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# The Effects of Org 2766 on the Performance of Sham, Neocortical, and Hippocampal-Lesioned Rats in a Food Search Task[1]

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HANNIGAN, J. H., JR. AND R. L. ISAACSON. *The effects of Org 2766 on the performance of sham, neocortical, and hippocampal-lesioned rats in a food search task.* PHARMACOL BIOCHEM BEHAV **23**(6) 1019–1027, 1985.—The behavioral effects of an $ACTH_{1-9}$ variant, Org 2766, given for one week postoperatively at a dose of 1 μg/rat daily, were evaluated in animals given hippocampal, neocortical, or "sham" lesions. After the week during which the injections were given, the animals were tested for 5 days in a food-search task in which food was hidden in two recessed holes in the floor. On the next day the ability of the rats to find food in these same two baited holes was tested in the presence of 14 additional holes that were not baited. On the following day, the animals were tested again, this time with all 16 holes baited. To assess the long-term effects of Org 2766 treatment, the animals were tested once again 2–3 months later in the same apparatus with 16 empty holes. In general, rats with lesions restricted to the neocortex were severely impaired in the task and were unaffected by prior treatment with Org 2766. Animals with hippocampal damage quickly learned the task and were hyperactive. During the test session with 16 baited holes they showed differential behavioral changes suggesting attentional deficits not seen in "sham" operated rats. These deficits were attenuated by prior Org 2766 treatment, whereas the lesion-induced hyperactivity was not. Treatment with Org 2766 impaired all aspects of performance of "sham" operated animals.

Hippocampal lesion Neocortical lesion Neuropeptides ACTH fragments Org 2766
Hormone-brain lesion interactions Attention

A major direction of current research on the effects of neuropeptides is the investigation of their possible therapeutic actions on neurological and psychiatric disorders (e.g., [10]). Peptide fragments and analogues of adrenocorticotropin (ACTH), in particular $ACTH_{1-10}$ and the substituted $ACTH_{4-9}$ variant, Org 2766 ($ACTH_{4-9}$ [methionine(O)-$^4$D-lysine$^8$phenylalanine$_9$]), are one class of neuroactive peptides that exert modulating influences on the central nervous system (CNS). Org 2766 is of interest because it can be more potent than $ACTH_{4-10}$ in some behavioral tests [8, 16, 46], possibly by virtue of a longer half-life in the CNS [45]. Like other short N-terminal fragments of ACTH, it is virtually devoid of steroidogenic, lipolytic, and melanotropic activity [32,45].

Org 2766 facilitates the growth of neurites in crushed rat sciatic nerve [2,3] and reduces some of the morphological correlates of aging in rat hippocampus [25]. In both preparations there are significant enhancements of behavioral recovery after treatment with the synthetic peptide that are correlated with the anatomical changes. Some behavioral changes after limbic system damage in rats are attenuated by ACTH treatment (e.g., [4]). Poplawsky and Isaacson [34] found that the hyper-emotionality that follows septal lesions in rats is returned to normal levels faster in Org 2766-treated animals than in saline-treated rats. In addition, the high levels of intertrial activity found after such lesions are reduced to near normal levels without affecting conditioned avoidance performance.

That the behavioral effects of hippocampal lesions could be attenuated by treatment with Org 2766 is suggested by known relationships between ACTH, hippocampal lesions, and dopamine systems in the basal ganglia, particularly the nucleus accumbens. First, the excessive grooming induced by intracerebroventricular (ICV) injections of ACTH [15] depends on the integrity of basal ganglia dopamine systems. Pharmacological manipulations that alter normal

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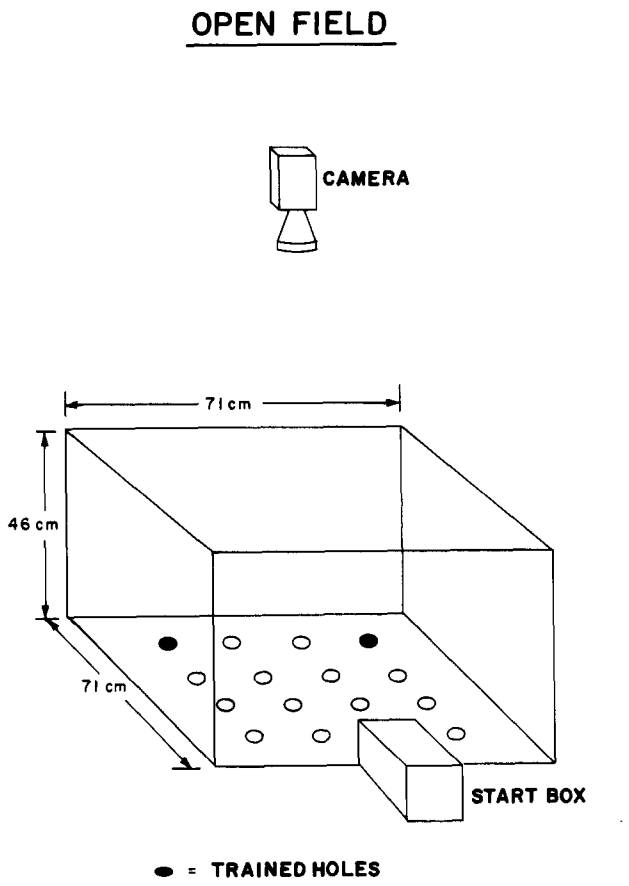


