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Water Immersion, Excessive Grooming, and Paper Shredding in the Rat

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After immersion in water or after intraventricular administration of $ACTH_{1-24}$, rats display excessive grooming behavior which in both instances can be suppressed by systemic administration of the opiate antagonist naloxone (1 mg/kg ip). If paper is glued to the walls of the observation cages, in addition to excessive grooming, water immersion or peptide administration induces paper-shredding behavior which can also be blocked by naloxone treatment. Opiate receptors seem to be involved in the mediation of both behavioral responses to water immersion. There is no relationship between scores for grooming and paper-shredding behavior. The paper-shredding behavior may be related to the piling of wood shavings often described as part of the nest-building behavior repertoire. The water immersion technique is a powerful environmental manipulation to induce grooming and may shed further light on the mechanism underlying novelty or peptide-induced excessive grooming in the rat.

Excessive grooming has been found to occur in the rat after the intracerebroventricular (icv) administration of ACTH, certain of its fragments, and β-endorphin (Ferrari, Gessa, & Vargiu, 1963; Gispen & Isaacson, 1981). It is also found after the relatively mild stress of handling, transportation, and placement in a novel observation chamber (Bindra & Spinner, 1958; Colbern, Isaacson, Green, & Gispen, 1978; Jolles, Rompa-Barendregt, & Gispen, 1979a). Both the neuropeptide and en-

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vironmental inductions of excessive grooming are reduced by systemic administration of the opiate antagonist naloxone in a dose-dependent manner (Gispen & Wiegant, 1976; Green, Isaacson, Dunn, & Lanthorn, 1979), and both manipulations are susceptible to the peripheral administration of haloperidol at high dose levels (Green et al., 1979; Wiegant, Cools, & Gispen, 1977). Evidence that environmental and central neuropeptide-induced excessive grooming arise from a common central factor comes from the observation that excessive grooming produced by handling, transport, and novelty is reduced or eliminated after the intraventricular administration of antiserum to ACTH (Dunn, Green, & Isaacson, 1979).

However, while the mild stress conditions associated with handling and transport induce excessive grooming in rats, not all stress conditions produce the excessive grooming. For example, electrical footshock reduces grooming (Jolles, Rompa-Barendregt, & Gispen, 1979b), and rats exposed to ether stress do not groom. Rats also fail to groom for an hour or more after the severe stress of 5 min restraint in an upside-down position (Hannigan, unpublished observation). Exposure to a cold atmosphere (5°C) for 30 min, on the other hand, does induce excessive grooming (Gispen & Isaacson, 1981).

In the course of studying grooming in brain-lesioned animals, Gispen and his colleagues noted that immersion in water for 50 sec or more induced excessive grooming in rats (unpublished observations). This method of inducing excessive grooming was not found effective after periods of water immersion less than about 1 min in duration. Excessive grooming after water immersion does not occur in the first 10 min after removal from the water but rather in the subsequent 50 min of observation (Gispen, Brakkee, & Isaacson, 1980). The excessive character of the grooming is a consequence of the extension of grooming bout durations that occur throughout the observation period. The first experiment was undertaken to pursue these preliminary observations and also to determine if such extensive grooming could be attenuated by naloxone at a dose previously shown to produce this effect on neuropeptide- and environmental-induced grooming.

In addition, preliminary observations indicated that the water immersion procedure often led to the animals' removing paper attached to the back wall of the observation chamber. Since this behavior was not observed in non-water-immersed animals, paper was made available in the observation boxes of approximately one-half of the animals in the several groups studied to explore the relation of this "paper-shredding" behavior to the stress of water immersion and to grooming.

The second experiment was designed to determine if the icv injections of ACTH would induce paper shredding in addition to excessive grooming, either alone or in conjunction with water immersion and, in addition, to determine if the effects of water immersion would be additive with

the ACTH treatment. In order to demonstrate possible additive effects, a relatively low dose of $ACTH_{1-24}$ was selected (0.1 μg). This dose produces a significant increase in grooming, but the amount is well below the maximal amount allowed by the scoring procedure.

GENERAL METHOD

Animals and Drugs

Male, Sprague–Dawley rats (Blue Spruce Farms, Altamont, N.Y.) weighing approximately 200–280 g were used. Animals were individually housed in wire-mesh cages on a 12–12 hr light–dark schedule (lights on 8 AM) and received Purina lab chow and water ad libitum. Testing was conducted between 9 AM and 5 PM with different treatment conditions balanced throughout this period. Ambient temperature in all test conditions was 24°C.

