opioid peptides detected in CSF and the pain relief resulting from stimulation of the periaqueductal gray matter.

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17. All procedures, risks, and benefits pertaining to the ventricular CSF collection during the electrode implantation surgery were explained to all patients participating in this study, and each of them signed a written informed-consent form.
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Intracerebral Adrenocorticotropic Hormone Mediates Novelty-Induced Grooming in the Rat

Abstract. *Intact male rats exhibited more grooming in unfamiliar testing chambers than in their home cages. Hypophysectomized rats showed a much reduced increase in grooming in these testing chambers. Intraventricular injections of antiserum to adrenocorticotropic hormone to intact rats decreased the grooming usually observed in the novel situation, whereas a similar injection of control serum did not produce this effect. Peripheral injections of the antiserum did not affect grooming. Since intraventricularly injected adrenocorticotropic hormone induces excessive grooming, these results suggest that the increased grooming observed in the novel environment may be at least partly due to the release of this hormone directly into the cerebral ventricular system.*

Naturally occurring self-grooming in animals has been characterized in a number of different ways by different investigators. Regardless of how it is interpreted, it frequently occurs as an afterreaction to goal-directed actions (*1*), stressful stimulation (*2*), conflict situations (*3*), and fear-induced flight behavior (*4*).

Increased grooming has been observed in the rat in response to local heating of limbic system structures, especially the hypothalamus and septum (*5*), and as an aftereffect of electrical stimulation of the hypothalamus or brainstem (*6*). Low doses of amphetamine (*7*) or morphine (*8*) also induce grooming in rats. Excessive grooming is observed in mice when they are terminating stereotyped responses induced by high doses of amphetamine, that is, 3 to 4 hours after administration (*9*). Whereas peripheral administration of adrenocorticotropic hormone (ACTH) does not induce grooming, intraventricular injection of ACTH elicits excessive grooming (*10–12*). This grooming is not directed toward any particular part of the body, but changes from one portion of the body to another and is complete and effective. It does not appear to be stereotyped, but is an excessively prolonged form of the natural grooming.

We recently observed that when rats were transferred to a novel observation chamber, there was a large increase in the time spent grooming relative to that observed in the home cage, even when similar handling was involved (*12*). To test the hypothesis that the increased grooming observed in the novel testing situation is mediated by ACTH, we examined grooming induced by a novel environment in intact and hypophysectomized rats and in intact rats injected intraventricularly with antiserum to ACTH.

Male Wistar rats (120 to 150 g) were supplied by Charles River (Wilmington, Mass.). Hypophysectomy was performed at the Charles River laboratories on 39-day-old (150 g) rats which were shipped the following day. Rats were housed singly in wire-mesh cages. They were given free access to Purina Lab Chow and water and maintained on a cycle of 12 hours of light and 12 hours of darkness (lights on at 8 a.m.). Ambient temperature under all experimental conditions was 22°C.

The rats were carried 20 m in their home cages to the experimental room and placed in a cage rack similar to the one used in the home room. Transfer to novel observation boxes made of glass and Plexiglas (30 by 30 by 20 cm) occurred in the experimental room. Each box was illuminated by a 7.5-W soft white bulb. The boxes were visually isolated from each other. A white-noise generator provided a continuous masking noise of approximately 65 dB. The rats were always observed in the experimental room with the observer separated from the animals by a one-way mirror. Observation commenced 15 minutes after transport to the experimental room, and continued for a total period of 50 minutes each day at the same time of day (12 noon to 2 p.m.). Whether rats were grooming or not was determined every 15 seconds as described (*11, 12*). On days on which more than one treatment was involved, the observer was unaware of the treatment to particular animals. For statistical analyses we used the Mann-Whitney U test, although in all cases comparable levels of significance were obtained from Student's t-test.

In the first experiment, intact and hypophysectomized rats were scored for grooming in their home cages on days 1 to 3. The rats were then observed in the novel boxes on days 4 to 7, and then again in their home cages on days 8 and 9. Intact animals exhibited a substantial enhancement in their grooming scores on transfer to the novel box (Fig. 1). Grooming in the novel box on day 4 was significantly different from that in the home cage on day 3 ($P < .002$). Average grooming scores for all days in the novel box were higher than those for all days in the home cage ($P < .002$). Over successive days in the novel box this enhanced level of grooming steadily declined, but remained significantly above levels of grooming exhibited in the home cage.