FIG. 1. Schematic diagram of the "open field" apparatus. In Sessions 1 through 5 only the two baited "trained" holes were available. In Session 6, all 16 holes were available, but only the "trained" holes were baited. In Session 7, all 16 holes were baited. None of the 16 holes was baited in Session 8.

dopaminergic activity in either caudate nucleus or nucleus accumbens also reduce the behavioral effectiveness of ICV ACTH [6,21], as does depletion of dopamine in nucleus accumbens [42]. Hippocampal lesions result in alterations of dopaminergic activity in nucleus accumbens [1, 35, 41], and specific pharmacological manipulations of basal ganglia dopamine systems can attenuate some of the behavioral effects of hippocampal damage [18,36]. Furthermore, hippocampal lesions attenuate excessive grooming [5], as might be expected, given that ACTH-induced excessive grooming is dependent on functional basal ganglia dopamine systems and that hippocampal lesions disrupt the functional state of those systems [41]. The effect of the lesion is due to a decreased sensitivity to the ICV peptide [9] that appears to be restored by intra-accumbens administration of a specific dopamine agonist [17].

Taken together, there is substantial evidence that behaviorally important neural changes that occur in response to hippocampal lesions involve ACTH-sensitive systems. We hypothesized that Org 2766 would facilitate behavioral recovery because it may be a more potent form of the endogenous types of ACTH [43,45], because ACTH and its fragments and variants are active in nucleus accumbens [23,37], and because it has been demonstrated previously that behavioral recovery from limbic damage is facilitated by Org 2766 [25,34].

The performance of rats with sham, neocortical, or hippocampal lesions was tested in a food search task after one week of daily treatment with either Org 2766 or control saline injections. The peptide treatment regime was chosen on the basis of similar doses and durations found to facilitate recovery from central [34] and peripheral nervous system damage [2,3] and to coincide with the period following the brain damage during which functionally significant reactive neural changes occur (e.g., [1, 35, 36, 41]). The behavioral task allowed relatively independent assessment of a variety of behaviors in rats that are often influenced by hippocampal lesions in other situations, including activity, search and exploration, attention, and memory [20, 27, 29, 31]. We also studied the relatively long-term effect of the Org 2766 treatment by testing the animals two to three months after the initial testing.

## METHOD

### *Subjects*

Forty-eight adult male Long-Evans strain hooded rats were used. They were housed individually in steel and wire-mesh cages for two to three weeks after arrival in the vivarium which was maintained on a 12 hr light/dark cycle with lights on at 0700 hr. The animals weighed between 200 and 240 g at arrival and were provided with ad lib lab chow and tap water until 24 hr before surgery, when they were deprived of both.

### *Surgery*

The rats were anesthetized with a solution of chloral hydrate (90 mg/kg), sodium pentobarbitol (56 mg/kg) and atropine sulfate (0.75 mg/kg). They were given either "sham," neocortical, or neocortical-plus-hippocampal lesions by aspiration as described by Isaacson and Woodruff [22]. Briefly, scalps were incised and holes drilled into the parietal bone plate. For rats in the "sham" groups, the wounds were closed at this point. For animals in the other groups, the skull defects were enlarged and dura mater opened. Neocortical lesioned groups had neocortex and corpus callosum overlying the hippocampus removed bilaterally, while, in addition, the hippocampal lesioned groups received large bilateral extirpation of hippocampus proper. After surgery, all rats were returned to the vivarium and ad lib lab chow and tap water.

### *Procedure and Apparatus*

Beginning on the first day after surgery, the animals in each lesion group were divided randomly into two groups receiving either control subcutaneous injections of 0.2 ml saline (0.9%) or 1 $\mu$g of ORG 2766 in 0.2 ml saline. The injections were given daily for the week following surgery. Beginning on the second day after surgery each animal was given 5 pellets of a commercial sweetened breakfast cereal ("Fruit Loops;" General Foods, White Plains, NY) in addition to the usual laboratory chow. They continued to receive the cereal pellets daily for the duration of the experiment. On the fourth day after surgery the rats were placed on a food-deprivation schedule. About 1 hr after the daily injections, 15 g of lab chow was given in addition to the cereal pellets. This deprivation schedule was maintained for the duration of the first portion of the experiment, i.e., through test Session 7.

Beginning on postoperative day 8 and continuing for 7 consecutive daily sessions, the rats were trained and tested on a simple food search task. The animals were transported

