg/kg intraperitoneally 30 min prior to the training trial. A preliminary experiment showed that this dosage was effective in producing a deficit in passive avoidance in the rat. Hydrocortisone-21-sodium succinate (Sigma Chemical Company) was used as an exogenous corticosteroid because it dissolves readily in physiological saline. The concentration was determined in such a way that the volume of injection was 1.0 ml/kg for subcutaneous injection and 5 μl per injection site for intracranial injection.

Bilaterally simultaneous intracranial injection was achieved by using two microsyringes (Hamilton Company) driven together by a syringe pump (Sage Instruments, Model 355). Each syringe was connected to a stainless-steel injection needle (30 ga) by a length of polyethylene tubing (PE 50). The stoppers were removed from bilaterally implanted cannulae, and injection needles inserted. The pump was then activated to deliver 5 μl of solution at a rate of 2 μl/min. In all animals, intracranial injection was completed within 5 min of footshock.

Experimental Design

There were 11 groups of rats (10 rats per group), each group receiving different injections. Group N was a normal control group which received no injection at all. The remaining 10 groups were injected with cycloheximide 30 min prior to the training trial. Group C was a cycloheximide-only group which received no further injection after the training trial. Groups Sc-0, Sc-1 and Sc-2 were subcutaneous injection groups, which received hydrocortisone succinate 0, 14 and 42 mg/kg, respectively, within 5 min of the training trial. Groups Hp-0, Hp-1 and Hp-2 were injected with physiological saline containing a total of 0, 13 and 40 μg hydrocortisone succinate, respectively, into the hippocampus. Groups Amg, Spt and Hth received similar injections into the amygdala, septal area and hypothalamus, respectively, at a dosage of 13 μg per structure (equivalent to Hp-1 dosage).

Histological Confirmation

Immediately following completion of the final behavior test, the animals with implanted cannulae were anesthetized with sodium pentobarbital (60 mg/kg, IP) and perfused intracardially with physiological saline followed by 10% Formalin. The brains were fixed in Formalin, sectioned and stained with thionin to verify the implantation sites.

Corticosteroid Analysis

An additional 4 groups of rats were used to determine the corticosteroid concentration in the blood. Collection began at 18:00 hr, the time of behavioral testing for the other groups, and took less than 2 min per rat. To determine the baseline level, rats (n = 5) were taken directly out of the animal colony to the collection room, anesthetized with ether and decapitated. The stress level was determined for rats (n = 7) which were individually placed in the small compartment of the avoidance apparatus, shocked as in the training trial, returned to the home cage and 10 min later anesthetized and decapitated. The remaining rats were first injected with cycloheximide (1.5 mg/kg, IP) and 30 min later processed to determine the baseline level (n = 5) and the stress level (n = 7) as in the uninjected animals.

Approximately 7 ml of trunk blood was collected from each rat and immediately centrifuged. Plasma was stored at $-15°$C. Fluorometric analysis was conducted according to the method of Mattingly [18] using a Turner Fluorometer (Model 110). Corticosterone (Sigma Chemical Company) was used as a standard.

RESULTS

Passive Avoidance

The mean latencies in Test 1 and Test 2 for each group are shown in Fig. 1. Numerical data were subjected to analysis of variance and post hoc comparisons using the method of Rodger [22]. There was no significant difference among the groups in Test 1 (white columns), $F(10,99) = 0.417$. The normal control animals (Group N) exhibited significantly longer latencies in Test 2 (stippled) than in Test 1, $t(9) = 5.15$, $p<0.05$, indicating that they were able to acquire the passive avoidance response in one trial and to retain it for 6 days. However, on Test 2 the cycloheximide-only group (Group C), readily stepped into the small compartment where they had been previously shocked, showing no sign of passive avoidance. These

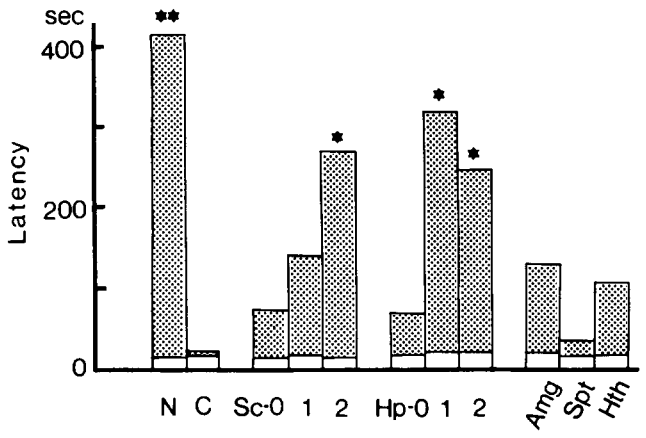

FIG. 1. Mean latencies in Test 1 (white) and in Test 2 (stippled). *Significantly different ($p<0.05$) from all unmarked groups. **Significantly different ($p<0.05$) from all other groups.

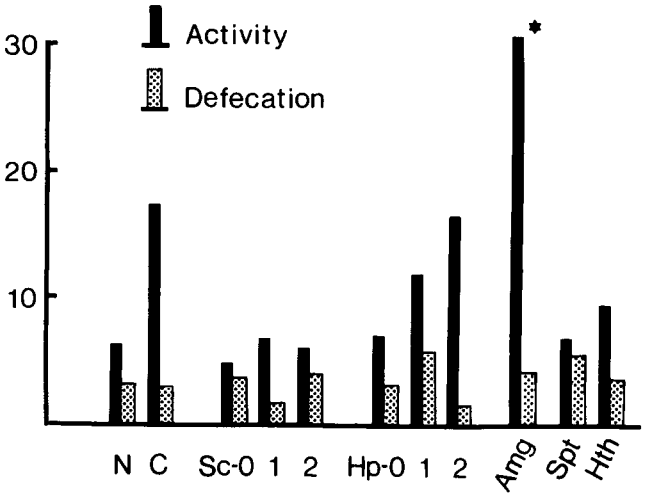

FIG. 2. Mean activity scores and defecation scores in Test 2. *Significantly different ($p<0.05$) from all other groups.

findings are in agreement with earlier reports on mice [11,19].

