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Intraventricular ACTH Reduces Social Interaction in Male Rats

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FILE, S. E. AND A. CLARKE. *Intraventricular ACTH reduces social interaction in male rats.* PHARMAC. BIOCHEM. BEHAV. 12(5) 711–715, 1980.—Intraventricular $ACTH_{1-24}$ (1.25 μg), $ACTH_{4-10}$ (1.6 μg) and $ACTH_{4-10}$:D-Phe (1.6 μg) resulted in significant reductions in the time that pairs of male rats spent in active social interaction. This decrease in social behaviour was not accompanied by a decrease in motor activity. The results are similar to those previously found with peripheral administration of ACTH and suggest that these behavioural effects of ACTH are centrally mediated. None of the ACTH fragments had a significant effect on the latency with which thirsty rats started drinking in an unfamiliar environment. The results are discussed in relation to a possible anxiogenic action of ACTH.

ACTH Social interaction Rats Anxiety

PERIPHERAL administration of adrenocorticotrophic hormone (ACTH) has been reported to reduce the time spent in active social interaction by pairs of male rats, whether the test arena is familiar or unfamiliar to the rats, or whether they are tested under high or low illuminance [9]. It is unlikely that these effects were due to the action of corticosteroids released by ACTH because: (a) the effects were found three mins after injection of ACTH (by which time there would be relatively little steroid release) but were no longer significant thirty mins after injection; (b) peripheral administration of corticosterone, the principal corticosteroid in the rat [2,3], did not produce the same effects as injected ACTH [10]; and (c) peripherally administered $ACTH_{4-10}$, which does not have steroid-releasing properties [22], had similar effects on social interaction to those found with $ACTH_{1-24}$ [5]. However, these arguments do not preclude a peripheral site of action of ACTH other than the adrenal cortex.

It was the purpose of this experiment, therefore, to examine the behavioural effects of centrally administered ACTH. If the reduction in social interaction observed with peripherally administered ACTH were due to a central action of the peptide, then it would be expected that very low doses given intracerebroventricularly (ICV) would also result in reduced social interaction. The doses of $ACTH_{1-24}$ and $ACTH_{4-10}$ that had significantly reduced social interaction when administered intraperitoneally were 50 and 40 μg/kg, respectively [5,9], i.e. approximately 10 and 8 μg/rat. The doses used in the present experiment were based on the results of pilot studies which indicated that about one tenth and one fifth of the peripheral doses of $ACTH_{1-24}$ and $ACTH_{4-10}$, respectively, would have behavioural effects when given centrally. Since the decrease in social interaction caused by peripheral administration of ACTH was not accompanied by a decrease in motor activity, and since treatment with anxiolytic drugs reversed the effects of ACTH on social interaction, it was suggested that ACTH might have an anxiogenic action [9]. A similar role for ACTH has also been suggested by Weiss *et al.* [22], who found that peripherally administered ACTH improved the poor avoidance performance shown by hypophysectomised rats. Because of these suggestions the effects of ICV ACTH were studied in two animal tests of anxiety, the social interaction test [7] and a drink-latency test [21], in which the latency for thirsty rats to start drinking in an unfamiliar environment was recorded.

METHOD

Animals and Surgery

Male hooded rats (Rattus norvegicus) from Olac Ltd. Bicester, weighing 100–120 g, were anaesthetised with sodium pentobarbitone (60 mg/kg, IP), and permanent indwelling cannulae were placed stereotaxically into the right lateral ventricle (2 mm posterior and 1.8 mm lateral to the bregma and 4 mm vertical to the dura). The cannulae were of surgical steel, 0.82 mm outside diameter, and were held in place with acrylic dental cement set onto four screws placed into the skull. Animals were allowed at least two weeks postoperative recovery time before the first experiment was begun, and were housed in groups of six in a room maintained at 25°C in a 11 hr light:13 hr dark cycle (lights on at 0700 hr). Food and water were freely available. At the time of testing, animals weighed 190–250 g.

Apparatus

Social interaction test. The test box was 60×60×36 cm with wooden walls and floor, above which a closed-circuit television camera was mounted at a height of 1.5 m. Rats in the box were observed on a television monitor situated in an

adjacent room. Two levels of illuminance were used: 338 and 22 scotopic lux, for the high and low light levels respectively. An automated measure of motor activity was derived from infra-red cells mounted in the walls of the box; a count being scored each time a beam was interrupted.

