epinephrine formation. It remains to be seen if the pro-opiomelanocortin fragments found in the human adrenal medulla do have a physiological role and if this role involves controlling the formation (*26*) or release of catecholamines or steroids from the adrenal gland.

Note added in proof: After submitting this report, we learned of a study in which ACTH-, β-endorphin-, and γ-MSH-like immunoreactive material were found in human adrenal glands.

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Normalizing Effect of an Adrenocorticotropic Hormone (4–9) Analog ORG 2766 on Disturbed Social Behavior in Rats

Abstract. *Short-term isolation increased the frequency of social interactions in rats tested in pairs, while pairs of rats placed in an unfamiliar test cage and subjected to a high level of illumination spent less time in active social contact. These changes in social behavior elicited by environmental manipulations were counteracted by treatment with the adrenocorticotropic hormone (4–9) analog ORG 2766. The peptide's normalizing effect may be mediated by endogenous opioid systems.*

It is usually in a social setting that people take psychopharmacological agents intended to alter behavior and feelings. The interactions between drug effects and social variables should thus be analyzed in detail (*1*). Social behavior can be affected by drugs, and social variables can influence the effects of medications. Furthermore, pathophysiological changes in social behavior and distressing symptoms associated with social interactions may indicate that medication is needed. However, any investigation in sociopharmacology is hampered by the fact that few animal models are available for social behaviors under standardized conditions. More information about changes in social behavior in response to environmental manipulation could improve our understanding of the pathophysiological aspects of social behavior.

Short-term isolation increases the frequency of social interactions in rats (*2*), while the time spent in active social contact decreases when rats are exposed to an unfamiliar test cage and a high level of illumination (*3*). We used these two test situations to analyze the influence of the adrenocorticotropic hormone (4–9) analog ORG 2766 on social behavior. Initially, this peptide was shown to be 1000 times more potent than ACTH-(4–10) in delaying extinction of pole jumping avoidance behavior (*4*). This increased potency could be generalized to other behaviors, including passive avoidance behavior and self-stimulating behavior elicited from the medial septal area (*5*). It was recently reported that the hippocampal morphological correlates of brain aging and the age-dependent behavioral impairment of rats were reduced by long-term treatment with ORG 2766 (*6*). As had been predicted from animal experiments, ORG 2766, like ACTH-(4–10), beneficially influenced motivational and attentional processes in clinical studies. In addition, ORG 2766 ameliorated the mood and increased the sociability of mentally retarded and elderly people (*7*).

In our first series of experiments (*8*), rats that had been housed alone for 7 days and group-housed rats were placed together in pairs and their social behavior was observed for 5 minutes. Most of the social interactions of the rats appeared to be amicable explorative behavior—biting, kicking, and fighting rarely occurred. The frequency of social interactions was significantly greater in isolated rats than in their group-housed partners (Fig. 1A). This was probably due to a specific effect on gregariousness, since ambulation was no different for the two rats, whether it was measured during or outside the social interaction test. Subcutaneous treatment of group-housed rats with ORG 2766 (0.1 μg/kg) 1 hour before the test did not significantly change the frequency of social interactions. However, identical treatment of isolated rats decreased the frequency of social interactions to the level seen in group-housed rats (Fig. 1A). The frequency of social interactions among the test partners of rats treated with ORG 2766 did not differ from that of control rats. Intraperitoneal injections of ORG 2766 (0.1, 5, or 50 μg/kg) 5 minutes before the test had a similar effect, that is, the frequency of social interactions in isolated rats was decreased to the level measured in group-housed rats. The ORG 2766 did not appear to affect nonsocial activities.

In our second series of experiments (*9*), pairs of rats tested in an unfamiliar environment under intense light (group 1) spent less time in active social contact than did rats tested in a familiar environment under low lighting (group 2) (Fig. 1B). The more explorative social behaviors, such as exploration of the partner,

social grooming, approaching, and following were those most decreased in group 1. There was less ambulation (a nonsocial activity) in group 1 rats, too. Intraperitoneal injection of ORG 2766 (50 μg/kg) 5 minutes before the test did not significantly affect the time spent in active social contact in group 2 animals. In contrast, ORG 2766 significantly increased active social contact in group 1 (Fig. 1B). In fact, ORG 2766 abolished the decrease in active social contact usually observed under these conditions. The effect of ORG 2766 was specific for social behavior, since ambulation was not affected by peptide treatment under either set of conditions. Lower doses of ORG 2766 (0.5 and 5 μg/kg) slightly increased the time spent in active social contact under both sets of conditions (*3, 10*).