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Grooming returned to initial home cage levels when rats were observed in their home cages on days 8 and 9. These results are essentially identical to those observed previously (*12*), except that scores for grooming in the home cage were slightly lower in that study, possibly because no transportation to the experimental room was involved. The progressive decline in grooming scores recorded during the first 3 days in the home cage was observed previously (*12*) and is probably caused by the rats' adapting to the effects of transport to the novel environment in the experimental room.

The experiment was performed approximately 1 week after operation on 16 hypophysectomized rats. The grooming scores of hypophysectomized rats increased slightly when they were tested in the novel box (Fig. 1). The grooming scores on day 4 in the novel box were not significantly different from those on day 3 in the home cage, but the average grooming score in the novel box (days 4 to 7) was significantly higher ($P < .002$) than that in the home cage (days 1 to 3). The grooming scores of hypophysectomized rats were significantly lower than those of intact rats on all days in the novel box ($P < .05$), but not when observed in their home cages. These results suggest that a pituitary factor is at least partly responsible for the novelty-induced grooming.

In a second series of experiments, intact rats were cannulated in the interventricular foramen of Monro with a plastic cannula as described (*11, 12*). Three days after implantation (day 1), 3 μl of saline was administered through the cannula before the rats were placed in the novel box. The grooming scores (Fig. 2) were similar to those exhibited by control animals in the first experiment. The administration of 3 μl of antiserum to ACTH (*13*) before testing on day 2 depressed the grooming score. The scores after administration of the antiserum were not significantly different from those obtained in the home cage in the previous experiment, but were significantly below those of intact control animals ($P < .01$) and their own scores on the previous day in the novel box ($P < .05$). On days 3 and 4, saline was administered intraventricularly to these animals before they were tested. The grooming scores were significantly lower than control on day 3 ($P < .02$) but not on day 4. Apparently the effects of the antiserum persisted for at least 1 day after administration.

After 2 days of rest, the antiserum-injected rats ($N = 10$) were tested again (day 7). In these tests five of the rats

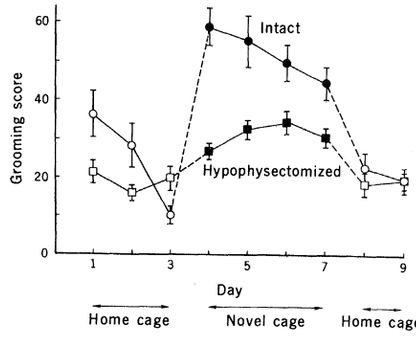

Fig. 1. Grooming by intact and hypophysectomized rats. Grooming was scored in the home cage but in a novel room on days 1 to 3 and days 8 to 9, and in the novel box on days 4 to 7. Symbols: ○, ●, intact rats ($N = 8$); □, ■, hypophysectomized rats ($N = 16$). Open symbols, grooming scored in the home cage; closed symbols, grooming scored in the novel box.

were injected with antiserum to ACTH and the other five received control serum. All were tested in the novel testing chamber. On the next day (day 8) the treatments were interchanged. The effect of the antiserum on day 7 was not significantly different from control ($P = .075$), but the difference scores for days 7 and 8 for each subject showed that ACTH-antiserum treatment resulted in lower grooming scores than did treatment with control serum [$P < .05$; (*14*)].