An analysis of variance on the Test 2 latency scores indicated significant differences among the groups, $F(10,99) = 3.946$. The post hoc comparisons showed that Groups Sc-0, Sc-1, Hp-0, Amg, Spt and Hth were not significantly different from Group C. The latencies of Group Sc-2, Hp-1 and Hp-2 (marked with an asterisk in Fig. 1) were significantly longer than those of the above groups, but still shorter than the latency of Group N ($p<0.05$).

The activity scores and defecation scores for Test 2 are shown in Fig. 2. Analysis of variance and post hoc comparisons [22] revealed that Group Amg showed a level of activity significantly higher than any other group during Test 2, $F(10,99) = 1.795$, $p<0.05$. Therefore, the short latency in this group may partly be due to the enhanced general activity. It should also be noted that the activity levels of Groups Hp-1 and Hp-2 were not lower than Group Hp-0, indicating that their longer latencies are not attributable to a depression in general activity. The number of feces defecated in Test 2 did not bear any systematic relation to the experimental treatments.

Injection Sites

The cannula tips in Groups Hp-0, Hp-1 and Hp-2 were located in CA3, CA4 or the dentate gyrus of the dorsal hippocampus. Among the three hippocampal groups, the injection sites were evenly distributed, and there appeared to be no systematic bias that could have confounded the behavioral results. In the amygdala, the tips were mostly in the basolateral and cortical nuclei. The septal sites were all in the lateral nucleus, and the hypothalamic sites were distributed in the anterior and dorsomedial nuclei or in the medial part of the lateral hypothalamic area near the fornix. No systematic relation was detected between the sites of injection and the behavioral results of the individual animals in Amg, Spt or Hth groups. Considering the volume of injected solution (5 μl of solution would easily diffuse across a few millimeters), the small variability in the location of cannula tips was probably not critical.

Corticosteroid Levels

The mean baseline level of corticosteroids in uninjected animals was 10.7 μg per 100 ml plasma, and footshock in the training situation increased it to 17.0 in 10 min. In the cycloheximide-injected animals, the baseline level was 11.3, and footshock-induced stress level was 13.9. Analysis of variance and post hoc comparisons [22] indicated that the increase in the steroid level was significant only in the uninjected animals, $F(3,20) = 2.470$, $p<0.05$.

DISCUSSION

The present experiment clearly demonstrated the importance of the hippocampus in detecting an increase in corticosteroid levels after an aversive experience. Injection of cycloheximide reduced the stress-induced secretion of corticosteroids and later produced a deficit in passive avoidance. However, if the cycloheximide-injected animals received a supplementary injection of an exogenous corticosteroid, either subcutaneously or into the hippocampus, the avoidance response was restored.

The importance of the hippocampus is evident from the effectiveness of a much smaller dose compared to the subcutaneous dose in counteracting the cycloheximide effect, the effective hippocampal dose being approximately 1/1000 (13 μg) the amount of the subcutaneous dose (11–16 mg per rat). The septal and hypothalamic injections were ineffective. Therefore, the hippocampal effect is specific to that structure, and not attributable to ventricular diffusion of the hormone. Whether or not the amygdala is also important in this regard was not clear from the present experiment. The role of the amygdala needs to be examined in a situation where the performance of the avoidance response is not influenced by an increase in general activity.

The corticosteroid used in the present experiment (hydrocortisone-21-sodium succinate) did not completely antagonize the cycloheximide effect even when the dosage was increased. Although the esterified form of hydrocortisone is readily soluble in water, its effect on hippocampal neurons may be weaker than that of corticosterone, which is the major endogenous corticosteroid in the rat.

According to McEwen and Wallach [16], corticosterone has a higher affinity for hippocampal neurons than hydrocortisone in the rat. If corticosterone had been injected into the hippocampus in an appropriate form, it might have antagonized the cycloheximide effect completely.

The results of the present experiment suggest that corticosteroids, secreted from the adrenal cortex after an aversive experience, act on the hippocampus to influence later passive avoidance behavior. A deficit in avoidance may be produced, not only by suppressing the hormonal secretion with cycloheximide, but also by saturating the hippocampus with the hormone well before the aversive experience. Bohus [4] implanted crystalline corticosterone into the dorsal hippocampus of rats and trained them in a passive avoidance task 3 hr later. In a test conducted 24 hr after the training, the implanted rats showed an impairment in passive avoidance. It appears that the presence of corticosterone at a high concentration in the hippocampus prevented the hippocampal neurons from detecting a stress-induced increase in the circulating level of corticosteroids.

The present study leaves a number of questions unanswered. One of them is how adrenalectomized animals acquire and retain passive avoidance response in the absence of corticosteroids. A possibility of state-dependent learning was suggested earlier [19], but a later experiment showed that cycloheximide does not produce state dependency [20]. Squire *et al.* [23] raised a question of why hormonal suppression with aminoglutethimide does not lead to an avoidance deficit. It may be that there is an alternative mechanism which produces an effect in the hippocampus similar to the effect of corticosteroids. Still another question is whether the hippocampal mechanism is involved in active avoidance as well as in passive avoidance. All these questions remain to be answered in future experiments.

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