

REDUCED BEHAVIORAL EFFECTIVENESS OF ACTH_{1–24} AFTER A SECOND ADMINISTRATION: INTERACTION WITH OPIATES

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SUMMARY

Repeated intraventricular administration of ACTH results in reduction of subsequent ACTH-induced grooming behavior. A previous injection of β -endorphin, (D-Phe⁷)ACTH_{4–10}, or morphine, also reduces the behavioral response to a subsequent injection of ACTH. The reduction in grooming as a result of repeated administration is not mediated by corticosteroid feedback and can be eliminated by pretreatment with the opiate antagonist naloxone.

In rats, intracranial administration of ACTH and related peptides induces a stretching and yawning syndrome (SYS) [4], which is preceded by a display of excessive grooming [5,10].

We have shown that this induction of grooming is dissociated from endocrine activities of ACTH since the response can be elicited by ACTH_{1–24} in rats which are either adrenalectomized, or hypophysectomized or castrated. Furthermore, excessive grooming was observed in intact subjects using small N-terminal fragments of ACTH (ACTH_{4–7}, (D-Phe⁷)ACTH_{4–10}) devoid of corticotropic effects [5,15] and the response could be elicited by injection of ACTH into the substantia nigra [14].

Peptide-induced excessive grooming can be suppressed by peripheral administration of specific opiate antagonists (naloxone, naltrexone; 6). Low doses of morphine also induce excessive grooming [1,6] and it was found recently [7] that LPH fragments are very potent in this respect. Fragments of LPH have opiate-like effects and affinity for opiate receptors [8]. N-terminal peptides of ACTH have also measurable, although much lower, affinity for rat brain receptors in vitro [12]. In view of these similarities to opioids it was decided to search for the possible development of tolerance to a behavioral effect of

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ACTH₁₋₂₄. When the animals were treated daily with 1 $\mu\text{g}/\mu\text{l}$ for 10 days, no reduction of effectiveness of the intraventricular injection of ACTH₁₋₂₄ was observed. Subsequent experiments, however, revealed a remarkable suppression of the excessive grooming response when a second injection of ACTH₁₋₂₄ was given 3–5 h after the first injection.

The methodology has been described previously [2,5–7]. Briefly, male rats (170 g) of an inbred Wistar strain had a plastic cannula implanted into the foramen intraventriculare one week prior to the experimental session. Immediately after intracerebroventricular (i.c.v.) injection of peptide or saline (3 μl) the rats were placed individually into glass boxes (24 \times 12.5 \times 14 cm) in a low-noise room, and 15 min later the behavioral analysis began. The behavioral procedure consisted of a sampling technique, the validity of which has been established previously [5]. During a 50 min period the observer determined every 15th sec whether the rat displayed an element of the maintenance repertoire: vibrating, washing, grooming, scratching, licking paws, licking tail. Since the predominant element recorded appeared to be grooming, we refer to grooming only.

The behavioral response is presented as the percentage of the maximum possible grooming score (200). As reported previously, a single injection of $\beta\text{-LPH}_{61-91}$ (β -endorphin) in low doses such as those used here, not only elicited grooming but also excitation in some rats, typified by quick movements of body and head, jumping, gnawing and bodyshakes [7].

It was also determined whether the animal displayed an element of the SYS: the occurrence of stretching (S) and yawning (Y) was recorded and the combined number of S + Y was taken as a measure of SYS activity [2]. The whole procedure was repeated at a fixed time interval after the first i.c.v. injection (injection, placement in cage and analysis).

In the first series of experiments we varied the time between the first and second injection of ACTH₁₋₂₄. The results are summarized in Fig. 1. ACTH₁₋₂₄-induced excessive grooming is reduced to 20–30% by a similar, preceding injection of ACTH₁₋₂₄. This equals the amount displayed by saline/saline and ACTH/saline treated animals. The inhibition is manifest from 1–8 h after the first injection. In contrast, pretreatment with saline does not alter the grooming response to the peptide (Fig. 1). At 10.5 h after the first injection, a second injection of ACTH induced considerable grooming again (55%). This score, however, is still significantly lower than normal ($P < 0.05$, Student-*t*, two-tailed). If the second injection of ACTH was delayed for 18 h, maximal excessive grooming (80–90%) was found again.