In both experiments, 1 mg/kg naloxone–HCl (Endo Labs, Garden City, N.Y.) or an equal volume of saline was injected intraperitoneally. In addition, rats in the second experiment received an intracerebroventricular (icv) injection of 0.1 μg/μl $ACTH_{1-24}$ (Organon Int. B. V., Oss, The Netherlands), or 1 μl of its vehicle, 0.001 *N* HCl in saline.

Apparatus

Each rat was observed in a 30-cm^3 Plexiglas chamber with white side walls, ceiling, and floor, a mirrored back, and a transparent front panel that allowed the viewing of the subjects. A line of small holes in the top back of each chamber provided adequate ventilation. These boxes were located in a dimly lit, quiet observation room with the observer present. Rats were visually isolated from each other. Chambers were rinsed with water between tests of different animals.

Behavioral Measures

In both experiments behavioral observation commenced immediately upon placement of each rat into its box and continued for 65 min after the water immersion or handling control treatment. A time-sampling technique previously described and validated by Gispen et al. (1975) allowed simultaneous observation of eight animals. Every 15 sec, the observer recorded any grooming in progress. The grooming repertoire was defined as: forearm vibration, face washing, body or genital grooming, scratching, licking paws, holding or licking tail, head, or body shakes. One point was given for each grooming occurrence with a maximum of 260 possible scores for the 65-min observation period.

Paper shredding was quantified using a sampling technique on the same 15th sec schedule as for grooming. Before the beginning of each observation session in which paper shredding was to be measured, a fresh square of Grass Instrument Company polygraph paper was securely taped to the back of the observation chamber. Except for the ventilation holes

near the top of the chamber, the paper covered the whole back of the box with the unlined white side toward the animal. A positive score was recorded each 15th sec the animal was biting, ripping, carrying, or pawing the paper.

The number of fecal boli found in each chamber at the end of the observation period was also counted, as were other unusual behaviors, including instances of coprophagy and nasal hemorrhages.

Experiment 1

Rats were taken to the testing area in their home cages. Each rat was removed from its cage, injected ip with naloxone (NLX) or saline (SAL) and returned to its cage. Ten minutes after the ip injection, each rat was either immersed in water (WET) or subjected to the handling control procedure (DRY). If assigned to the WET condition, the rat was placed in a plastic bucket half-filled with water (22°C) in which the animal could not stand on the bottom and keep its head above water. In the DRY condition, the rat was placed in a similar container without the water. For each condition, the rat was removed from the bucket after 50 sec and placed in the nearby observation chamber.

Paper was available in the observation chambers of half the animals.

Experiment 2

Naive rats were implanted with a polyethylene cannula into the lateral ventricle 7 days prior to their initial behavioral test. Both the surgery and icv injection into the unanesthetized conscious rat have been described elsewhere by Brakkee, Wiegant, and Gispen (1979). However, in order to prevent water from entering the cannula during water immersion, the procedure was modified such that a shortened, blunt 27-gauge needle with the hub removed was used to plug the cannula immediately after the icv injection.

In the initial test, each rat was removed from its home cage, given an icv injection of $ACTH_{1-24}$ or saline, the plug inserted, an ip injection of NLX or SAL administered, and the rat returned to its home cage. This procedure took approximately 45 sec. Ten minutes later, each rat was placed into the bucket of water (22°C). After 50 sec, the plug was removed from the cannula, the animal placed into the observation chamber, and its behavior recorded.

Twelve days later, each rat was again subjected to the same treatment described above except there was no water in the bucket. Paper was present in all test situations for all animals.

RESULTS

Despite the incompatibility of paper-shredding and grooming behaviors, having paper in the observation boxes did not affect the amount

of excessive grooming exhibited for either the saline or naloxone pretreated animals in the first experiment. Therefore, these groups were combined for the analysis of grooming scores. The means and standard errors of the means (SEM) for the immersed and nonimmersed groups are shown in Fig. 1. For both the WET and DRY conditions, the naloxone-pretreated animals gave grooming scores lower than those of the saline-pretreated groups ($p < .01$ and .05, respectively, Student's t test).