Drink-latency test. The test box was 28×28×26 cm with Perspex walls and a metal grid floor, into which a water spout 3 cm long protruded, 7 cm from the floor. The light level was 9.2 scotopic lux.

Drugs

Social interaction test. $ACTH_{1-24}$ (Synacthen, CIBA Ltd.) was injected in a volume of 5 μl, corresponding to a dose of 1.25 μg per rat. The control rats received injections of 5 μl of de-ionised water. $ACTH_{4-10}$ (Organon, 0163) and $ACTH_{4-10}$:D-Phe ($ACTH_{4-10}$ in which the L-phenylalanine at position 7 is substituted by D-phenylalanine, Organon, 0164) were dissolved in de-ionised water, and animals each received a dose of 1.6 μg in a volume of 2 μl. The control rats received injections of 2 μl of de-ionised water. All injections were intraventricular.

Drink-latency test. $ACTH_{1-24}$ was injected in volumes of 2 μl and 5 μl, corresponding to doses of 0.5 μg and 1.25 μg per animal. $ACTH_{4-10}$ and $ACTH_{4-10}$:D-Phe were administered in volumes of 2 μl, corresponding to doses of 1.6 μg per rat. Control animals received either 2 μl or 5 μl injections of de-ionised water.

Procedure

Social interaction test. Six days prior to testing, the animals were singly housed. Rats were randomly allocated to one of four test conditions: low light familiar, high light familiar, low light unfamiliar or high light unfamiliar. Similarly, rats were randomly allocated to either control or $ACTH_{1-24}$ groups. Six pairs of rats were tested in each condition and each drug group, except the high light familiar and low light unfamiliar control groups, where only five pairs were tested. A pair of animals did not differ from each other in weight by more than 10 g, and both members of a pair always received the same drug treatment.

For each of the two days immediately prior to testing, the rats allocated to the familiar groups were placed singly in the test box for ten minutes in the appropriate light level. Rats allocated to the unfamiliar test conditions were placed in the test room for ten minutes in the appropriate light level, but remained in their home cages.

Pairs of rats were tested in an order randomised for drug treatment and test conditions, between 0730 hr and 1130 hr. Each ten min session began immediately following the intraventricular injections. Pairs of rats were placed together in the test box, and the time spent in active social interaction was scored by two observers, one of whom had no knowledge of either the test condition or the drug state of the animals. The behaviours scored as active social interaction included: sniffing, following, grooming, wrestling, boxing, nipping, mounting, jumping on and crawling under or over the partner. Time spent lying or sitting in body contact with the partner was scored separately as passive contact. At the end of each session, boluses were counted and removed, and the box was wiped with detergent then dried.

Six days later, 18 pairs of rats were re-tested under low light familiar conditions, 12 pairs of which had previously been tested in this condition and 6 pairs which had previously been tested under low light unfamiliar conditions, but had also received a 15 min familiarisation period the day before the second test. The pairs of rats were randomly assigned to control, $ACTH_{4-10}$ or $ACTH_{4-10}$:D-Phe groups, and there were six pairs in each group. This test condition was selected for studying the effects of the short peptides because it is the one in which the reduction in social interaction from peripherally administered ACTH is greatest.

Drink-latency test. The animals from the Social Interaction test were randomly allocated to one of six drug groups, such that there were ten animals in each group, except the 5 μl control group were there were six. The animals were water-deprived for 24 hr prior to the testing, which took place two days after the Social Interaction test. Animals were tested between 1000 hr and 1430 hr, immediately after the intraventricular injections. Rats were placed singly into the test box and the times taken to (1) sniff/touch the spout, and (2) start drinking were recorded. A cut-off time of ten minutes was used. Boluses were counted and removed after each test, and the presence of urine noted.

Finally, intraventricular cannulae were localised by post-mortem inspection of rat brains, two minutes after the intraventricular injection of 10 μl of 2% (w/v) pontamine sky blue dye, which was injected under deep sodium pentobarbitone-induced anaesthesia.