In all experiments in which we found the reduction in grooming, no reduction in SYS was observed. This observation is in line with previous experiments in which we showed that the effects of ACTH on grooming and SYS can be dissociated by lesioning of limbic-mid-brain structures [2,7]; it suggests that ACTH-induced grooming and ACTH-induced SYS are governed by different brain mechanisms.

In all further experiments we used a 4 h interval between the first and the

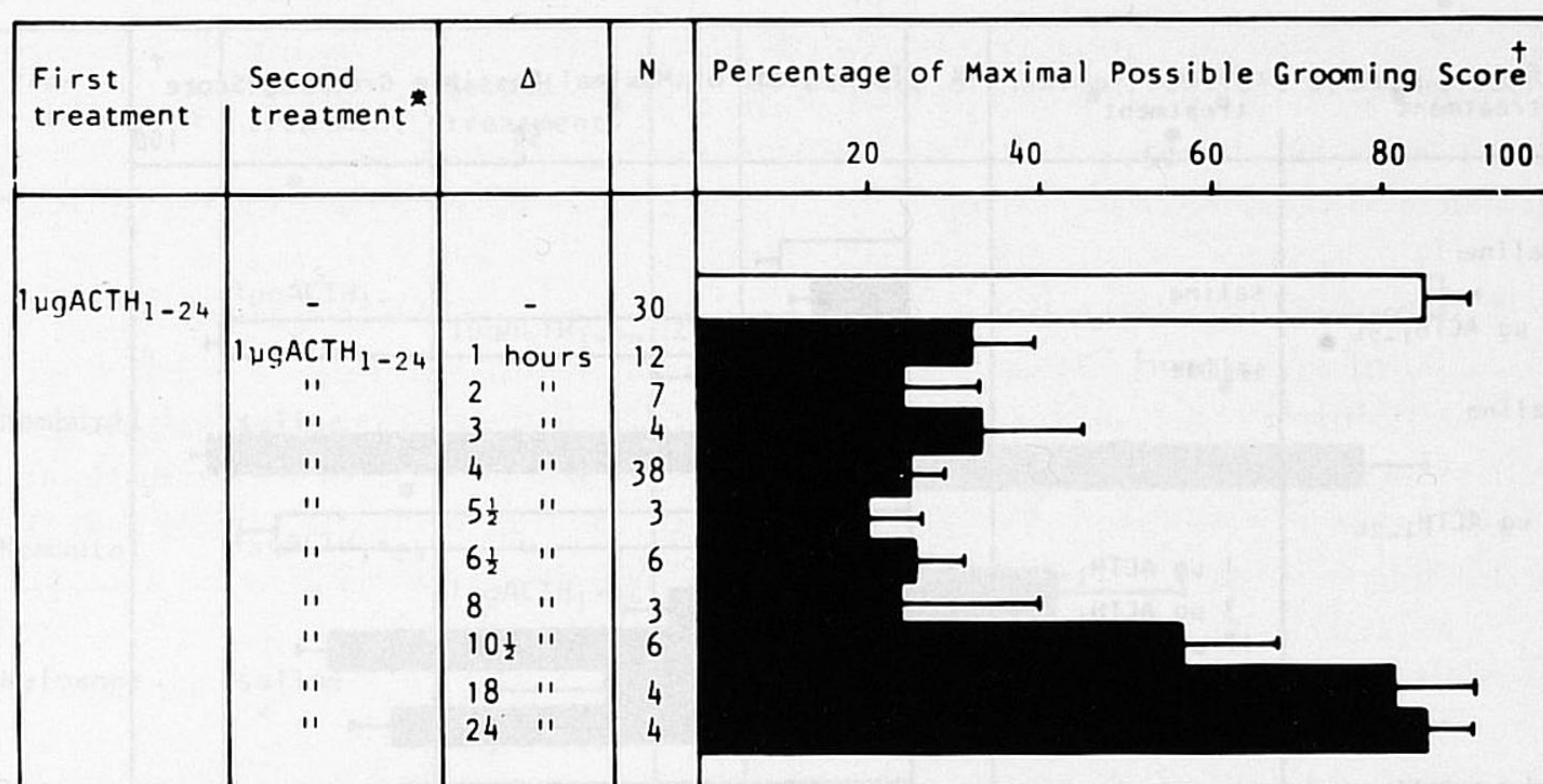


Fig. 1. ACTH₁₋₂₄-induced excessive grooming in the rat: effect of repeated administration. *First treatment (indicated by the open bar) consists of an intracerebroventricular (i.c.v.) injection of 3 μ l. Second treatment (indicated by the dark bar) is an i.c.v. injection of 3 μ l at varying intervals after first treatment. Δ = Time interval between first and second injection of ACTH₁₋₂₄; N = Number of animals; [†] Mean \pm S.E.M.

