BEHAVIORAL AND NEURAL BIOLOGY 29, 481–486 (1980)

BRIEF REPORT

Hypophysectomy and Novelty-Induced Grooming in the Rat

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In an attempt to resolve an apparent conflict in the literature concerning novelty-induced grooming, an experiment was undertaken in which half of the hypophysectomized or sham-operated groups of rats were subjected to stressful experiences for 2 days after surgery. The animals were tested for excessive grooming on three subsequent days using standard procedures. They were also tested for locomotion and exploration in a modified open field, before a final grooming test after water immersion. The results indicate that the 2 days of stress procedures did not affect the behavior of either group of animals. The sham operates' grooming scores did not change with repeated testing, but those of hypophysectomized animals did. It appears that when tested initially or after a 7-day interval from a previous test, hypophysectomized animals have higher grooming scores than the controls. They also had higher locomotion scores in the open field. Similar and excessive amounts of grooming were found after water immersion in the hypophysectomized and sham-operated animals. The results partially resolve the conflict between previous studies by pointing out the significance of procedural variables for the behavior of hypophysectomized rats.

The placement of rats in novel observation cages produces a prolonged display of excessive grooming behavior (Bindra & Spinner, 1958; Dunn, Green, & Isaacson, 1979; Jolles, Rompa-Barendregt, & Gispen, 1979a), although the amount is substantially less than produced by the intraventricular injection of $ACTH_{1-24}$ (Gispen, Wiegant, Greven, & de Wied, 1975). Although recent studies have suggested brain biosynthesis of ACTH-like peptides (see Krieger, Liotta, Hauser, & Brownstein, 1979), there is also evidence indicating a specific transport of pituitary ACTH into the brain (Mezey, Palkovits, de Kloet, Verhoef, & de Wied, 1978).

0163-1047/80/080481-06$02.00/0

The question of the effect of removal of the pituitary was considered important because the answer could shed light on whether neuropeptides released from the pituitary play a role in determining novelty-induced grooming. Dunn et al. (1979) reported data that made it appear that they did. In that study rats were hypophysectomized via the parapharyngeal route at Charles River Laboratories the day before shipment and were tested 1 week after being received in laboratories at the University of Florida. The intact, control animals showed a considerable increase in grooming when tested in the novel cages, while the hypophysectomized rats, as a group, evidenced only a very small increase. On the other hand, Jolles et al. (1979b) found substantial increases in grooming when hypophysectomized (transauricular route) rats were tested in a novel environment after transport and handling. Their comparison was made between independent groups of hypophysectomized animals, each tested once, while Dunn et al. (1979) evaluated a change in grooming of the same animals after a change in conditions (home cages vs novel cage). However, in all of the conditions reported by Jolles et al. (1979b), grooming scores in the hypophysectomized animals were greater than those found by Dunn and his co-workers.

A number of procedural differences may account for the discrepancies between the studies (the route of surgery, genetic differences, the prior, presumably stress experiences of the animals, and testing of the subjects, etc.) Therefore, we undertook to try to resolve some of these issues.

Forty male Wistar rats of an inbred strain (TNO, Zeist, The Netherlands) weighing about 120–130 g were used. Twenty were hypophysectomized by the transauricular route. Twenty were subjected to sham surgery. At the conclusion of the experiments, absence of pituitary tissue in the sella turcica was determined by macroscopic inspection; loss of body and adrenal weight were taken as signs of a correct hypophysectomy (Gispen, van der Poel, & van Wimersma Greidanus, 1973).

Prior to the surgery the animals were group housed. After surgery half of each group of animals was subjected to presumably stressful experiences, and on the second day after surgery the animals were individually housed. All animals were on a 12:12 day–night cycle with lighting onset at 8:00 AM. The data obtained from all sham operates and from the 14 hypophysectomized animals that remained in good health throughout the experiment were evaluated

A series of presumably stressful experiences were given to half of each group of animals on postoperative Days 2 and 3. They consisted of 30 min in a cold room maintained at 4°C. This occurred at 10:00 AM. At 11:00 AM, masking tape was wrapped around the animal's head and forepaws and the animals were replaced in their home cages. Tape and tape remnants remaining on the animals were removed 30 min later. At 2:00 PM the animals were forced to swim in water (22°C) for approximately 3 min. At

4:00 PM groups of five animals were placed in a chemical shaker run at slow speed for 5 min.

On the 4th, 7th, and 14th days after surgery the animals were observed for 60 min in the grooming observation situation described by Gispen et al. (1975). In this test situation animals are transported in their home cages to an unfamiliar, sound-isolated testing room and placed in small (24 × 12.5 × 14-cm) observation chambers. Their behavior is scored by observers outside the room watching the animals through a "one-way" glass window. Usually, behaviors are scored for the occurrence of grooming on a time-sampling basis every 15 sec beginning 10 min after the animals are placed in the small chambers. In the present experiment observations were made for 60 min after the animals were placed in the chambers, but the first 10 min of the observation period was considered separately from the subsequent 50 min, the more common observation period. Using this time-sampling method, maximum scores of 40 and 200 grooming counts could be obtained in the 10-min and 50-min observation periods, respectively. Grooming is scored whenever the animal is exhibiting any class of facial, forepaw, flank, or genital grooming (see Gispen et al., 1975). On the 15th day after surgery the animals were tested in the locomotion–exploration apparatus described by Isaacson and Green (1978). Separate measures were made of locomotion, grooming, and the placement of the animal's head into holes in the floor (hole poking) over the 5-min test period. The open field was located in a different observation room from that used for grooming. On the 16th day after surgery, the stressed and nonstressed groups were each divided into groups that were either immersed in water (22°C) for 3 min or placed in a dry container for the same amount of time. After these experiences, the animals were again tested in the small grooming observation chambers in the unfamiliar room.

