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# Repeated Intraventricular Injections of ACTH 1–24: The Effects of Home or Novel Environments on Excessive Grooming[1]

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Rats implanted with cannulas in the intraventricular foramen were tested after repeated daily injections of ACTH 1–24 or saline. Animals were either observed in their home cages or transported to an experimental room and observed in a "novel" Plexiglas chamber. Animals treated with the ACTH peptide evidenced an excessive, natural-type grooming which did not differ in the two experimental situations. No sign of adaptation of the ACTH-induced grooming was found over 10 consecutive days of testing. Saline-treated animals evidenced more grooming in the novel experimental chambers than when observed in their home cages. Some suggestion of adaptation of the grooming response was found in the saline-treated animals tested in the novel chambers. The results suggest that ACTH 1–24 induces a specific grooming response that is not dependent on being tested in a novel environment.

The excessive grooming that follows the intraventricular injection of ACTH peptides has been well documented (e.g., Colbern *et al.*, 1977; Ferrari *et al.*, 1963; Gispen *et al.*, 1975). This grooming appears to have a "natural character" and is easily distinguished from the stereotypical grooming which is seen with dopaminergic agonists and amphetamine (Ayhan and Randrup, 1973). That is, grooming induced by ACTH follows a normal sequence from vibration of the forelimbs to face washing, then body grooming, scratching, and licking. In drug-induced stereotypical grooming, animals usually exhibit only one element of grooming which is concentrated to a very specific area of the body.

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The significance of the excessive grooming is not easily explained on the basis of its utility for the animal or the type of circumstances that seem to induce it. Observation of natural bouts of grooming occur upon waking from sleep and after eating and drinking (Bolles, 1960). It also occurs after the presentation of unexpected stimulation (Fentress, 1968a,b) and after a fear-inducing event (Fentress, 1965). It has also been observed in conflict situations (Hinde, 1975).

The excessive grooming behavior induced by ACTH peptides is usually found a few minutes after the animal has been transferred to a novel and restricted environment. Usually, this environment is a small plastic box in a room at some distance from the animals' normal living quarters. Under these environmental conditions, a small amount of grooming occurs even without peptide administration.

The increased amount of grooming observed after transfer to the novel testing situation depends, at least in part, on the intregrity of the pituitary gland, since it is attenuated after hypophysectomy or administration of antiserum to ACTH (Dunn *et al.*, 1978). It may be that the action of the ACTH fragments effective in inducing grooming is to enhance and prolong the grooming induced by the transfer to the novel testing situation.

The present study was undertaken to extend previous observations by determining and comparing the effect of repeated injections of ACTH 1–24 when animals were observed both in the novel environment usually used for such experiments and in the animals' home cages. As a control condition, the effect on grooming of transport to the experimental room and placement in the novel situation after intraventricular administration of saline was compared with the effects of intraventricular administration of saline in the home cage.

## METHODS AND MATERIALS

*Animals and surgery.* Twenty-six male Wistar rats (Charles River Laboratories) weighing between 140 and 175 g at the start of the experiment were used. The animals were individually housed on a 12-hr light–dark cycle in a colony room which was separated from animals being used in other experiments. Food and water were provided *ad libitum*.

Using clean, surgical technique, each rat was anesthetized with Nembutal (45 mg/kg), and a plastic cannula (Portex 25, o.d. = 0.7 mm) was lowered into the interventricular foramen of Monro according to the following König and Klippel (1967) coordinates: AP, −0.8; L, 1.0; and DV, 4.5 mm down from the skull. The cannula was then fixed in place using dental cement and each rat received an injection containing approximately 60,000 units of Bicillin.

*Injections.* Just prior to the daily behavioral test, each animal, according to its group assignment, received an intraventricular (ivt) injection of

either 1 μg of ACTH 1–24 in 1 μl (Organon Int. BV., Oss, The Netherlands) or 1 μl of the 0.0001 *N* HCL and saline solution used to dissolve the peptide. The injections were made via a microliter syringe (Unimetrics Corporation, Type 5010R) affixed with a 30-gauge blunt-tipped needle. The rat was held by the experimenter and its eyes were shielded with a folded cloth while the needle was lowered into the cannula to the point where the tip of the needle bisected the 45° bevel cut of the plastic cannula. Each rat was then either transported to a different room and placed into a novel observation box or placed back into its home cage for observation. The observer did not know what injection treatment an animal had undergone.