second injection. We found that the inhibitory effect of the first ACTH injection can be overcome by increasing the dose in the second injection (Fig. 2). Thus, 10 μ g ACTH₁₋₂₄ nearly completely restores the grooming score to its normal value (75%). Suppression of ACTH-induced grooming could also be obtained by using β -endorphin or (D-Phe⁷)ACTH₄₋₁₀ in the first injection. Likewise, ACTH₁₋₂₄ can inhibit β -LPH₆₁₋₉₁-induced grooming and also under these circumstances of a dose-dependent counteraction of this inhibition could be demonstrated (Fig. 2). If 0.1 μ g morphine-HCl was injected, a subsequent injection of 1 μ g ACTH₁₋₂₄ after 4 h induced a normal amount of grooming. However, 0.5 μ g morphine-HCl inhibited the effect of the second ACTH₁₋₂₄ injection by 40% (data not shown).

It is unlikely that the present results can be explained in terms of 'habituation to the experimental situation', as saline/ACTH treated rats display normal grooming (Fig. 2). Besides, ACTH-induced grooming is not dependent on novelty and handling stress (J. Jolles, J. Rompa-Barendregt and W.H. Gispen, in preparation; D. Colbern, W.H. Gispen and R.I. Isaacson, in preparation). The observation suppression is not related to the actual display of excessive grooming 4 h previously: we tested animals that at the time of the first ACTH-injection were under anaesthesia (sodium ethyl (1-methylbutyl) barbiturate, Nembutal[®] Abbott, Amsterdam; 30 mg/kg i.p.; injection 15 min before i.c.v. injection of ACTH₁₋₂₄). The animals were under complete anaesthesia during the first 60 min after injection but after 2 h no behavioral after-effects of the anaesthesia were observed. The results showed that also in such animals the grooming activity after the second injection is reduced to control levels (Fig. 3).