The effect of water immersion on paper shredding and the suppression of that effect by naloxone are illustrated in Fig. 2. Because of the fact that some animals did not show the response, as well as the variability in scores of those that did, paper shredding is described by the percentage of the animals within the groups to exhibit any degree of paper shredding. After immersion into 22°C water, 55% of the rats with paper available and which were injected with saline exhibited paper shredding, whereas after naloxone treatment only 14% exhibited this behavior. Only 12.5% of the rats in the DRY condition with saline touched paper, whereas none in the DRY-NLX group did. There was no relationship between scores for grooming and paper-shredding behavior. Overall, the naloxone-treated animals deposited more boli than did the saline-treated animals (Median test, $p < .05$).

The means and standard errors of the means (SEMs) of the grooming scores of all groups in Experiment 2 are shown in Fig. 3. A mixed design analysis of variance was performed on the grooming data and indicated that there were significant main effects of the icv peptide administration ($F(1, 37) = 27.59$, $p < .001$), systemic drug injection ($F(1, 37) = 15.52$, $p < .001$), and water immersion ($F(3, 37) = 24.94$, $p < .001$). Planned comparisons (Duncan's Multiple Range Test) showed that these effects were due to an increased grooming score after ACTH, a decrease after naloxone injection, and an increase after water immersion. There were no significant interactions.

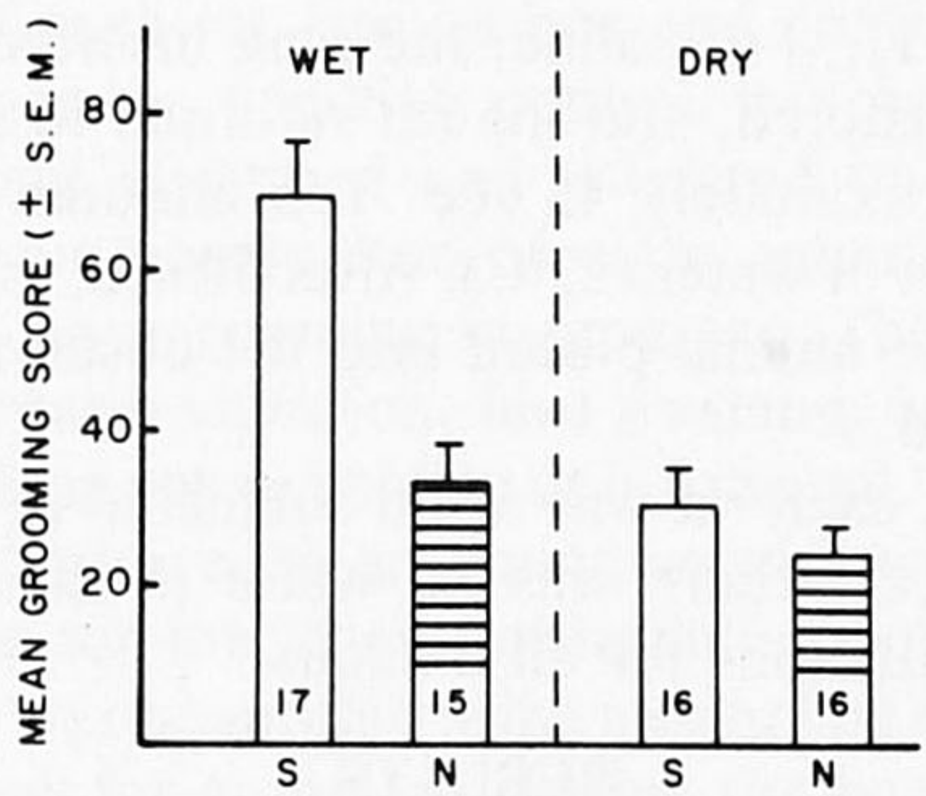

FIG. 1. Mean total grooming scores (and SEMs) for each group of immersed (WET) and nonimmersed animals (DRY) in Experiment 1. S and N indicate saline or naloxone pretreatments. Numbers within bars indicate the number of animals in each group.