To determine whether the antiserum might have acted peripherally, we injected (subcutaneously) another group of intact rats with 100 μl of tenfold diluted antiserum to ACTH or control serum. Both groups of rats exhibited similar grooming scores in the novel environment (ACTH antiserum, 38 ± 5; control serum, 40 ± 5) indicating that the effect of the antiserum on grooming was not directly exerted peripherally. We cannot exclude the possibility that the intraventricular antiserum to ACTH acted centrally to alter a peripheral factor that

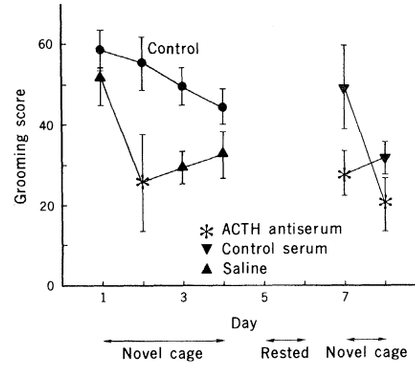

Fig. 2. Grooming by intact rats injected intraventricularly with antiserum to ACTH. Symbols: ●, no antiserum; ▲, saline; *, antiserum to ACTH; and ▼, control serum.

influenced the grooming. However, it is unlikely that such a peripheral factor was ACTH or corticosterone, since peripheral administration of ACTH or corticosteroids does not induce grooming (*11*) and corticosteroids did not alter grooming in the novel cage (unpublished observations).

Our experiments show that the increased grooming observed when rats are transferred to a novel testing chamber can be attenuated by prior hypophysectomy or by intraventricular application of antiserum to ACTH. Previous observations in this laboratory and by others have shown that ACTH administered intraventricularly induces excessive grooming (*10–12*), even in hypophysectomized rats (*11*). Thus, it seems likely that novelty-induced grooming is induced, at least in part, by the release of ACTH into the brain. A similar suggestion has been made for the pigeon in which stress and intraventricular ACTH produce similar behavioral responses (*15*).

Adrenocorticotropic hormone that can be measured both by bioassay and radioimmunoassay has been demonstrated in cerebrospinal fluid (*16*) and throughout the brain (*17, 18*). It has been suggested that this cerebral ACTH emanates from the adenohypophysis and is carried up the pituitary stalk in portal blood vessels (*16, 19*). This would be consistent with the finding of a relatively high concentration of ACTH in the median eminence and, to a lesser extent, throughout the hypothalamus (*17, 18*). Also, it was recently shown that a radioactive ACTH analog injected into the anterior pituitary later appeared in the brain, especially in the hypothalamus. Infundibular section prevented this appearance (*20*). However, the amount of ACTH in the brain is not altered by hypophysectomy, even long after the surgery (*18*). And, although this has been attributed to incomplete hypophysectomy (*21*), hypothalamic neurons with axons descending into periventricular brainstem regions have recently been shown to contain ACTH by immunohistofluorescence (*22*).

The function of the intracerebral ACTH is not known, nor is it known whether the cerebral content of ACTH is altered by stress. It is not clear whether the ACTH in the cerebrospinal fluid arises only from the pituitary or also from the cerebral ACTH-containing neurons. It is possible that the intracerebral ACTH not depleted by hypophysectomy accounts for the small increase in grooming we observed in hypophysectomized rats after transfer to a novel box. Alter-

natively, this response could have been due to residual adenohypophysial tissue not completely removed by the hypophysectomy or to other unknown factors (*21*).

We believe that ACTH acting within the central nervous system mediates the increased grooming observed in the novel environment. The presence of antiserum to ACTH in the ventricular fluid attenuates this response. Our results provide good evidence that the secretion of a peptide hormone into the brain can mediate a complex behavioral response. This response is observed physiologically under conditions known to cause release of ACTH. These findings support those of others suggesting that the cerebrospinal fluid functions to transport intracerebrally secreted hormones to their sites of physiological and behavioral action (*23*).

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Accumulation of a Tetrahydroisoquinoline in Phenylketonuria

Abstract. *3′,4′-Deoxynorlaudanosolinecarboxylic acid (DNLCA), a tetrahydroisoquinoline derived from dopamine and phenylpyruvic acid, has been detected by computerized mass fragmentography in urine of phenylketonuric children and in urine and brain of rats with experimentally induced hyperphenylalaninemia. Levels of DNLCA in brain of treated animals were more than tenfold higher than controls, and the excess tetrahydroisoquinoline appeared to accumulate in the cerebellum and cortex. DNLCA is a noncompetitive inhibitor of dopamine β-hydroxylase (inhibition constant, K_{i_s} = 0.42 mM) and is taken up by the brain.*

Recently we demonstrated the presence of a tetrahydroisoquinoline alkaloid, 3′-*O*-methylnorlaudanosolinecarboxylic acid (MNLCA) (*1*) in parkinsonian patients treated with L-dopa (*2*). We now report on the occurrence of a related catecholamine derivative in phenylketonuric children and in rats with an experimentally induced hyperphenylalaninemia.