*Behavioral testing*. On the sixth day following the cannulation procedure, behavioral testing began. Grooming behavior was measured using a time sampling technique developed by Gispen and his colleagues (Gispen *et al.*, 1975). If the animal was exhibiting an element of the grooming repertoire at the time of the sample (i.e., forelimb vibration, face washing, scratching, licking the paws, tail grooming, or body shakes), a positive grooming score was recorded. Samples were taken every 15th second for 50 min beginning 15 min after the ivt injection. Therefore, a maximum grooming score of 200 was possible for each animal during the 50-min observation period.

Animals were divided into two groups: one to be tested in the novel environment, the other to be tested in the home cage. Each of these groups was further divided so that approximately half of the animals would receive repeated ivt injections of ACTH 1–24 and the other half would repeatedly receive the saline control.

Animals being tested in the novel environment were taken in their home cage from the colony room to where they were injected with either ACTH 1–24 or saline and then were placed into a dimly lit, novel, glass box (20 × 30 × 30 cm) in a room with a low level of white noise. The animals were observed through a one-way mirror by an observer in an adjacent room.

Animals being tested in their home cages were taken from their cage, injected with either ACTH 1–24 or saline, and returned to their cage on the rack in the colony room. The cages measured 24 × 17 × 17 cm and were made of stainless steel with wire-mesh fronts and bottoms and solid sides and backs. The animals' behaviors were easily recorded by an observer sitting behind a blind in the colony room.

In both environments, the animals in the ACTH 1–24 group received ivt injections of 1 μg of ACTH 1–24 for 11 consecutive days commencing on Postoperative Day 6 and ending on Postoperative Day 16.

The animals in the saline group received nine consecutive ivt injections of 1 μl beginning on Postoperative Day 6 (Test Days 1–9). However, on Test Day 9, the animals which had been tested in the home environment

were switched to their home cage. Remaining in their recently switched environments, on Test Days 10 and 11 all the saline animals received ivt injections of 1 $\mu$g of ACTH 1–24.

*Histology.* At the conclusion of the experiment, each rat was injected intraventricularly in the same manner described before with 2 $\mu$l of a methylene blue and saline solution. Immediately following the injection, each animal was anesthetized with Nembutal and the brain was intracardially perfused first with saline and then with 10% formalin. The brain was carefully removed from the skull and sliced coronally down the cannula tract. If the cannula placement was correct, the stain had circulated through the ventricular system and was easily detected in all forebrain ventricular spaces. However, if the cannula was not in the correct position, the stain was not observed in the ventricles but rather was seen in the tissue surrounding the tract.

## RESULTS

Given the small volume injected (1 $\mu$l) into animals in this study and the repeated injection procedures, occasionally an animal would not receive an effective injection of the peptide. This could be caused by a temporary malfunction of the microsyringe, a transient clogging of the implanted cannula, or improper administration against strong cerebrospinal fluid pressures. Approximately 18% of the injections of the ACTH peptides were behaviorally ineffective. This "miss rate" of effective injections is somewhat higher than that found in other studies (usually an 8% "miss rate" is found) using 1-$\mu$l injections in this laboratory and others. However, no animal had ineffective injections on more than 2 out of the 11 test days, and no 2 consecutive test days of ineffective injections ever occurred. On the day after an ineffective injection, scores were as high as the median response of that animal to other "effective" injections. Therefore, to provide the most representative data from animals with effective peptide injections, the highest grooming score for 2 consecutive days of testing was taken as the basis for statistical analysis.

Figure 1 shows the median grooming scores for the four groups of subjects. Friedman analyses were applied to these data for the first 8 days of testing for the groups receiving saline and for all test days for those receiving the ACTH peptide. No significant change in the scores of any of the groups was found. However, the group receiving the intraventricular injection of saline in the novel environment approached statistical significance. A Wilcoxon matched-pair comparison indicated a reduction in the scores obtained on Day 1 or 2 relative to those obtained on Day 7 or 8 ($P < 0.05$), but the validity of this test must be considered questionable in the absence of a significant effect over all test days using the Friedman procedure.