There were no differences in grooming scores between the hypophysectomized or sham-operated animals that were stressed or not stressed on the 2 days following surgery. These postoperative days were the same days on which the animals used by Dunn et al. (1979) were presumed to have received stressful experiences: the rats had been shipped and placed in new laboratory conditions on the second and third day after hypophysectomy. The present results suggest that the stress experienced by these animals on those days is not likely to account for the differences in novelty-induced grooming found between the Dunn et al. and Jolles et al. studies.

Subsequent analyses were made between the total number of hypophysectomized and sham operates without regard to prior stress conditions. Statistical tests were performed using nonparametric statistics because of possible problems associated with a lack of homogeneity of variance between treatment groups which would be exacerbated by unequal sample sizes. The grooming scores of the sham-operated control

rats did not change significantly over the repeated testing days during either the first 10-min observation period or the subsequent 50-min observation period (Table 1, Days 4, 7, and 14). The 50-min test scores were comparable to those routinely observed after novelty by Gispen and his co-workers (Gispen et al., 1975; Jolles et al., 1979a). It should be noted that these novelty-induced grooming scores are substantially lower than those routinely obtained by Isaacson and his co-workers (e.g., Dunn et al., 1979; Isaacson & Green, 1978). A Friedman analysis of variance indicated significant differences over the tests given to the hypophysectomized animals on the 4th, 7th, and 14th days after surgery for the 50-min observation period ($p < .05$). On the 4th postoperative day the grooming scores obtained in the first 10 min were greater than those of the sham operates as well ($p < .01$, Mann–Whitney U). The fact that grooming scores of this group were higher at this first test is in agreement with the data of Jolles et al. (1979b). On the 7th day, however, the second time the rats had been transported to the observation room there was a significant reduction in their grooming scores ($p < .01$, Sign test) to about the level of the sham operates. Based on these results, and those of other tests given later in the experiment, it would appear likely that experience with the novel grooming test area decreases the tendency of hypophysectomized animals to give high grooming scores in novel conditions in a subsequent test under the same conditions. The animals tested by Dunn et al. (1979) had been placed in the novel testing room, but in their home cages, for 3 consecutive days before a test was made in the novel observation cages. If this interpretation is correct, then the effect of previous exposure to the novel area of testing must be diminished over the course of 7 days, since the hypophysectomized rats showed increased grooming levels when tested at Day 14 (Table 1). On the 15th day after surgery when the animals were tested in the modified open field, enhanced locomotion and hole poking was observed in the hypophysectomized rats compared to the

TABLE 1

Means (SD) of Time-Sampled Grooming Scores Observed in First 10 min (1st 10) and Subsequent 50 min (Next 50) of Observation for the Hypophysectomized and Sham-Operate Groups

	Test day					
	4		7		14	
Group	1st 10	Next 50	1st 10	Next 50	1st 10	Next 50
Hypophysectomy	18.21 (6.85)	43.14 (16.97)	15.79 (9.54)	16.64 (11.20)	14.00 (5.59)	33.07 (16.88)
Sham operates	12.6 (5.60)	28.40 (17.84)	13.2 (5.94)	21.45 (12.95)	14.8 (6.64)	20.85 (14.75)

TABLE 2
The Means (SD) of Locomotion, Hole Poking, and Grooming Recorded in the Modified Open Field on the 15th Day after Surgery

Group	Behavior		
	Locomotion	Hole poke	Grooming
Hypophysectomized (N = 14)	129.57 (17.58)	14.21 (3.02)	6.38 (3.11)
Sham (N = 20)	109.65 (17.04)	10.2 (4.88)	4.85 (4.20)

sham operates (Table 2, $p = .04$, exact χ^2 test for both behaviors). This confirms previous observations by Gispen et al. (1973), who found that hypophysectomized animals showed increased motor activity over several testing days in isolated conditions. In this earlier study testing was done near the midpoint of a reversed day–night cycle. Coupled with the present results, it suggests that the hyperactivity of the hypophysectomized animals may be independent of the light–dark cycle.

On the 16th day after surgery half of each group were immersed in water before observation for grooming. The water treatment increased the grooming scores of animals in both groups ($p < .01$, Sign test; see Table 3). The hypophysectomized animals exhibited more grooming after water immersion than did the sham operates in the 50-min observation period but not in the initial 10-min observation interval. A more detailed discussion of the effects of water immersion can be found in Gispen and Isaacson (1980).

Although these experiments do not explain the role of pituitary gland in novelty-induced grooming, they add to our knowledge of the effect of

TABLE 3
Means (SD) of Time-Sampled Grooming Scores Observed in the First 10 min (1st 10) and Subsequent 50 min (Next 50) after Water Immersion (Water) or Control Procedures (Dry) on the 16th Day after Surgery for Hypophysectomy and Sham-Operate Groups

Group	Water		Dry	
	1st 10	Next 50	1st 10	Next 50
Hypophysectomy	35.00 (5.92)	85.86 (23.84)	14.67 (4.18)	21.83 (6.72)
	N = 8		N = 6	
Sham	35.67 (3.20)	65.33 (13.73)	20.62 (4.65)	29.25 (13.14)
	N = 12		N = 8	

procedural variables on the behavior of hypophysectomized animals. The results indicate that hypophysectomized animals exhibit enhanced or decreased novelty-induced excessive grooming depending on the prior testing regime given the animals. The question of whether the excessive grooming found after exposure to novel circumstances is mediated by ACTH remains clouded. Even though antibodies to ACTH reduce novelty-induced grooming (Dunn et al., 1979), animals without the pituitary gland can exhibit such grooming, at least under some circumstances.

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