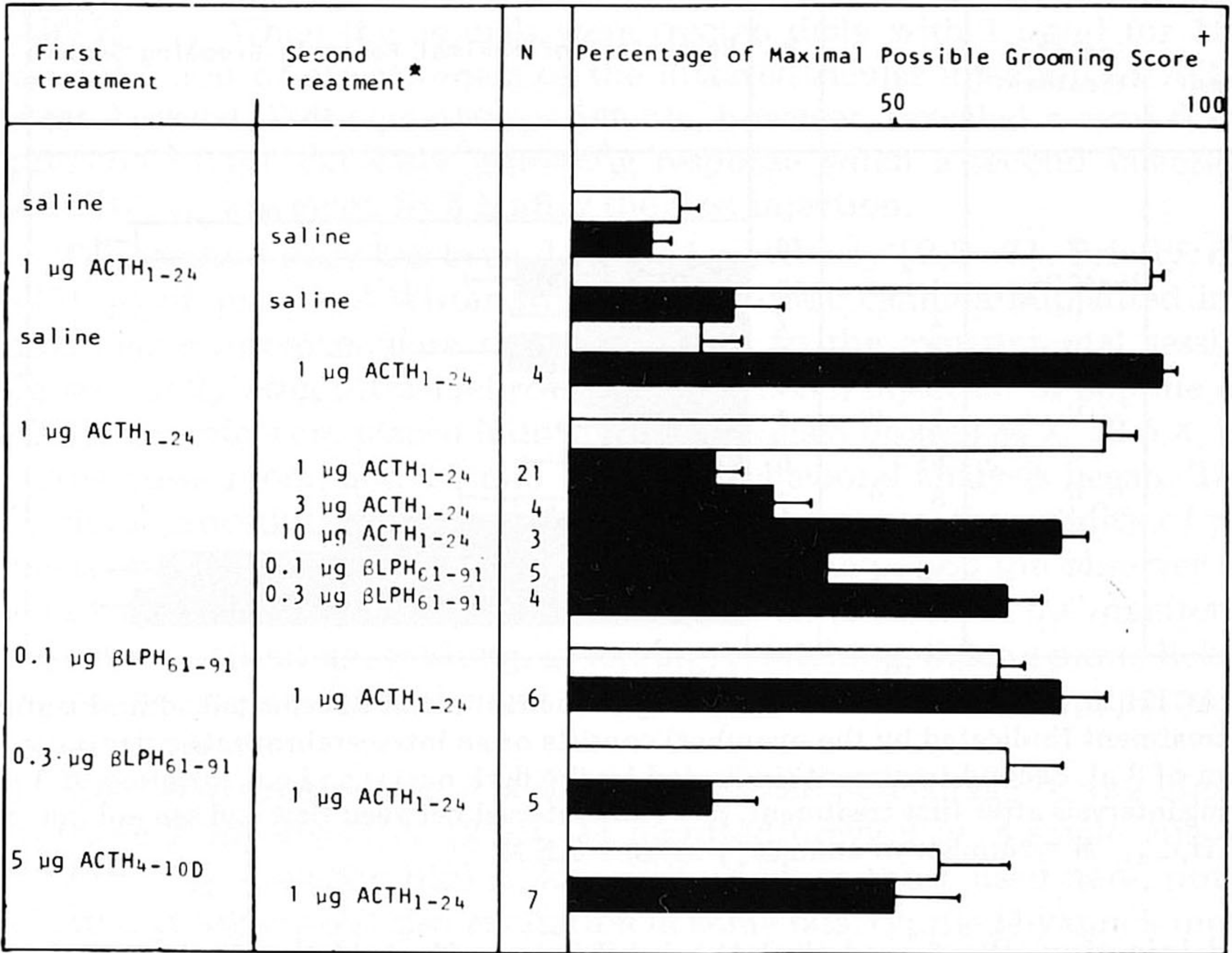


Fig. 2. Interaction of peptides and its effect on excessive grooming. *First treatment (indicated by the open bar) consists of one i.c.v. injection of 13 µl. Second treatment (indicated by the dark bar) is an i.c.v. injection of 3 µl 4 h after first treatment. N = Number of animals; † Mean ± S.E.M.

Thus it seems that the observed phenomenon is independent of the actual display behavior during the first observation period.

Clearly, a component sensitive to opiates and opioids is involved since both β -endorphin and morphine can induce the inhibition. This was further explored by injecting the specific opiate antagonist naloxone (1 mg/kg s.c.) 5 min prior to the first injection of ACTH₁₋₂₄, followed 4 h later by a second injection of ACTH₁₋₂₄. As Fig. 3 shows, naloxone not only inhibits grooming in the first treatment as expected [6,7] but also prevents the inhibitory effect of the first ACTH injection of grooming. It may be that naloxone does not allow ACTH₁₋₂₄ to occupy the relevant site of action (receptor). Yet, it can not be excluded that the neuronal substrate involved in peptide-induced grooming in fact consists of a pathway with separate ACTH₁₋₂₄ and opioid sensitive components [14,15]. To assess whether or not the inhibitory effect is related to the steroid feedback exerted by the activated pituitary-adrenal system, we repeated part of the experiments using adrenalectomized rats (bilateral surgery was performed 36 h prior to the test). As can be seen in Fig. 3 adrenalectomized animals have a normal grooming score after the first injection and show a similar effect as intact animals. This finding indicates that feedback by corti-