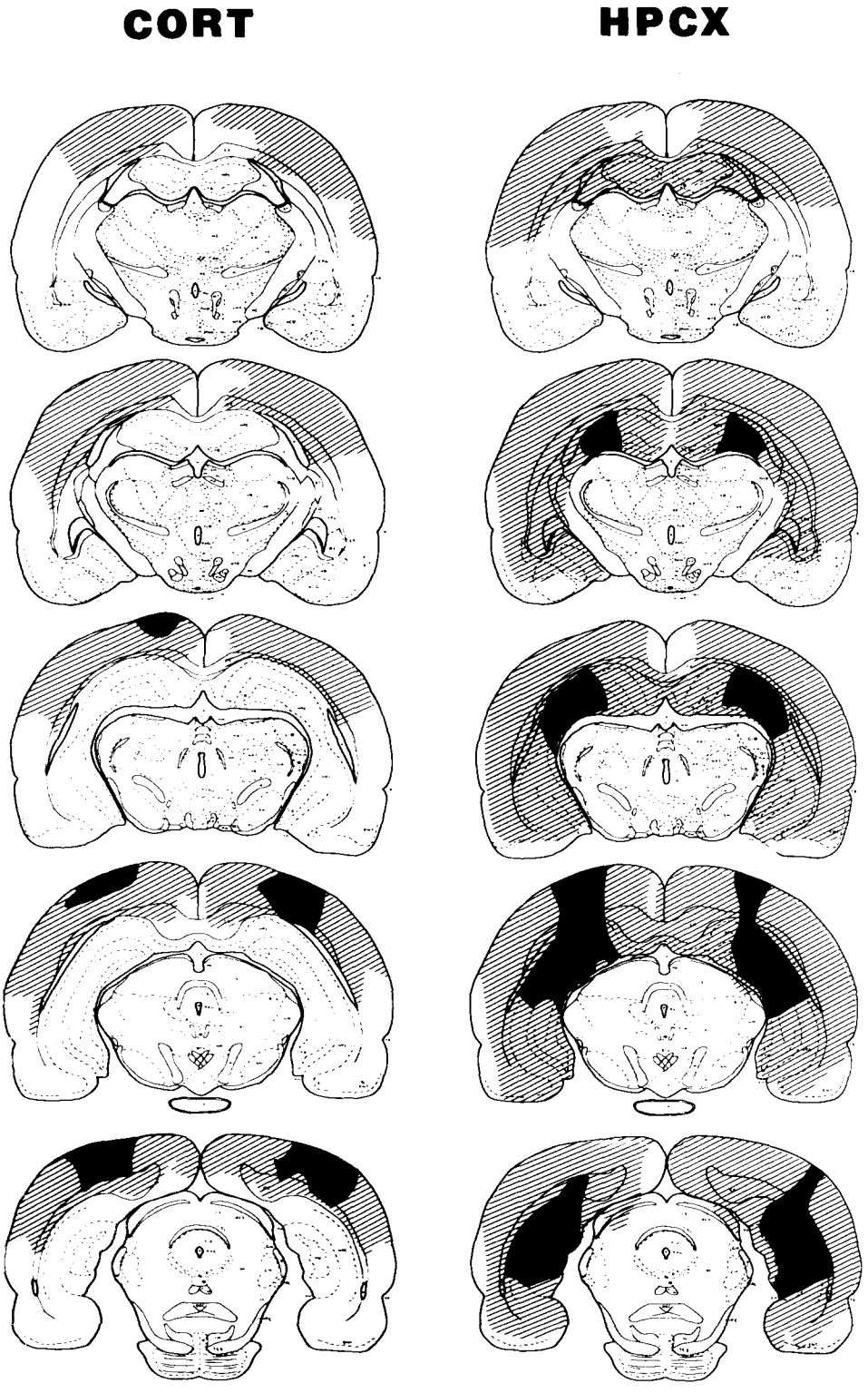


FIG. 2. Serial reconstructions of the extent of damage in neocortical and hippocampal lesioned groups. Areas covered by stripes were damaged in at least one animal in each group. The dark areas were damaged in every animal in each group.

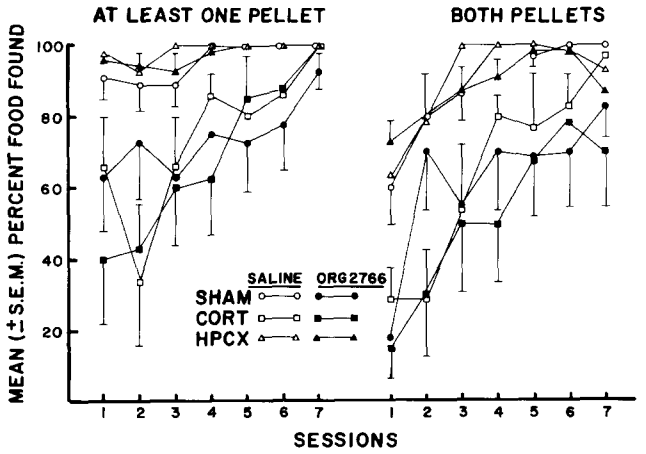


FIG. 3. Mean (±S.E.M.) percent of trials in a session where at least one food pellet was found in a "trained" hole (left) and mean percent of trials where both pellets were found (right).

in their home cages from the vivarium to the testing suite. The animals were observed in an "open field" apparatus located in a quiet and dimly lit (red light) observation room. The "open field" was a white Plexiglas box with a smaller "start box" (22×13 cm) attached in the center of one side at floor level and separated from the open field by a guillotine door. A rendering of the apparatus and its dimensions are provided in Fig. 1. There were 16 3.2-cm diameter holes cut in a uniform 4×14 matrix in the floor of the apparatus. On Sessions 1 through 5 (training) all but the two corner holes opposite the wall with start box entrance were covered with a white Plexiglas sheet. On Sessions 6 through 8, this sheet was removed, leaving all 16 holes open.