RESULTS

Social Interaction Test

The social interaction and motor activity scores for the control rats and those injected with $ACTH_{1-24}$ were subjected to analyses of variance. As can be seen from Fig. 1, $ACTH_{1-24}$ produced a significant reduction in active social interaction, $F(1,38)=8.8$, $p<0.05$. This effect cannot be attributed to a sedative action of the peptide, as the level of motor activity was not significantly different in the ACTH and control groups, $F(1,38)=1.0$ and nor was the passive contact significantly increased, except in the high light unfamiliar test condition (see Table 1). The administration of $ACTH_{1-24}$ did not significantly alter the mean number of boluses produced, which was very low for both groups. It can also be seen from Fig. 1 that the rats injected with $ACTH_{1-24}$ showed relatively little change in social interaction as the test conditions were manipulated, but this was probably an artifact of their very low levels of response.

$ACTH_{4-10}$ and $ACTH_{4-10}$:D-Phe significantly reduced the mean time spent in active social interaction in the low light, familiar test condition, $t(10)=2.3$, $p<0.025$ and $t(10)=2.1$, $p<0.05$, respectively, see Fig. 2. These decreases in social interaction were not accompanied by significant reductions in motor activity, $ts(10)=0.6$ and 1.0, for $ACTH_{4-10}$ and $ACTH_{4-10}$:D-Phe, respectively. However, the time spent in passive contact was significantly increased by administration of the short peptides (see Table 2), which does suggest that they may have had some sedative action, since sedative drugs produce a dose-related increase in passive contact [6]. The increase in passive contact was most marked with $ACTH_{4-10}$:D-Phe, and it is this fragment that was found, after peripheral administration, to reduce motor activity in the Social Interaction test [5].

Drink-Latency Test

As can be seen from Table 2, none of the ACTH frag-

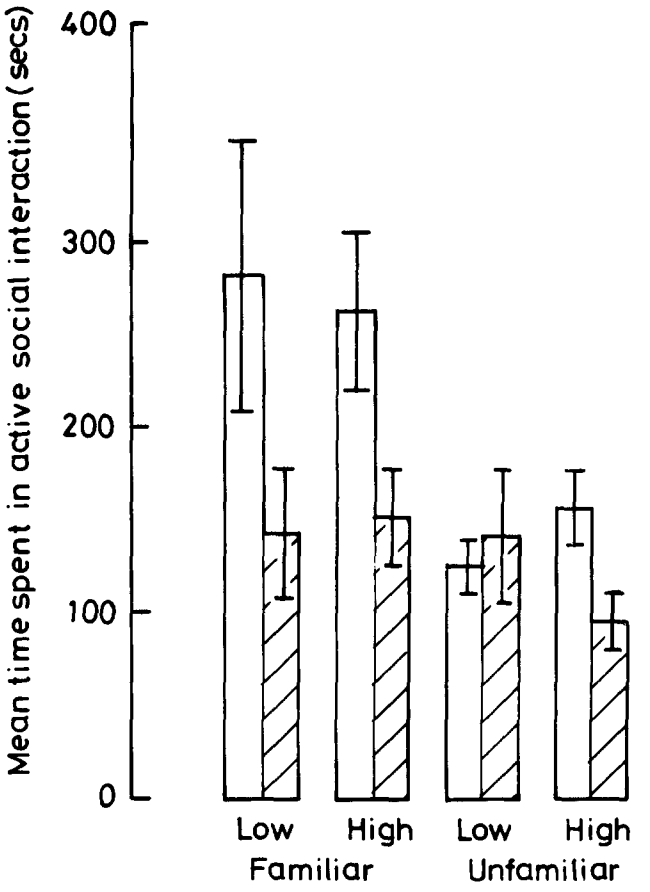

FIG. 1. Mean (± SEM) time spent in active social interaction in four different test conditions (low and high light, familiar and unfamiliar test box), by rats injected with $ACTH_{1-24}$ (1.25 μg, ICV ▨) or de-ionised water, (□).

ments significantly affected the time to first sniff/touch the water-spout, the latency to start drinking, or the difference between these two latencies. None of the peptides significantly affected the mean number of boluses dropped or the occurrence of urination in this test. The data from this test were analysed with the non-parametric U-test, because they were not normally distributed and the variances of the different groups were not homogeneous. The standard errors are included in Table 2 simply as a rough guide to the extent of variation in the scores.