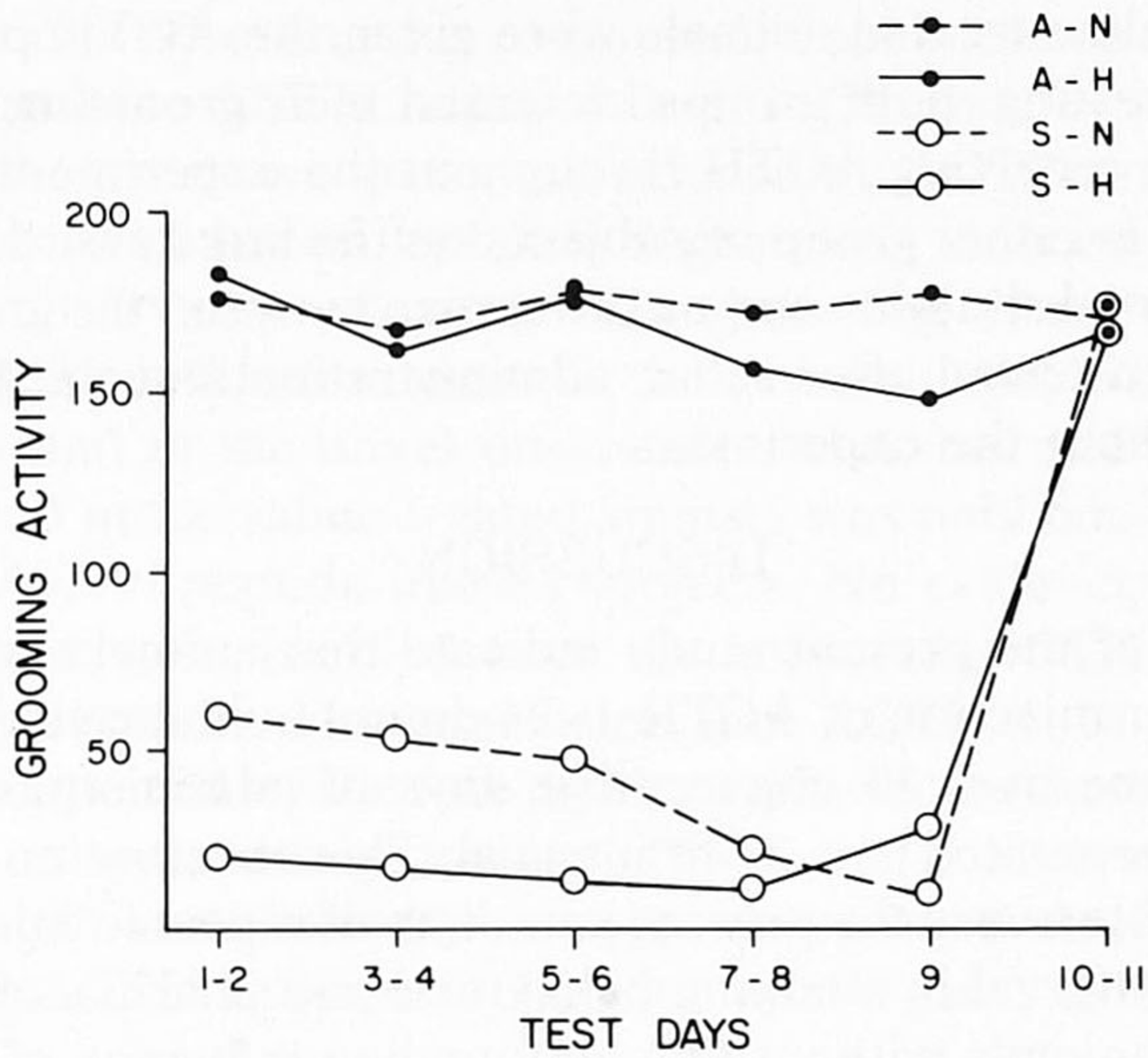


FIG. 1. Median grooming scores for the four groups of animals. As described in the text, the highest grooming score was obtained over 2 consecutive days of testing for the first 8 days of testing and for Days 10 and 11 when animals treated previously with saline were given ACTH 1–24 before testing. On Test Day 9, the saline-treated animals were tested in the environment different from that in which they were previously tested. (A-N) ACTH-treated animals tested in novel observation box ($n = 6$); (A-H) ACTH-treated animals tested in home cage ($n = 4$); salinex-treated animals tested in novel observation box ($n = 6$); (S-H) saline-treated animals tested in home cage ($n = 5$).