During training Sessions 1 through 5, both of the holes were baited with a single "Fruit Loop" (0.083 g±0.006). One rat at a time was placed in the observation room in its home cage for 3–5 min to allow it to adapt to the dim light conditions. The rat was then placed in the start box. One minute later the door to the large arena was opened. The rat was allowed a total of 3 min to enter the open field and find and eat the 2 cereal pellets. If a rat was eating at the end of a 3-min trial, it was allowed to finish. Otherwise, the rat was removed as soon as it finished eating the second pellet and then returned to the start box for a 30-sec intertrial delay. The experimenter rebaited the holes and the procedure was repeated for 5 trials per session. The animals were trained in this way for 5 consecutive daily sessions. The decision to terminate "training" after 5 sessions was made empirically. After 5 sessions there were no longer significant differences between groups in several measures of task performance (e.g., search time and mean percent of trials per session in which at least one food pellet was found; see below for behavior definitions). The changes in the task requirements in Sessions 6, 7, and 8 were designed to reveal possible deficits in attention and memory. In Session 6, all 16 holes were open but only the same two holes on which the rats were trained (the "target" holes) were baited. In Session 7, all 16 holes were baited on each of the 5 trials.

After its 5 daily trials, the rat was returned to its home cage, the open field was cleaned with a dilute Lysol disinfectant solution. The rats were returned to the vivarium and fed their rations of lab chow and cereal pellets 1 hr later. After completion of Session 7, all rats were returned to ad lib lab chow. Eight to 12 weeks later the rats were again placed on the food deprivation schedule for 48 hr and tested for a single 5-min trial (Session 8) with all 16 holes in the open field accessible but with no food available.

An experimenter remained in the testing room throughout each trial. Observations were made via a closed-circuit video system by trained observers located in a nearby room. The observers encoded the animals' behavior as audio signals on "touch-tone" keypads. The analogue signals were recorded and decoded for digital analysis that approximated continuous measures of behavior with a 0.1-sec time sampling interval. The following behavioral measures will be reported:

*(1) Latency to leave start box.* Movement of the animal's whole body into the open field.

*(2) Locomotion.* Movement of greater than 50% of the animal's head and body in one of the 16 grid squares superimposed on the video monitor.

*(3) Rearing.* Frequency and duration of lifting both forepaws off the floor with a vertical extension of the trunk.

*(4) Hole pokes.* Placement of the animal's nose into one of the holes in the open field. The frequency and duration of hole pokes were measured, as well as recording which hole was searched and whether or not a pellet was found.

*(5) Search time.* The total duration of a trial minus latency to leave start box.

*(6) Relative activity.* Since the amount of time individual animals spent in the open field varied, locomotion alone represented only the amount of linear movement required to complete the task. Relative activity was defined as the sum of locomotion and rearing frequency, divided by search time.

*(7) Latency to find first pellet.* Time required to find one pellet measured from entry into the open field.

*(8) Latency to find second pellet.* Time required to find the second pellet in a trial measured from when the rat finished eating the first pellet.

*(9) Revisits.* Frequency of hole pokes to holes where the pellet had already been found within a trial.

*(10) Failures.* Frequency of hole pokes to baited holes where the pellet was not found.

These data were analyzed using unbalanced, mixed design analyses of variance with up to two crossed factors (Lesion and Peptide treatment) and two repeated and nested factors (Trials within Session).

### *Histology*

Two to four weeks after Session 8 the animals were deeply anesthetized and perfused transcardially with saline (0.9%) followed by a 10% formalin-saline solution. The brains were fixed in formalin for at least 3 days before being sectioned at 80 $\mu$ while frozen, saving every fifth section through the area around the lesion. The sections were stained with cresyl violet and the extent of the lesion evaluated.

## RESULTS

### *Histology*

Schematic serial reconstructions of the lesions provided in Fig. 2 represent the extremes of group lesion variability. The striped areas were damaged in at least one rat in each group. The dark shaded areas were damaged in every animal in each group. There were no differences in the size or extent of lesions between the saline and Org 2766-treated animals. Three CORT and six HPCX rats suf-

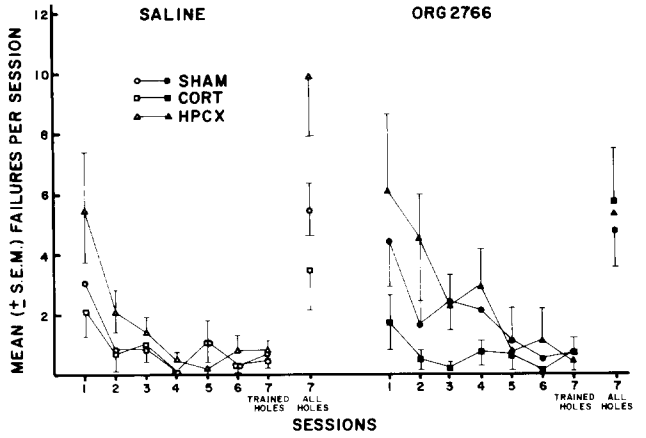


FIG. 4. Mean (±S.E.M.) frequency of failures per session. For Session 7, both the total numbers of failures and the frequency of failures to "trained" holes alone are shown.

fered minor damage to underlying archicortical, and diencephalic and midbrain areas, respectively. None of the damage beyond primary target areas was extensive and there were no differences between SAL and ORG groups. Quantitative estimates of lesion volume did not correlate significantly with behavior measures and no animals were excluded from further analyses on the basis of extraneous damage.