DISCUSSION

The results presented in this paper have shown that the central administration of low doses of $ACTH_{1-24}$, $ACTH_{4-10}$ and $ACTH_{4-10}$:D-Phe causes a reduction in active social interaction, an increase in passive contact, but without a concomitant reduction in motor activity. Thus the central administration of these peptides has similar effects in the social interaction test to those observed after peripheral administration. Although the spread of ACTH from the cerebrospinal fluid to the blood plasma (and hence a peripheral action) cannot be discounted this is unlikely to account for the observed effects: the doses we used were far lower than those

TABLE 1

MEAN SCORES (IN SECONDS) FOR THE TIME SPENT IN PASSIVE CONTACT IN THE VARIOUS TEST CONDITIONS. THE NUMBERS IN PARENTHESES ARE THE SIGNIFICANCE LEVELS FROM MANN-WHITNEY U-TESTS (ONE-TAILED) FOR THE COMPARISON BETWEEN EACH DRUG GROUP AND ITS RELEVANT CONTROL (n.s.=NOT SIGNIFICANT)

Test condition	Water (5 μl)	$ACTH_{1-24}$ (1.25 μg)
Low light familiar	0.00	3.83 (n.s.)
High light familiar	0.60	6.66 (n.s.)
Low light unfamiliar	0.00	11.50 (n.s.)
High light unfamiliar	0.83	5.00 ($p<0.05$)

	Water (2 μl)	$ACTH_{4-10}$ (1.6 μg)	$ACTH_{4-10}$: D-Phe (1.6 μg)
Low light familiar	2.14	10.14 ($p<0.01$)	20.8 ($p<0.01$)

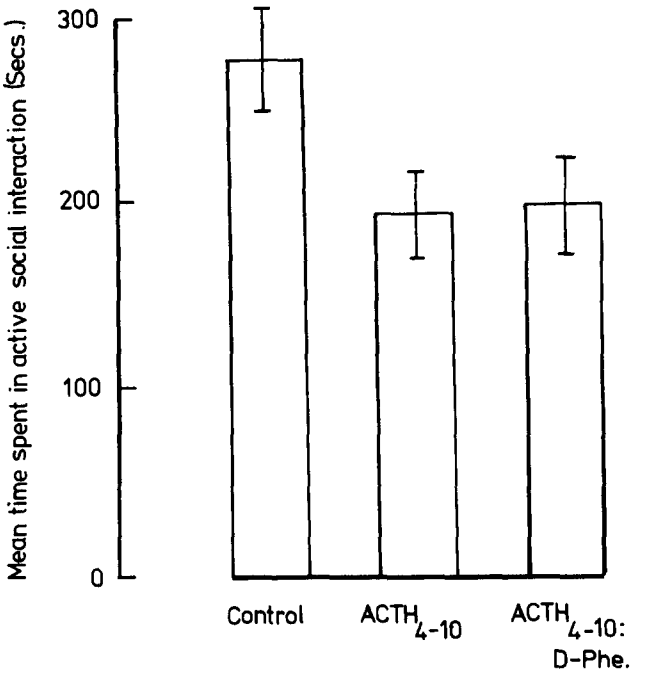

FIG. 2. Mean (± SEM) time spent in active social interaction in low light familiar test conditions, by rats injected with de-ionised water (control), $ACTH_{4-10}$ (1.6 μg) or $ACTH_{4-10}$:D-Phe (1.6 μg).

TABLE 2

MEAN LATENCIES (IN SECONDS) TO FIRST SNIFF/TOUCH THE WATER-SPOUT, TO START DRINKING, AND THE MEAN DIFFERENCE BETWEEN THESE SCORES. NONE OF THE SCORES FROM THE ACTH GROUPS DIFFERED SIGNIFICANTLY FROM THEIR RELEVANT CONTROLS WHEN THE DATA WERE ANALYSED BY MANN-WHITNEY U-TESTS. STANDARD ERRORS OF EACH MEAN ARE GIVEN ONLY AS A ROUGH GUIDE TO THE EXTENT OF VARIATION OF THE SCORES

Substances administered	Sniff/touch	Drink	Difference
Water (2 μl)	16.75 ± 3.83	65.66 ± 8.73	54.50 ± 7.56
$ACTH_{1-24}$ (0.5 μg)	17.40 ± 4.00	68.20 ± 13.20	50.80 ± 13.35
$ACTH_{4-10}$ (1.6 μg)	29.80 ± 13.52	71.40 ± 10.38	40.60 ± 11.88
$ACTH_{4-10}$: D-Phe (1.6 μg)	10.11 ± 3.41	66.80 ± 19.54	61.33 ± 19.98
Water (5 μl)	51.83 ± 26.81	134.00 ± 29.24	82.17 ± 22.23
$ACTH_{1-24}$ (1.25 μg)	29.30 ± 10.18	91.50 ± 18.85	63.20 ± 13.75

used to produce an effect when given peripherally, and testing took place immediately after injection.