Since the Friedman analyses for all groups did not find significant differences among the scores over the test days, the median score was obtained from each animal for all days of testing for the ACTH-treated groups and for the first 8 days of testing from the saline-tested group. Mann–Whitney $U$ tests made with these scores, indicated that the saline-treated animals tested in the novel cages groomed more than those tested in their home cages ($P < 0.01$) but that there were no differences between ACTH-tested animals observed in their home cages and in the novel observation boxes. Animals receiving ACTH in either situation groomed more than those receiving saline in either situation. (Mann–Whitney $U$, $P < 0.01$). There was no overlap in the distribution of median scores of the ACTH and saline groups over the first 8 days of testing.

On Test Day 9, when the animals receiving intraventricular injections of ACTH in their home cages were moved to the novel environment for observation, no change in grooming was observed. However, every animal that had received saline in the home cage increased its grooming score relative to its median grooming score on the first 8 days of testing when transferred to the novel observation chamber ($P < 0.05$, Wilcoxon signed-rank test).

When the saline-treated animals were given the ACTH peptide on the last 2 days of testing, both groups increased their grooming scores to the levels of those receiving ACTH throughout the experiment. There were no differences in either group of subjects on the last 2 test days related to the environmental situation and no difference between the groups that had previously been tested after saline administration and those tested after ACTH throughout the experiment.

## DISCUSSION

The results of the present study indicate that animals given repeated intraventricular injection of ACTH 1–24 do not evidence signs of adaptation or tolerance over 11 consecutive days of administration when the injections are separated by a 24-hr interval. This observation suggests that it will be possible to use the present paradigm of repeated injections at this interinjection interval in studying behavioral and pharmacologic manipulations of the animals without the confounding influence of the development of tolerance. Animals that had received intraventricular saline for 9 days prior to receiving ACTH 1–24 immediately groomed to the same extent as those that had been receiving ACTH over the same number of days. This indicates that the cannula implantation procedure and previous injections of saline had not altered the responsiveness of the mechanisms responsible for the grooming response.

In a recent study (Jolles, Wiegant, and Gispen, personal communication), it was found that, when the ACTH 1–24 injections were separated by less than 24 hr, a decrease in behavioral responsiveness to the second injection was observed. However, in that study as in this, injections made at intervals of 24 hr showed no signs of behavior tolerance or sensitivity.

The fact that animals which had received intraventricular saline over the first 8 days and had been placed in a novel environment groomed more than those injected similarly but observed in their home cages suggests that grooming can be stimulated by environmental changes associated with transport to the experimental room and placement in the novel observation chamber. Switching the saline-injected animals from their home cage to the novel observation chamber produced an increase in their grooming scores to a level comparable to those found in animals repeatedly tested in the novel observation chambers. Similarly, the animals that had received saline and had been tested in their home cages also adjusted their grooming scores to show a decreased level of grooming, comparable to that of the animals which had been repeatedly tested in their home cage.

Taken in conjunction with other studies, the data from the saline-treated animals suggest that bouts of grooming may occur in the rat after periods of arousal or activation produced by handling, transportation to an unfamiliar location, or placement in a novel situation. At least under

certain conditions of testing this response is dependent on the integrity of the pituitary and the ACTH released from it (Dunn *et al.*, 1978).

However, the excessive grooming induced by ACTH does not seem to be merely a prolongation of the bouts of grooming induced by the transfer to the novel observation chamber. The grooming of the animals tested after the ACTH peptide treatment in their home cages was fully equivalent to that found in the novel observation chamber and the amount of grooming found in the saline-treated animals was only one-quarter of that found in the ACTH peptide-treated subjects. No evidence for adaptation of the response was observed over the repeated ACTH treatments in either testing situation. The conclusion that ACTH 1–24 directly elicits grooming is supported by the fact that hypophysectomized animals which fail to show exaggerated grooming after transfer to a novel chamber (Dunn *et al.*, 1978) can be induced to groom by intraventricular administration of the ACTH fragment (Gispen and Wiegant, 1976).

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