3′,4′-Deoxynorlaudanosolinecarboxylic acid (DNLCA) was prepared by Pictet-Spengler condensation of dopamine and phenylpyruvic acid, a reaction that can occur in low yields spontaneously under physiological conditions (*1, 2*). DNLCA was characterized as its hydrochloride: melting point, 239° to 241°C (decomposes); ultraviolet wavelength of maximum absorption (λ_{max}; 1 *N* HCl) 285 nm (log molar absorptivity 3.55); nuclear magnetic resonance spectrum [$(CD_3)_2SO$-CF_3COOH] chemical shift, 6.6 (singlet H-5), 7.1 to 7.6 (multiplet remaining aromatic H atoms), 2.6 to 4.0 (multiplet αH, H-3, H-4); mass spectra *m/e* 299 (M^+ 1.7 percent), 281 (11 percent), 255 (39 percent), 254 (91 percent), 253 (30 percent), 208 (100 percent), 164 (67 percent), 162 (65 percent), 91 (50 percent). Using a computerized gas-liquid chromatography–mass spectrometric method (GC-MS) (*2–4*), we have found DNLCA in the urine of four phenylketonuric children ranging in age from 5 to 15 years (45, 100, 69, and 60 ng/ml, respectively). Although all of the children were on a normal diet, the average of their DNLCA concentrations was twice that of three age-matched controls (35, 34, and 34 ng/ml). Urinary metabolite patterns were obtained by paired-ion, reversed phase, high-pressure liquid chromatography (*5*). A direct correlation was observed between the concentration of urinary phenylpyruvate and DNLCA.

Additional data were obtained by injecting rats with *p*-chlorophenylalanine

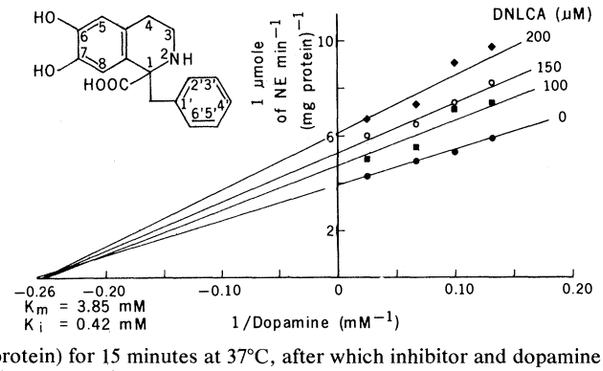

Fig. 1. Incubation mixtures contained 200 μl of 1*M* potassium phosphate buffer (*p*H 6.2), 50 μl of 20 m*M* pargyline, 50 μl of 0.2*M* ascorbate, 200 μl of 0.25 m*M* *p* - chloromercuribenzoate, 50 μl of 0.2*M* sodium fumarate, and 80 μl of a catalase solution (1 mg protein/ml, 27,000 units) which was added last. This solution was incubated with 40 μl of enzyme (bovine adrenal dopamine β-hydroxylase, 5 to 10 units per milligram of protein) for 15 minutes at 37°C, after which inhibitor and dopamine were added to give the final concentrations as indicated. After 40 minutes the reaction was terminated by addition of 200 μl of 25 percent aqueous trichloroacetic acid. Samples were then taken to dryness, dissolved in a mixture of methanol and 1 percent aqueous acetic acid (3:7 by volume), and subjected to paired-ion, reversed phase, high-pressure liquid chromatography as described (*5*). Norepinephrine (NE) concentrations were estimated from peak height analysis with an ultraviolet spectrophotometric detector (*5*). Kinetic constants were obtained by the method of least squares.

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