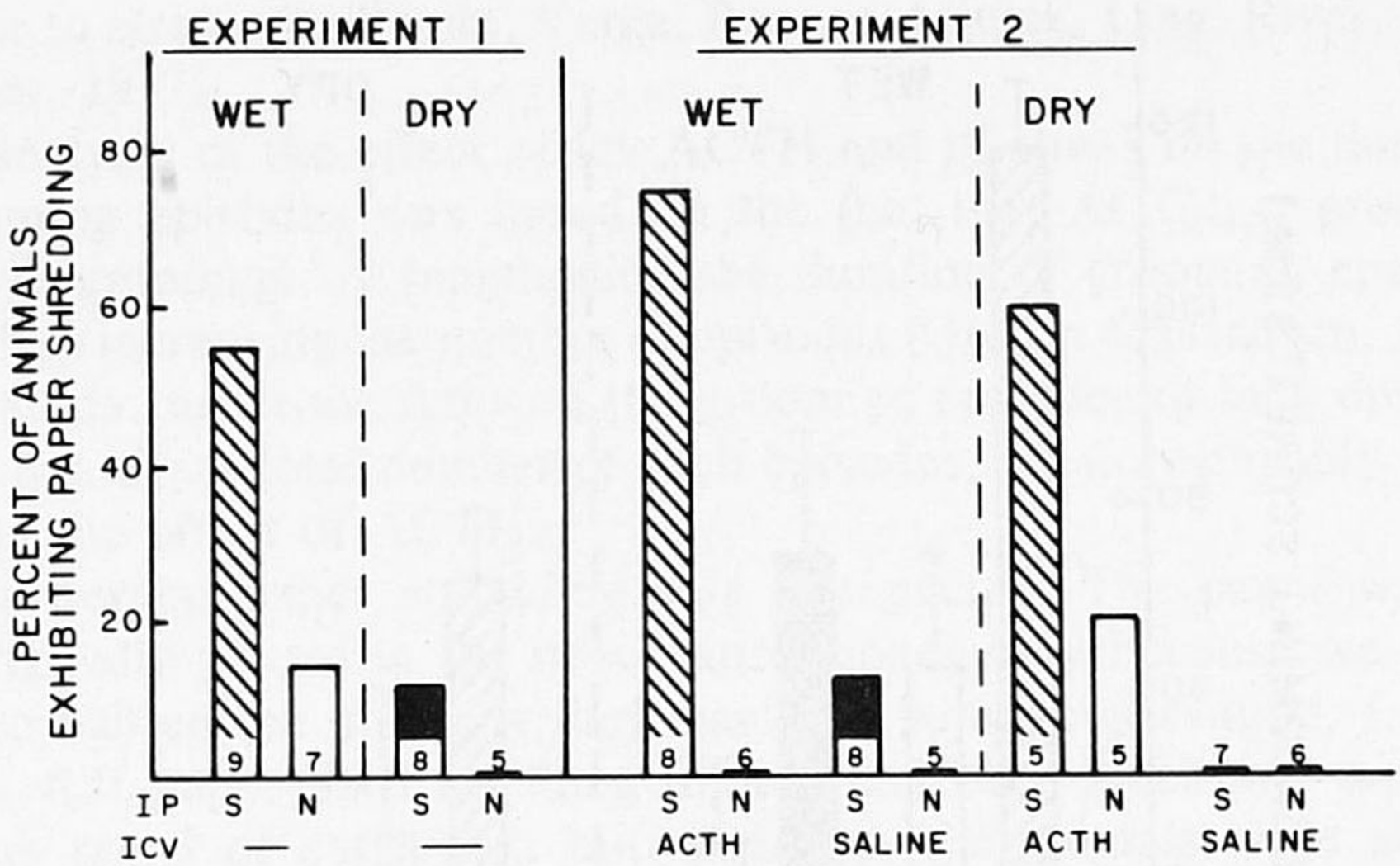

FIG. 2. The percentage of animals in each group that exhibited paper shredding during the test periods. (IP) Intraperitoneal injections given before testing; (S) saline; (N) naloxone; (ICV row) intraventricular injection treatments; (ACTH), 0.1 μg $ACTH_{1-24}$ in 1 μl fluid. Saline: 1 μl saline vehicle. Numbers in or above bars represent the number of animals in each group.

Naloxone did not change grooming scores of animals in the DRY condition after the icv injection of saline ($F < 1.0$). The effect of the naloxone treatment on the water-immersed animals receiving either icv saline or ACTH was to reduce the number of grooming episodes of long duration. For example, of those animals receiving icv saline with systemic naloxone pretreatment, only 12% of the grooming episodes had durations longer than five consecutive sample periods (an estimated 75 sec), while those pretreated with saline had 29% of their episodes of this duration or longer. The animals with icv ACTH and systemic saline pretreatment had 71% of their grooming episodes longer than five consecutive sample periods, while those with the icv ACTH pretreated with naloxone had only 39% of their grooming bouts in that range. There were no differences in the number of grooming bouts initiated by the four groups.