These findings show that the social behavior of pairs of rats can be measured reliably when testing conditions are standardized. The results also show that relatively slight environmental modifications lead to dramatic changes in rat social behavior. Thus manipulating the environment before the test (short-term isolation) resulted in a > 50 percent increase in the frequency of social interactions, and manipulation during the test (unfamiliar surroundings and intense light) halved the times spent in active social contact. These findings may contribute to our understanding of the processes involved in pathophysiological changes in social behavior and may lead to animal models for disturbed social behavior.

ORG 2766 normalized the social behavior of rats after it had been changed by environmental manipulations. Both the increase in social interactions due to short-term isolation and the decrease due to unfamiliar surroundings and intense light were antagonized by ORG 2766. The peptide thus regulates social contact in rats. The attenuating effect of ORG 2766 on the increase in social interactions induced by short-term isolation is mimicked by antidepressant drugs particularly (nortriptyline, clomipramine, and mianserin) (*11*). The antagonizing influence of the peptide on the decrease in social contact due to unfamiliarity and intense illumination is similar to the effect of benzodiazepines (*3*). These effects of ORG 2766 may thus be related to the antidepressant action and positive effects on mood found in clinical studies with this peptide (*7*).

The following evidence prompted us to study the involvement of opioid systems in the action of ORG 2766 on social behavior: (i) endogenous opioid systems are implicated in social behavior (*12*); (ii) ACTH and related peptides have been reported to interfere with the action of morphine and with endogenous opioid systems (*13*); and (iii) the inhibiting but not the facilitating effect of ORG 2766 on passive avoidance behavior and electrical self-stimulation behavior elicited from the medial septum at threshold current intensities are mediated by a naltrexone-sensitive brain opioid receptor system (*14*). In experiment A rats that had been isolated for 7 days were tested with group-housed partners; the isolated rats were injected subcutaneously with naltrexone (450 μg/kg) or ORG 2766 (0.05 μg/kg) or both 1 hour before the social interaction test. In experiment B pairs of rats were injected with naltrexone (450 μg/kg, subcutaneously) or ORG 2766 (50 μg/kg, intraperitoneally) or both at various periods before testing under intense light in an unfamiliar test cage. ORG 2766 decreased the frequency of social interactions in the isolated rats and increased the time spent in active social contact in unfamiliar surroundings under intense light (Table 1). Naltrexone did not significantly affect social behavior, although active social contact was slightly increased in experiment B. In both experiments the influence of ORG 2766 was completely antagonized by treatment with naltrexone (Table 1), indicating that the effect of ORG 2766 on social behavior is mediated by a naltrexone-sensitive receptor system.

Thus the influence of ORG 2766 on

Table 1. Antagonizing effect of naltrexone (450 μg/kg) on the influence of ORG 2766 on the social behavior of rats. In experiment A the frequency of social interactions between a rat that had been isolated for 7 days and a saline-treated, group-housed rat was determined for 5-minute periods. In experiment B the time spent in active social contact by pairs of rats was measured for 5 minutes in an unfamiliar cage under intense light. Values are means ± standard errors. Numbers of observations are given in parentheses.

Treatment 1 hour before test	Treatment 1 hour (A) or 5 minutes (B) before test	Experiment A, social interactions (number in 5 minutes)	Experiment B, active social contact (number in 5 minutes)
Saline	Saline	56 ± 4 (6)	78 ± 3 (7)
Naltrexone	Saline	61 ± 4 (7)	89 ± 5 (9)
Saline	ORG 2766 [0.05 μg/kg (A) or 50 μg/kg (B)]	45 ± 3 (7)*	92 ± 6 (7)*
Naltrexone	ORG 2766	65 ± 5 (7)†	67 ± 5 (7)†
Analysis of variance		$F(3, 23) = 4.36$, $P < 0.05$	$F(3, 26) = 5.17$, $P < 0.01$

*Significantly different from the corresponding value for control rats ($P < 0.05$, Student's t-test). †Significantly different from the corresponding value for rats treated with saline and ORG 2766 ($P < 0.01$).