One interpretation of the reduction in social interaction produced by ACTH is that the peptide exerts an anxiogenic action [9]. Since ACTH was without effect in the drink-latency test, the possibility exists that ACTH has a specific inhibitory effect on social behaviour and that the reversal of this by chronically administered benzodiazepines [7] and an acute, low dose of ethanol [8] is unrelated to the anti-anxiety effects of these drugs. However, we feel that such a conclusion would be premature because there has been no validation of the drink-latency test, and the action of benzodiazepines on drinking in a novel environment is thought unlikely to correlate only with the anti-anxiety effects of these drugs [21]. The large variances of the data obtained from this test (see Table 2) also suggest that group sizes considerably larger than ten would be needed to show significant drug effects. It seems likely, therefore, that this test is less sensitive to anxiolytic and anxiogenic substances than is the Social Interaction test.

Many of the behavioural effects of ACTH can be, and indeed have been, interpreted as the result of increased arousal, motivation or fear. For example, enhanced acquisition of avoidance responses and delayed extinction in several tasks [24], and the induction of grooming, where grooming has been suggested to serve as a mechanism for de-arousing the organism after activation by ACTH [16]. The effects of $ACTH_{4-10}$ on shortening the lapses due to fatigue in a reaction-time task could also be due to an increase in arousal [1,12]. The re-instatement of hormonal responses that were present during training, e.g. by post-trial injection of ACTH, can enhance memory formation [11, 14, 18], an effect that can be accounted for by actions on retrieval mechanisms or by changes in fear/anxiety. Indeed, it may be that ACTH acts to increase arousal in a general way and that the stimuli present in the experimental context determine the emotional quality of the change. It has been suggested [19] that ACTH improves selective attention, i.e. enhances attention to relevant stimuli and improves the ability to ignore irrelevant stimuli. Although this may be the case in some tasks this suggestion is not supported by the finding that ACTH-injected rats take significantly longer to cease responding to an irrelevant auditory stimulus [4]. It would also seem at variance with the finding that ACTH reduces exploration in novel situations [4,15].

However, it is likely that ACTH, as well as other peptides, has multiple actions and that these can be independent of each other [17]. Certainly there is evidence that the effects of injected ACTH can depend on the endogenous levels at the time of injection [13,18]. This could certainly account for our finding of a large decrease in social interaction in the low light, familiar test condition and a smaller reduction in the more stressful conditions. It is also likely that the levels of social interaction were reduced by ACTH to near their minimum level and thus the failure to get a large reduction in the high light, unfamiliar test condition was due to this floor effect.

Unless there is an inverted 'U' function relating social interaction to peptide dose, our results suggest that $ACTH_{1-24}$ is more potent at reducing social interaction than is $ACTH_{4-10}$; this difference is particularly marked when the doses are considered in relation to the molecular weights of the two peptide fragments. A similar result was reported by Smotherman and Levine [20] where $ACTH_{1-39}$ was found to be more potent than $ACTH_{4-10}$ at delaying extinction of a learned taste aversion. Also, $ACTH_{1-24}$ given at the interventricular foramen induces excessive grooming, whereas $ACTH_{4-10}$ does not [25]. In contrast, $ACTH_{4-10}$ is more potent than $ACTH_{1-24}$ at delaying extinction of a pole-jump response. Thus the structure-activity relationship for ACTH

and behaviour does differ for different behaviours and this gives further support to the suggestion that at least some of the multiple actions of ACTH are independent of each other.