Paper shredding was observed in 75% of the water-immersed animals receiving central ACTH and peripheral saline and in none of the seven animals given central ACTH and peripheral naloxone in the WET conditions (see Fig. 2). When ACTH was given centrally and saline systemically in the DRY condition, 60% displayed paper shredding. All animals in the DRY condition exhibited paper-shredding scores below those shown after immersion. There was no significant change for the DRY condition in the number of boli produced by naloxone vs saline-treated rats nor by $ACTH_{1-24}$ vs saline-treated rats.

DISCUSSION

The results indicate that water immersion produces excessive grooming beyond that usually found under novel testing conditions. The effect was

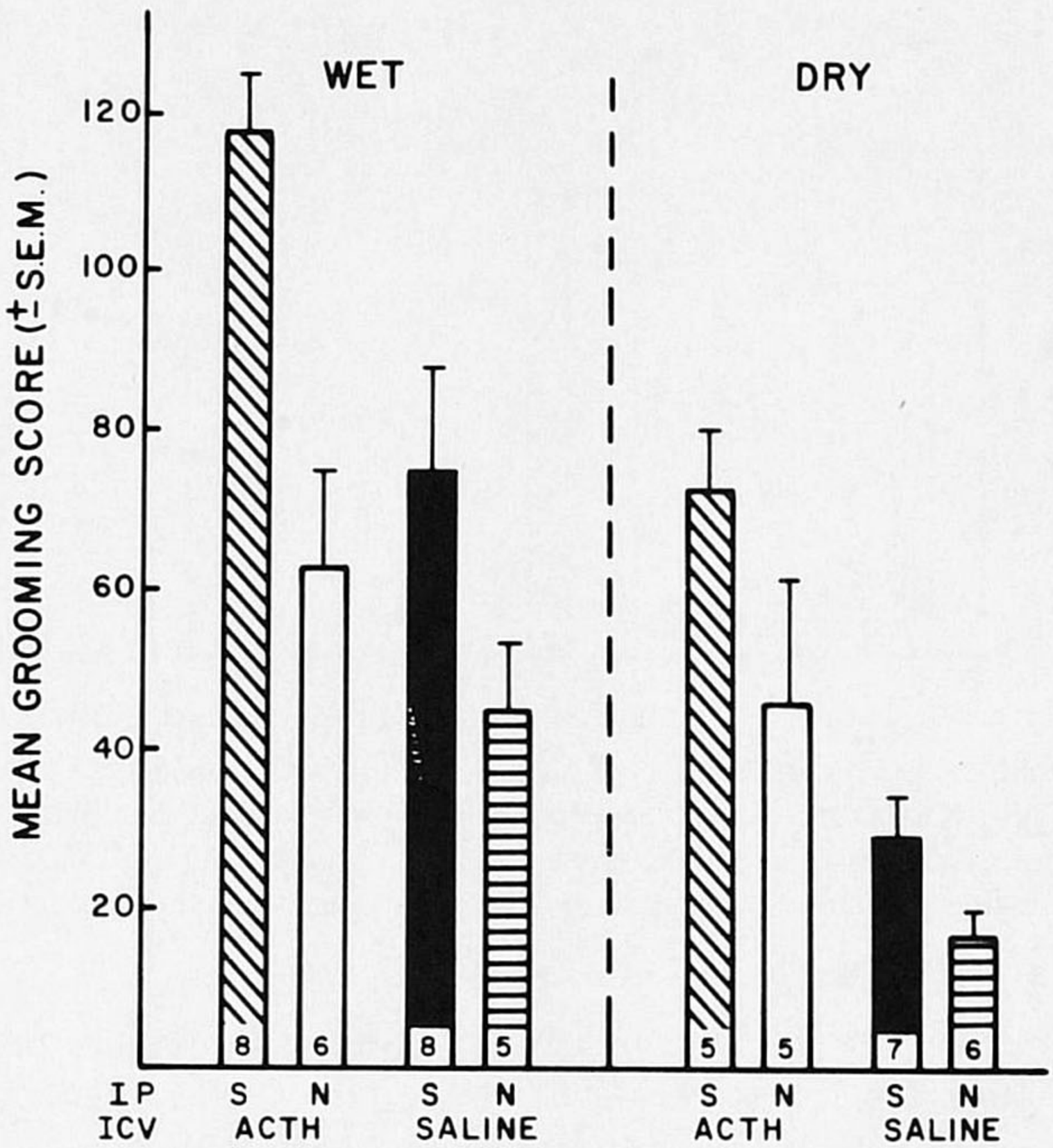

FIG. 3. Mean total grooming scores (and SEMs) for each group after water immersion (WET) or nonimmersion (DRY) and after icv injection of ACTH or saline in Experiment 2. (IP row) Intraperitoneal treatments given before testing; (S) saline; (N) naloxone; (ICV row) intracerebroventricular treatments; (ACTH) 0.1 μg in 1 μl fluid; (S) 1 μl of saline vehicle. Numbers in bars indicate the number of subjects in each group.