To estimate possibly differential effects of food deprivation on each group, the body weights of the animals in the food search task were compared to separate groups of sham, neocortical, or hippocampal lesioned rats. These animals were treated similarly to the rats in the food search task, including Org 2766 treatment, except that they were maintained on an ad lib food schedule and were not trained in the behavioral task. Among the saline-treated groups, the SHAM but not CORT or HPCX groups significantly increased body weight following surgery, $F(12,144)=2.25$, $p<0.025$. Following treatment with Org 2766, there was an increase in body weight for all groups, $F(6,138)=27.21$, $p<0.001$. Using the weight gains in these groups as a baseline, the mean body weights for the animals in the food search task were 82 to 85% of what each group would have been expected to weigh one week after surgery. There were no differences between groups in percent body weight lost during deprivation.

*Behavior: Sessions 1 Through 7*

The effects reported are the sums or means of the scores obtained on the 5 trials given each training session.

*(1) Latency to leave start box.* The HPCX-SAL group left the start box more quickly than either the SHAM-SAL or CORT-SAL groups, $F(2,20)=10.49$, $p<0.002$, and the CORT-SAL group was slower than the SHAM-SAL group, $F(1,12)=5.23$, $p<0.04$. All groups left the start box more quickly in later sessions, $F(6,120)=10.43$, $p<0.001$. The CORT-SAL group decreased faster than the others, $F(12,120)=1.84$, $p<0.05$. Among the Org 2766 treated groups, the HPX-ORG group was quicker to leave the start box than the other groups, $F(2,22)=5.34$, $p<0.02$. However, unlike their saline-treated counterparts, the SHAM-ORG and CORT-ORG groups were not different from each other.

*(2) Locomotion and relative activity.* Hippocampal lesioned rats had higher locomotion scores than the SHAM or CORT groups across the first five training sessions, $F(2,42)=4.26$, $p<0.05$. All groups decreased the amount of movement required to complete the task over training, $F(4,168)=20.45$, $p<0.001$. The two HPCX groups decreased locomotion faster than the other groups, $F(8,168)=3.16$, $p<0.01$, so that by Session 5 there were no differences among the groups. There was a significant increase in locomotor behavior between Sessions 6 and 7, $F(1,42)=7.96$, $p<0.01$. There were no differences in the increases in locomotion between saline- and Org 2766-treated groups. In addition, all groups were equivalent in rearing frequency and mean duration per rearing bout. All groups decreased rearing frequency across sessions, $F(6,252)=25.17$, $p<0.0001$. There was, however, a significantly greater overall tendency for the SHAM-ORG and CORT-SAL groups to enter the center of the apparatus (athigmotaxia) than for the other groups, $F(12,252)=3.10$, $p<0.001$.

There were no significant group differences in the amount of time required to complete the task. All groups followed the same temporal pattern across sessions, taking less time toward the end of training, and increasing search time again in Sessions 6 and 7, $F(6,252)=28.58$, $p<0.001$, when the total number of holes was increased to 16.

Among the saline-treated animals, the SHAM-SAL and HPCX-SAL groups increased relative activity over the course of training but the CORT-SAL group did not. This group was less active than the other saline treated groups, $F(2,20)=10.91$, $p<0.001$. The HPCX-SAL group was more active than the SHAM-SAL group, $F(1,14)=5.21$, $p<0.05$, over training. Overall, the Org 2766-treated groups were proportionally less active than the SAL-treated groups, $F(1,42)=4.53$, $p<0.05$, but all Org 2766-treated groups increased relative activity over the first 5 training sessions, $F(4,88)=12.78$, $p<0.001$. The HPCX-ORG group was more active than the other ORG 2766-treated groups, $F(2,22)=9.92$, $p<0.001$.

In Session 6 there was a significant overall decrease in relative activity compared to Session 5, $F(1,42)=11.38$, $p<0.001$, and the two hippocampal lesion groups were more active than the control groups, $F(2,42)=5.70$, $p<0.01$. In Session 7, there was a decrease in activity relative to Session 6 for the saline-treated animals, $F(1,20)=19.06$, $p<0.001$.

*(3) Food search.* Figure 3 illustrates the mean percent of subjects which found food pellets in each session. (The values for Session 7 represent only the percentages of subjects that found the pellets in the originally "trained" holes.) In Session 1, the animals in both of the hippocampal lesion groups and those in the SHAM-SAL group found at least one pellet on about 88% of the trials and both pellets on about 62% of the trials. The CORT-SAL, CORT-ORG, and SHAM-ORG groups, on the other hand, found at least one pellet on only 57% of the trials and both pellets on only about 22% of the trials.

Over subsequent sessions, the patterns of improvement in finding at least one pellet per trial depended on both lesion type and petide pretreatment (3-way interaction: $F(12,252)=4.36$, $p<0.001$). Both HPCX groups and the SHAM-SAL group found at least one pellet on 100% of the trials by Session 3 or 4, whereas the two CORT groups reached that level only on the last session. Surprisingly, the SHAM-ORG group never reached 100% success in finding at least one pellet per trial. Nonetheless, by Session 5 there were no significant differences among the groups. Treatment