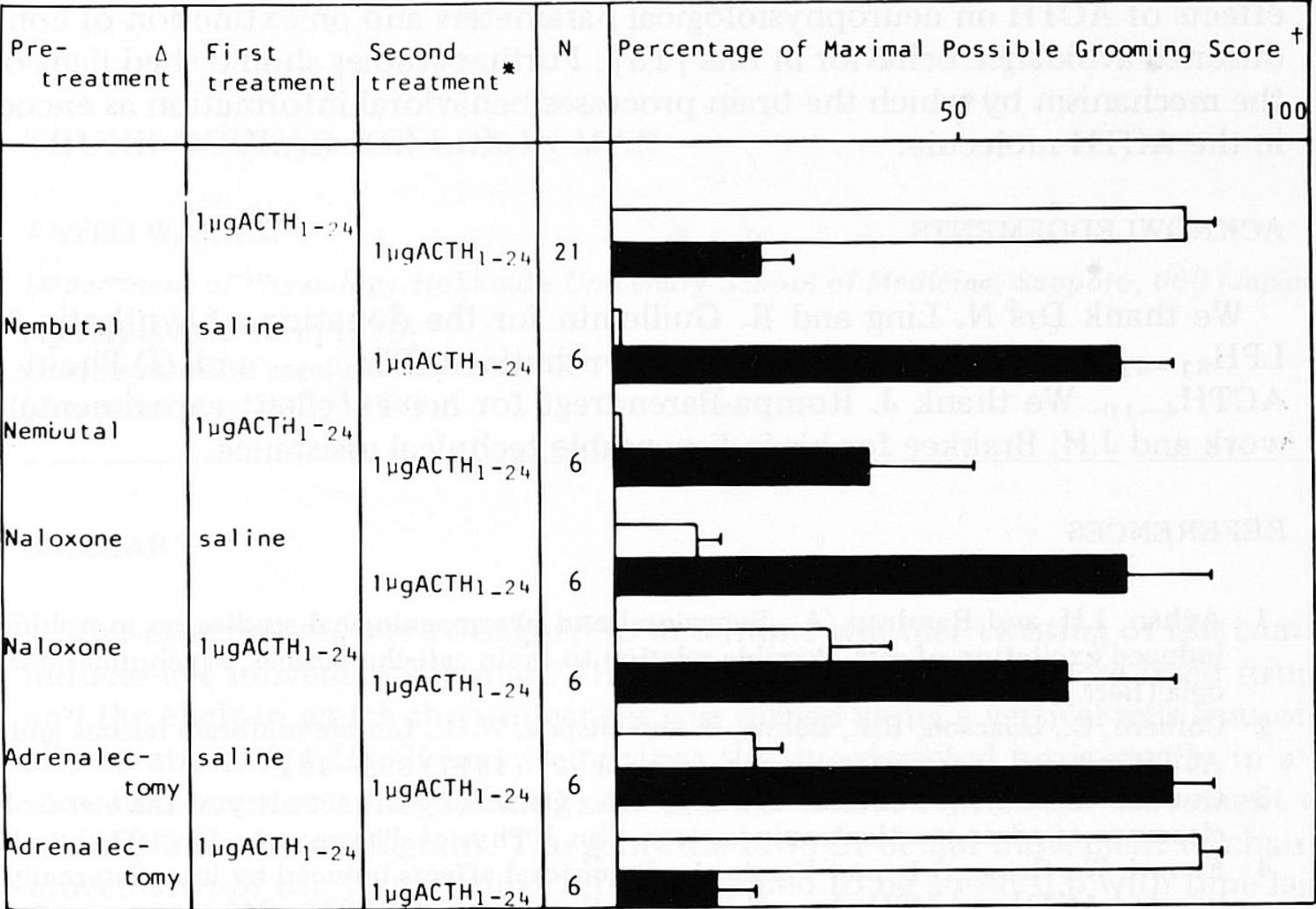


Fig. 3. Effect of different kinds of pretreatment on reduction in ACTH₁₋₂₄-induced excessive grooming. Δ Nembutal pretreatment (30 mg/kg i.p.) was 15 min before first treatment. Naloxone pretreatment (1 mg/kg s.c.) was 5 min before first treatment. Adrenalectomy pretreatment was 36 h before first treatment. *First treatment (indicated by the open bar) consists of an i.c.v. injection of 3 µl. Second treatment (indicated by the dark bar) consists of an i.c.v. injection of 3 µl 4 h after the first treatment. N = Number of animals; †Mean ± S.E.M.

costeroids is unlikely to be involved in the expression of the inhibitory effect. The literature of the biological half-life of ACTH points to a value that is in the order of minutes rather than of hours [3,11]. Yet, even after a 10.5 h interval a significant lower grooming response was obtained. By increasing the dose of ACTH in the second injection after a 4 h interval, the behavioral response reached normal levels again. For these reasons it seems unlikely that ACTH still present from the first injection could be responsible for the observed inhibition. We speculate that the observed reduction of effectiveness of the second injection relates to cellular responses to ACTH, resulting for instance in receptor (de)sensitisation, acute tolerance [9], inhibition or excitation of neuronal pathways. However, at present the information on the substrate involved is insufficient and does not allow further interpretation of our data. It remains to be seen to what extent our observations bear on the known longer term

effects of ACTH on neurophysiological parameters and on extinction of conditioned avoidance behavior in rats [13]. Further studies should shed light on the mechanism by which the brain processes behavioral information as encoded in the ACTH molecule.

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