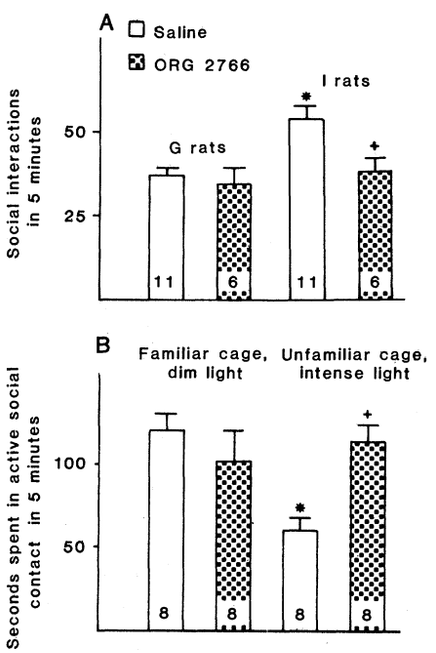

Fig. 1. Effects of ORG 2766 on changes in rat social behavior induced by environmental manipulations. (A) Frequency of social interactions between pairs of group-housed (G) and isolated (I) rats (*8*). The animals were injected subcutaneously 60 minutes before the test with saline (0.5 ml) or ORG 2766 (0.1 μg/kg). Data are given for the control rats (G and I rats injected with saline) and for G and I rats treated with ORG 2766. The social interactions of the partners of rats treated with ORG 2766 did not differ from those of control animals. (B) Time spent in active social contact by pairs of rats. Scores of both rats in a pair were combined (*9*). The animals were injected intraperitoneally with saline (0.5 ml) or ORG 2766 (50 μg/kg). Two identically treated rats were tested under two different test conditions (familiar cage, dim light and unfamiliar cage, intense light). Numbers in the bars show number of rats (A) and number of pairs (B) tested. Two-way analyses of variance revealed a highly significant interaction between treatment and test condition [(A) $F(1, 30) = 8.22$, $P < 0.01$; (B) $F(1, 28) = 13.52$, $P < 0.01$)]. (*) Significantly different from the value for G rats treated with saline (A) or for saline-treated rats tested in familiar surroundings under dim light ($P < 0.01$, Student's t-test). (+) Significantly different from the value for saline-treated rats tested under the same conditions ($P < 0.02$).

social behavior may be due to altered activity of brain endorphin systems, a possibility consistent with evidence that endogenous opioid systems are involved in social behavior (*12*). These results may help to explain the normalizing effect of ORG 2766 on disturbed social behavior in rats and its beneficial influence on sociability and mood in elderly people. It is possible that ORG 2766 may act to normalize disturbances in the endogenous opioid control of social behavior.

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8. Male Wistar rats weighing 180 to 200 g were housed individually (I rats) or in groups of five per cage (G rats) for 7 days before experimentation. Testing was performed by placing one I rat and one G rat together in a Perspex observation cage (70 cm long by 70 cm wide by 50 cm high) illuminated with red lights (low light condition) for 5 minutes. The behavior of the rats was analyzed later by individuals who did not know the group to which the rats belonged. The sum of the different social activities was taken as a measure of the total amount of social interactions of one animal. These activities included exploration of the partner, crawling over one another, mounting, social grooming, biting, approaching, following, and fighting. The animals were treated subcutaneously 60 minutes or intraperitoneally 5 minutes before the test as follows: both rats in a pair received saline (controls); the G rat received saline and the I rat ORG 2766; or the G rat received ORG 2766 and the I rat placebo. The primary structure of ORG 2766 is H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-OH.
9. Rats were housed in a dimly lit (20 to 40 lux) room during the 14 days before testing, first in groups of five per cage and then individually starting 6 days before the test. The animals were then assigned randomly to one of two test conditions. They were either placed singly in the observation cage for 5 minutes under dim red light (0.4 to 1 lux) on each of the 2 days preceding the social interaction test or they were placed in the observation cage while still in their home cage on the 2 days before experimentation and subjected to white light at 572 lux. On the test day these rats were injected intraperitoneally with saline or ORG 2766, and, after 5 minutes, two identically treated rats were placed in the observation cage. The time that the rats spent in active social contact was measured for 5 minutes (*3*).
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Isolation of Lamellar Bodies from Neonatal Mouse Epidermis by Selective Sequential Filtration