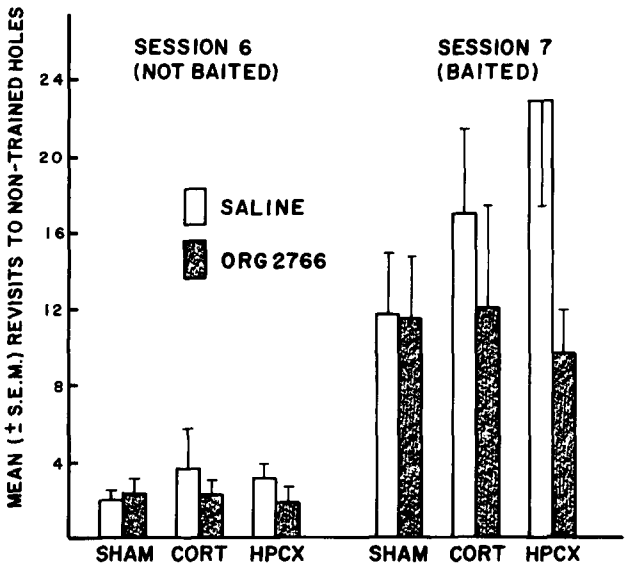


FIG. 5. Mean (±S.E.M.) frequency of revisits to the "non-trained" holes in Sessions 6 and 7.

with the peptide fragment influenced the SHAM animals more than those with cortical or hippocampal lesions, $F(2,42)=3.49$, $p<0.05$.

Success in finding both pellets increased for all groups across sessions, $F(6,252)=26.33$, $p<0.001$. The cortical lesion impaired finding 2 pellets relative to the SHAM-operated animals that received saline, $F(1,12)=8.76$, $p<0.025$, whereas after treatment with Org 2766, the CORT and SHAM groups did not differ significantly and both performed poorly relative to comparable groups receiving saline. In Session 7, when there were 16 pellets available, Org 2766 treatment reduced success in finding both the first, $F(1,42)=4.33$, $p<0.05$, and second pellets in "trained" holes, $F(1,42)=5.16$, $p<0.05$, for all lesion groups. Further, rats treated with Org 2766 found fewer total pellets in Session 7 than did saline-treated animals, $F(1,42)=5.31$, $p<0.05$.

The latency to find the first pellet in a "trained" hole decreased across sessions for all groups, $F(6,252)=22.69$, $p<0.0001$. In general, it took longer for the two cortical lesion groups to find the first pellet than either the SHAM-SAL group or the hippocampal lesion groups. However, the sham operated animals treated with Org 2766 were as slow to find the first pellet as the cortically lesioned animals. Overall, the Org 2766-treated groups took longer to find a pellet in one of the "trained" holes in Session 7, when all holes were baited, $F(1,42)=5.89$, $p<0.02$. These differences in latencies to find the first food pellet, which had essentially identical patterns for finding the second food pellet, are inflated by high proportions of rats in the CORT-SAL, CORT-ORG, and SHAM-ORG groups that had trials in which no pellets were found at all. When this occurred, the rats were assigned the maximum latency score of 3 min for the trial. When such trials were excluded from the analyses, there were no overall differences between groups in latencies to find either the first or second pellet.

*(4) Hole poke frequency.* Initially, hippocampally lesioned rats explored more holes than either of the CORT groups or the SHAM-ORG group, $F(2,42)=8.36$, $p<0.001$, but by Session 5 all groups were searching each of the 2 holes just once and were finding both food pellets. Naturally, when more holes were available in Sessions 6 and 7, the frequency of total hole pokes increased, $F(2,42)=110.93$, $p<0.001$. In Session 7, the HPCX-SAL group explored more holes than the SHAM-SAL and CORT-SAL groups, $F(1,21)=4.32$, $p=0.05$. The HPCX-ORG group explored fewer holes than the HPCX-SAL group, $F(1,16)=5.15$, $p<0.05$, and was not different from the other Org 2766-treated groups.

*(5) Failures.* Rats made errors by not searching a baited hole, by failing to retrieve food from a baited hole after it was searched, or by revisiting holes from which food had been taken. All groups made fewer failures to retrieve the food from baited holes in later sessions, $F(6,262)=11.31$, $p<0.0001$; see Fig. 4. Both of the HPCX groups had more failures than the other groups on the first few training sessions, $F(12,252)=2.03$, $p<0.025$. Overall, Org 2766 administration did not affect failures. The addition of 14 holes in Sessions 6 and 7 did not alter failures at "trained" holes. In Session 7, the HPCX-SAL group failed to retrieve pellets more often than the combined saline treated groups, $F(1,21)=6.45$, $p<0.025$, whereas there was no difference in the number of failures found among groups treated with Org 2766.

It is possible that the increased frequency of failures in the HPCX-SAL group was due to greater opportunities to fail since this group searched more holes. To test this possibility, failures were also assessed as the proportion of failures per opportunity to fail (i.e., per hole poke into a baited hole). The HPCX-SAL group failed proportionally more often than the combined SHAM-SAL and CORT-SAL groups, $F(1,21)=5.71$, $p<0.05$, in Session 6. There were no significant differences among the groups treated with Org 2766.

*(6) Revisits.* Across sessions, all groups reduced the number of revisits to holes in which food had been found previously within a trial, $F(6,252)=2.50$, $p<0.05$. There were no differences among the groups in revisits to "trained" holes. In Session 6, there were no differences among groups in the frequencies of revisits to the 14 added holes. In Session 7, although all groups revisited the added holes more frequently than in Session 6, $F(1,42)=53.44$, $p<0.0001$, the HPCX-SAL group revisited these additional "non-trained" holes more frequently than did controls. However, this was not found in the HPCX animals treated previously with Org 2766 (Lesion × Peptide interaction: $F(2,42)=4.15$, $p<0.025$; see Fig. 5). The differences in revisits were not due to greater numbers of holes poked by the HPCX-SAL group since the interaction was also significant when revisits were assessed as a proportion of total hole pokes, $F(2,42)=3.91$, $p<0.05$.