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REFERENCES

1. Branconnier, R. J., J. O. Cole and G. Gardos. $ACTH_{4-10}$ in the amelioration of neuropsychological symptomatology associated with senile organic brain syndrome. *Psychopharmacology* **61:** 161–165, 1979.
2. Bush, I. E. Paper-chromatographic study of the secretion of the adrenal cortex in various mammalian species. *J. Physiol.* **115:** 12P, 1951.
3. Bush, I. E. Species differences in adrenocortical secretion. *J. Endocr.* **9:** 95–100, 1953.
4. File, S. E. ACTH, but not corticosterone impairs habituation and reduces exploration. *Pharmac. Biochem. Behav.* **9:** 161–166, 1978.
5. File, S. E. Effects of $ACTH_{4-10}$ in the Social Interaction test of anxiety. *Brain Res.* **171:** 157–160, 1979.
6. File, S. E. ACTH as a mediator in anxiety. In: *Neuropsychopharmacology,* edited by B. Saletu. Oxford: Pergamon Press, 1979, pp. 439–448.
7. File, S. E. and J. R. G. Hyde. Can social interaction be used to measure anxiety? *Br. J. Pharmac.* **62:** 19–24, 1978.
8. File, S. E., J. R. G. Hyde and M. Pool. Effects of ethanol and chlordiazepoxide on social interaction in rats. *Br. J. Pharmac.* **58:** 465P, 1976.
9. File, S. E. and S. V. Vellucci. Studies on the role of ACTH and of 4-HT in anxiety, using an animal model. *J. Pharm. Pharmac.* **30:** 105–110, 1978.
10. File, S. E., S. V. Vellucci and S. Wendlandt. Corticosterone—an anxiogenic or anxiolytic agent? *J. Pharm. Pharmac.* **31:** 300–305, 1979.
11. Flood, J. F., M. E. Jarvik, E. L. Bennett and A. E. Orme. Effects of ACTH peptide fragments on memory formation. *Pharmac. Biochem. Behav.* **5:** Suppl. 1, 41–51, 1976.
12. Gaillard, A. W. K. and A. F. Sanders. Some effects of $ACTH_{4-10}$ on performance during a serial reaction task. *Psychopharmacologia* **42:** 201–208, 1975.
13. Gold, P. E. and R. van Buskirk. Enhancement and impairment of memory processes with post-trial injections of adrenocorticotrophic hormone. *Behav. Biol.* **16:** 387–400, 1976.
14. Gold, P. E., R. P. Rose, C. W. Spanis and L. L. Hankins. Retention deficit for avoidance training in hypophysectomized rats: Time dependent enhancement of retention performance with post-training ACTH injections. *Hormones Behav.* **8:** 363–371, 1977.
15. Issacson, R. L. and E. J. Green. The effect of $ACTH_{1-24}$ on locomotion, exploration, rearing and grooming. *Behav. Biol.* **24:** 118–122, 1978.
16. Jolles, J., J. Rompa-Barendregt and W. H. Gispen. ACTH-induced excessive grooming in the rat: The influence of environmental and motivational factors. *Hormones Behav.* **12:** 60–72, 1979.
17. Kastin, A. J., R. D. Olsen, A. V. Schally and D. H. Coy. Minireview. CNS effects of peripherally administered brain peptides. *Life Sci.* **25:** 401–414, 1979.
18. Kendler, K., J. W. Hennessy, W. P. Smotherman and S. Levine. An ACTH effect on recovery from conditioned taste aversion. *Behav. Biol.* **17:** 225–229, 1979.
19. Sandman, C. A. and A. J. Kastin. Pituitary peptide influences on attention and memory. In: *Neurobiology of sleep and memory,* edited by R. R. Drucker-Colin and J. L. McGaugh. New York: Academic Press, 1977, pp. 347–359.
20. Smotherman, W. P. and S. Levine. ACTH and $ACTH_{4-10}$ modification of neophobia and taste aversion responses in the rat. *J. comp. physiol. Psychol.* **92:** 22–33, 1978.
21. Soubrie, P., L. de Angelis, P. Simon et J. R. Boissier. Effets des anxiolytiques sur la prise de boisson en situation nouvelle et familiere. *Psychopharmacology* **50:** 41–45, 1976.
22. Weiss, J. M., B. S. McEwen, M. T. Silva and M. Kalkut. Pituitary-adrenal alterations and fear responding. *Am. J. Physiol.* **218:** 864–868, 1970.
23. de Wied, D. Effects of peptide hormones on behavior. In: *Frontiers in Neuroendocrinology,* edited by W. F. Ganong and L. Martini. London, New York: Oxford University Press, 1969, pp. 97–140.
24. de Wied, D. Minireview. Peptides and Behavior. *Life Sci.* **20:** 195–204, 1977.
25. Wiegant, V. M., D. Colbern, Tj. B. van Wimersma Greidanus and W. H. Gispen. Differential behavioral effects of $ACTH_{4-10}$ and (D-Phe^7)$ACTH_{4-10}$. *Brain Res. Bull.* **3:** 167–170, 1978.