Abstract. *Isolation of epidermal lamellar bodies has presented a challenge because pressures required to homogenize keratinocytes can destroy these organelles and because the lamellar body readily releases its contents during prolonged isolation procedures. In an attempt to isolate lamellar bodies, sheets of intact stratum corneum and stratum granulosum were obtained from neonatal mice with highly purified staphylococcal epidermolytic toxin, disrupted, and passed through a series of filters. The final filtrate was rich in intact lamellar bodies and contained variable amounts of ribosomes and other vesicular structures. Availability of a highly purified lamellar body preparation from postnatal epidermis should help to clarify the role of this organelle in epidermal function. The technique of selective, sequential filtration represents a new approach to cell fractionation that may have wide applications in cell biology and biochemistry.*

Orthokeratinizing epithelia contain a distinctive ovoid organelle, the lamellar body (membane-coating granule, Odland body, keratinosome) in the upper spinous and granular layers (Fig. 1) (*1*). Coincident with cornification, these organelles secrete their distinctive disclike contents into the intercellular spaces (*2*). Because ultrastructural and cytochemical studies have shown that lipids (*3*), sugars (*4*), and hydrolytic enzymes (*5*) are present in these organelles, they have been considered the primary source of materials for skin barrier function (presumably through their lipids) (*3*, *6*) and for cohesion and desquamation (pre-

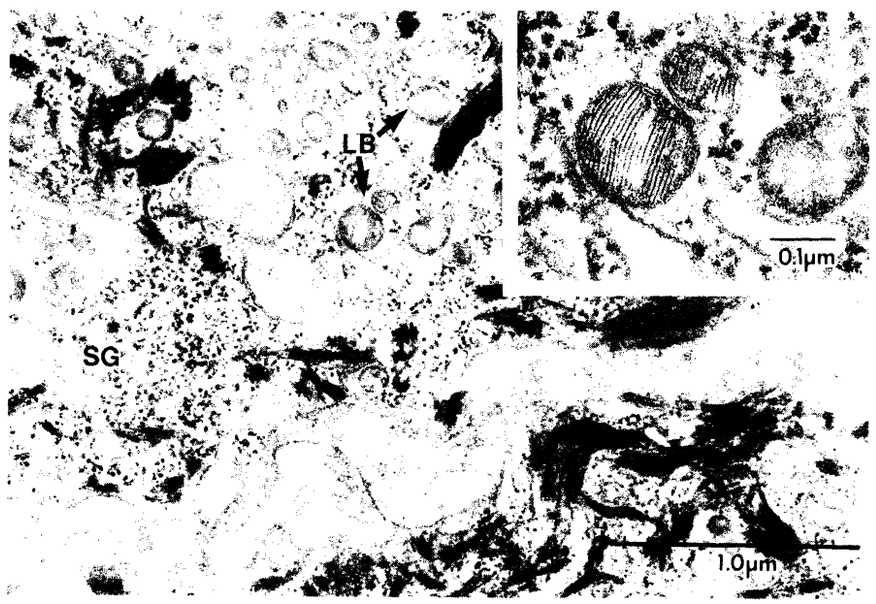

Fig. 1. Thin section through the stratum granulosum (*SG*) of neonatal mouse whole epidermis. The cytoplasm contains aggregates of intermediate filaments, keratohyalin granules, free ribosomes, occasional mitochondria, and abundant lamellar bodies (*LB*). The latter are ovoid, membrane-delineated organelles with a mean diameter of 0.10 to 0.20 μm. In cross sections they display stacks of parallel discs, but in grazing sections the contents look amorphous (inset). The granular cell is notably devoid of endocytic vesicles and contains only sparse rough and smooth endoplasmic reticulum.