*(7) Mean hole poke duration.* Assuming a rat must spend some minimum time investigating a hole to determine whether a pellet is present, reductions in the duration of hole pokes could be associated with increased failures in finding pellets. Over the course of training, the SHAM and HPCX groups, but not the CORT groups, decreased the mean duration per hole poke, $F(8,168)=2.44$, $p<0.025$. Since their successes were increasing, this implies an increased search efficiency in these animals. There were no differences in durations among the groups in Session 6 at either "trained" (baited) or "non-trained" (non-baited) holes. In Session 7, when all holes were baited, mean hole poke durations were analyzed as a function of what happened as a result of each hole poke: The pellet either had been found already (a "revisit" to an empty hole), or if food was present, it was either found (a

"hit") or not (a "failure"). There were no overall differences between mean hole poke durations for revisits and hits, but those hole pokes that were failures were of significantly shorter durations than those for revisits and hits failures, $F(2,121)=26.58$, $p<0.002$.

*(8) Retest: Session 8.* One Org 2766-treated animal with a neocortical lesion died before completion of Session 8. To assess the long-term effects of treatment with Org 2766, the remaining animals were observed for a single 5-min trial, eight to twelve weeks after the completion of Session 7, using the open field with 16 unbaited holes. The two CORT groups were slower to leave the start box than the other groups, $F(2,41)=5.30$, $p<0.009$. The two HPCX groups had higher locomotion scores, $F(2,41)=19.56$, $p<0.0001$, and higher relative activity than the SHAM and CORT groups, $F(2,41)=9.83$, $p<0.0003$. There were no differences in rearing frequency, mean duration per rearing bout, search time, total hole poke frequency, or mean duration per hole poke among all groups. Prior treatment with Org 2766 did not influence any of these behaviors.

Figure 6 illustrates the mean proportion of total hole pokes into the holes that had been baited on the initial sessions. If there was no preference between these "trained" holes and the other 14 holes then only 12.5% of all hole pokes would be expected to occur at the "trained" holes. The SHAM-SAL and HPCX-SAL groups searched the previously "trained" holes more frequently than would have been expected by chance alone (SHAM-SAL: $\chi^2(1)=12.88$, $p<0.001$; HPCX-SAL: $\chi^2(1)=6.51$, $p<0.01$). All other groups were near chance levels. The proportions of "trained" holes visited by the SHAM-ORG group and the HPCX-ORG group were significantly less than for their respective saline-treated groups (SHAM: $\chi^2(1)=7.14$, $p<0.01$; HPCX: $\chi^2(1)=5.56$, $p<0.025$). In general, all of the Org 2766-treated groups acted as if they did not remember the holes to which they had been trained.

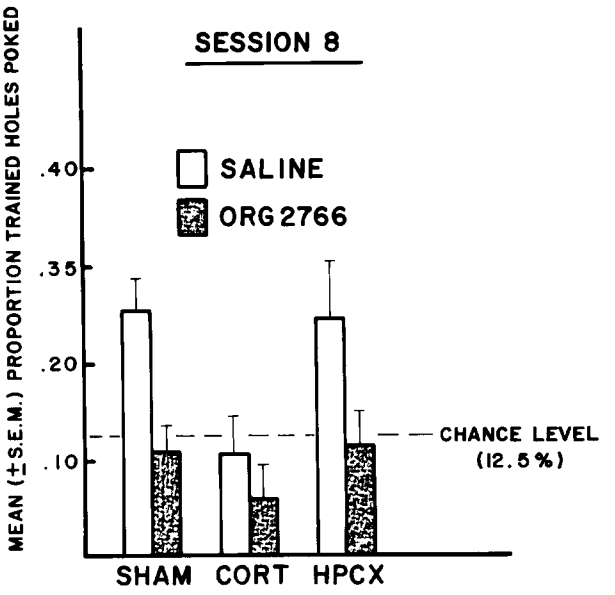


FIG. 6. Mean (±S.E.M.) proportion of the frequency of hold poke into "trained" holes per total frequency of hole pokes.

## DISCUSSION

In this experiment, rats with hippocampal lesions learned the food search task, whereas rats with neocortical damage alone did not. It must be emphasized that the treatment with Org 2766 was terminated 24 hours before the start of training. The drug was given the animals only during the first week following surgery. Therefore, the interpretation of peptide effects must be on the basis of essentially longer-lasting effects of the relatively short course of treatment with the peptide. This prior treatment with Org 2766 severely impaired performance of sham controls but had little or no effect on the behavior of rats with neocortical damage. The peptide treatment given to animals with hippocampal damage improved performance on some measures and impaired it on others. The overall pattern of significance following Org 2766 treatment shows that some of the specific behavioral changes related to attention deficits in hippocamally lesioned rats are attenuated, but the Org 2766 also reduced their performance to chance levels in Session 8 a few months after training.

By a variety of measures, hippocampally lesioned rats were generally more active than control groups, consistent with previous findings [24,36]. Even so, they were able to learn this simple food search task as easily as saline-treated shams as measured both by the percentage of pellets found and the time required to find them. Treatment with Org 2766 did not affect the efficient performance of the hippocampal-lesioned animals.