suppressed by 1 mg/kg of naloxone, thus suggesting that the excessive grooming behavior in this experiment is similar to ACTH-induced (Gispen & Wiegant, 1976) and mild stress-induced excessive grooming (Green et al., 1979) with respect to the suppressive effect of naloxone. The effect of the naloxone is not likely due to a change in general activity since grooming was not different after ip naloxone injection, relative to ip saline, in animals measured in the DRY condition after icv saline. Naloxone has also been shown to decrease excessive grooming at doses that did not affect locomotion, exploration, or rearing in an open field (Green et al., 1979). We have also found that the effects of systemic naloxone are specific to excessive grooming and do not affect rearing in the small observation boxes used in this study (unpublished observations). Based on evidence obtained from studies with isolated guinea pig ileum, it seems that the stress of water immersion stimulates the release of β-endorphin (Bodycote & Chesher, 1979) and presumably of ACTH, since the two neuropeptides are thought to be contained in the same pituitary cells (Mains & Eipper, 1978), and may be concomitantly released in

response to stress (Guillemin, Varga, Rossier, Minick, Ling, River, Vale, & Bloom, 1977).

Our analysis of the effect of icv ACTH and of stress on the duration of grooming episodes was based on the fact that $ACTH_{1-24}$ produces excessive grooming by lengthening the duration of grooming episodes rather than increasing the number of episodes (Gispen & Isaacson, 1981). In this study, naloxone reduced the grooming episodes of long duration but did not affect total number of such episodes, thus presumably counteracting the effect of ACTH.

The observed paper shredding was unexpected. The paper was, in fact, originally placed in the observation boxes only because we felt it useful to lighten the normally dark back for better observation. In pilot studies, stiff paper used for filing folders had been glued and taped to the back panel of each box, but, even so, the animals were able to remove and shred them when tested after water immersion. The paper-shredding behavior is reminiscent of the piling of wood shavings seen when rats have access to such shavings and often described as representing nest-building behavior (Grota & Ader, 1969; Zarrow, Denenberg, & Sachs, 1972). Whether the paper-shredding behavior represents attempts at nest building or not remains to be determined. The reduction of paper shredding by naloxone would suggest the involvement of opiate receptors in the mediation of the response, although the mechanisms of naloxone's effects on the inhibition of hormonally induced behaviors is not clearly established. Paper shredding is not likely to be an attempt to build a nest for warmth even though the water immersion may have lowered body temperature, since naloxone which itself lowers body temperature in rats (Goldstein & Lowery, 1976) blocked the response.

The paper shredding observed after water immersion or after the central administration of the ACTH fragment could be due to the release of prolactin, a hormone that has been related to nest-building behavior for both male and female rats (Zarrow et al., 1972). If this were the case, the decrease in this behavior after prior treatment with naloxone would be anticipated since naloxone reduces the prolactin released by either restraint or by heat (Van Vugt, Bruni, & Meites, 1978), although spontaneous release is only reduced by low doses of naloxone, not those exceeding 1 mg/kg (Shear, Frederickson, Dininger, & Jackson, 1977).

Because the number of fecal boli produced during testing could be related to the stress of the experience and the antagonism of opiate receptors by naloxone, they were counted in each condition of both experiments. In the first experiment, naloxone-treated animals produced more boli than their nontreated counterparts. This was not found in the second experiment, however. It is possible that the icv administration of ACTH in this second experiment counteracted the naloxone-induced effects.

The amount of grooming exhibited after water immersion is higher than usually found after transport, handling, and observation in a novel environment as found in other experiments (Colbern et al., 1978; Jolles et al., 1979a), and higher than in animals observed under our DRY testing conditions. Both Jolles et al. (1979a) and Colbern et al. (1978) found that novelty-induced excessive grooming habituated with repeated testing, in contrast to ACTH-induced grooming which does not, provided that the interval between tests is at least 12–18 hr (Jolles et al., 1978).

Water immersion seems to provide a relatively powerful environmental manipulation to induce excessive grooming that should be further investigated.

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