The behavioral sequelae of hippocampal lesions in rodents, particularly those changes related to food search and maze performance, have contributed to the development of several theories of hippocampal function. For example, the deficits are thought to represent impairments in particular types of memory [29,31], or in attentional processes [27, 28, 33, 44]. In the present experiment, saline-treated hippocampal lesioned rats failed proportionally more often to find food than did saline-treated sham controls only when the 14 additional baited holes were added in Session 7. This deficit can not be interpreted as a memory deficit, since these animals failed even when they had found and poked their heads into the food-baited holes. These failures under the novel condition of unexpectedly having all holes baited can be explained better as the animals had difficulty in adequately attending to the food pellets. Their difficulty was not in remembering where they or the pellets were located. Prior treatment with Org 2766 attenuated this apparent attentional deficit in the hippocampal-lesioned rats.

Whereas the results on failures suggest an attentional deficit, the increased frequency of revisits to holes where food had already been found may suggest an impairment in "working" memory [30,31]. This effect also was seen only during the unexpected presentation of additional baited holes and treatment with the $ACTH_{1-9}$ variant attenuated the apparent impairment in "working" memory.

In sum, we think the results demonstrate an attentional deficit in the hippocampal-lesioned rats under certain conditions. These functional disturbances in attention after hippocampal lesions were restored to control levels by prior treatment with Org 2766. This proposed action of Org 2766 on attentional processes is consistent with interpretations by others (e.g., [39]). However, the specific behavioral action of the peptide remains unclear since work with intact animals demonstrates effects on learning and memory, as well as attention (e.g., [11, 12, 26]).

Consistent with other studies (e.g., [29,31]), "reference" memory seems to be unaffected since the hippocampal-

lesioned rats found the food without difficulty and in Session 8 demonstrated a preference for "trained" holes. However, the lesioned rats (and the control groups) treated with Org 2766 did not show the preference for "trained" holes. Org 2766 treatment disrupted behaviors thought to be dependent on "reference" memory in both sham and hippocampal-lesioned rats.

By almost all indices, animals with neocortical lesions were severely impaired in performing the task. Although they eventually found some of the food pellets, they did not demonstrate a preference in Session 8 for previously "trained" holes. Deficits in food search tasks have been reported for animals with neocortical damage (e.g., [19]) and the present results are of interest because the deficits were not shared by the animals with hippocampal damage, animals which also sustained neocortical lesions at least as large as those in the neocortical lesion groups (cf., Fig. 2). This is consistent with other reports demonstrating that animals with neocortical lesions alone are impaired in learning situations in which neocortical-plus-hippocampal lesioned animals are not (e.g., [40]). We believe this is an important issue. It suggests that in ways yet to be determined, a hippocampus functioning in the absence of neocortical influences can be more debilitating than the combined loss of neocortical and hippocampal processing.

Treatment with Org 2766 did not particularly predispose the neocortical-lesioned animals to behave any differently than their saline-treated counterparts. However, treatment with Org 2766 produced behavioral effects that made sham operates largely indistinguishable from rats with neocortical damage. Such effects in locomotion were not found in adult rats following single injections of Org 2766 [23,46] nor after chronic administration in rat pups tested at 13 days of age, 24 hours after the last of 12 daily injections of Org 2766 [38].

It is possible also that the alterations in performance produced by Org 2766 could be explained on the basis of altered emotional reactions. In general, the peptide fragment has produced increased gregariousness and decreased anxiety in people [14,39]. These enhancements of social interactions in humans by Org 2766 are paralleled by facilitated social interactions in animals [13]. Even large doses did not seem to impair or reduce self-stimulation in rats [23]. A marked reduction in hyperemotionality in rats with septal area destruction has been demonstrated following Org 2766 treatment [34]. The increased tendency of sham-operated animals treated with the peptide to enter the center of the open field (athigmotaxia) in the present experiment suggests that these rats may have experienced a reduction of the fear or anxiety that may have been induced by handling and by being placed into the test apparatus. It is possible that a reduction in emotional responsiveness produced by Org 2766 would improve performance under conditions in which enhanced emotionality contributes to impaired performance. In the present study the animals with hippocampal damage were impaired when food was unexpectedly presented in all 16 holes. Their responses to this unexpected "reward" may have been an enhanced emotional reaction [7] which was reduced in animals given the prior treatment with the Org 2766. A similar effect may have occurred in the sham-operated animals treated with the neuropeptide fragment. In this case the Org 2766 may have reduced their emotional reactions below an optimal level required for maximal efficiency in the test situation. However, these possible explanations are only speculations at this time.

In summary, hippocampal-lesioned animals were exceptional in successfully finding food in the two-hole search task, and were not disturbed by the addition of extra empty holes. Some impairments indicative of attentional deficits were observed when these animals were confronted unexpectedly with all 16 holes filled with food "reward." A relatively brief treatment regime with the $ACTH_{1-9}$ variant Org 2766 specifically attenuated these deficits, while not changing lesion-induced hyperactivity. Rats with neocortical lesions were severely impaired in all aspects of the task and these deficits were not attenuated by Org 2766 treatment. Surprisingly, the Org 2766 treatment produced substantial impairments in performance in the sham-operated animals. We think the present study demonstrates that the $ACTH_{1-9}$ analogue Org 2766 can specifically influence behaviors related to attentional processing in animals with varying states of neural integrity. In general, there is much potential for future research into the diagnosis and attenuation of the functional deficits following CNS damage by treatment with ACTH-related peptides.

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