mitted recording of eight light-indifferent spontaneous units and 23 light-inhibitable units. Section of the pineal organ, in addition to optic tract section in the seven frogs mentioned, eliminated light-inhibitable units. Of 15 units recorded in such doubly lesioned animals, 14 were light-indifferent and one was questionably light-inhibitable. It is particularly significant that, in the experiment in which shielding of the ventral surface of the brain from light blocked photic inhibition in the pars intermedia of seven frogs, the eyes were intact.

The extremely long (approximately 30-second) latency of the electrical response in the pars intermedia to external changes in illumination suggests that a humoral step is involved in the response. Since the recording of the final electrical phenomenon is apparently from axonal structures within the pars intermedia, it is most likely that the humoral step is located outside the gland. The nature and locus of such a humoral step is unknown. Its existence can only be presumed from these data, but a search for it is very important.

Electrophysiological techniques, applied here to the study of the pars intermedia, have yielded information that characterizes several previously unsuspected properties of the vertebrate pigmentary response. These data open completely new avenues of inquiry and we hope that they will stimulate and orient further fruitful research into the mechanisms for pigmentary adaptation.

KIYOSHI OSHIMA
AUBREY GORBMAN

Department of Zoology,
College of Arts and Sciences,
University of Washington,
Seattle 98105

References and Notes

1. J. Bagnara, in *Structure and Control of the Melanocyte, 6th International Pigment Cell Conference*, A. Della Porta and J. Muhlbock, Eds. (Springer, Berlin, 1966), p. 16; M. Fingerman, *The Control of Chromatophores* (Macmillan, New York, 1963).
2. A. Enemar and B. Falck, *Gen. Comp. Endocrinol.* **5**, 577 (1965); W. Etkin, in *Neuroendocrinology*, L. Martini and W. F. Ganong, Eds. (Academic Press, New York, 1967), vol. 2; T. Ito, *Neuroendocrinology* **3**, 25 (1968); F. C. Iturriza, *Gen. Comp. Endocrinol.* **6**, 19 (1966); ———, *ibid.*, in press; C. L. Ralph and S. Sampath, *ibid.* **7**, 370 (1966).
3. S. Taleisnik and M. E. Tomatis, *Endocrinology* **81**, 819 (1967); ———, *Amer. J. Physiol.* **212**, 157 (1967).
4. F. C. Iturriza, *Gen. Comp. Endocrinol.* **4**, 492 (1964); L. C. Saland, *Neuroendocrinology* **3**, 72 (1968).
5. K. Oshima and A. Gorbman, *Gen. Comp. Endocrinol.* **7**, 482 (1966).
6. K. Yagi and H. A. Bern, *ibid.* **5**, 509 (1965); H. Morita, T. Ishibashi, S. Yamashita, *Nature* **191**, 183 (1961); M. V. L. Bennett and S. Fox, *Gen. Comp. Endocrinol.* **2**, 77 (1962).
7. Aided by a research grant NB04881 from NIH.

28 August 1968

Pituitary-Adrenal Influences on Fear Responding

Abstract. *In a passive avoidance situation, hypophysectomized male rats show less fear than normal rats, whereas adrenalectomized rats show greater fear than normals. These results probably occur because hypophysectomized rats lack adrenocorticotrophic hormone, which increases arousal or emotionality, whereas adrenalectomized animals lack certain adrenal steroids, which inhibit excitatory effects. The results indicate that adrenocorticotrophic hormone and certain adrenal steroids have opposite effects in regulating fear-motivated behavior.*

Administration of adrenocorticotrophic hormone (ACTH) can maintain fear-motivated behavior in normal rats (*1*). Miller and Ogawa (*2*) demonstrated that ACTH prolonged shuttle avoidance responding in adrenalectomized subjects, an indication that this hormone can affect behavior independently of the adrenals. In the experiment reported now, we examined the possibility that ACTH itself has an "excitatory" or "arousing" effect, particularly in augmenting fear responses. We also considered that one action of adrenal steroids released by ACTH might be to shut down or inhibit this excitement, since the pituitary-adrenal system would then influence excitability via a rather elegant feedback circuit—stressors would cause the release of an excitatory hormone (ACTH) that would initiate its own "shut-off" mechanism (steroids). Certain steroids might act by reducing excitability directly, or by inhibiting ACTH since steroids inhibit ACTH secretion when either their blood concentration increases (*3*), or they are introduced directly into the brains of rats (*4*). Moreover, observations that plasma concentration of corticosterone in rat is not maximum until approximately 15 to 60 minutes after stress begins (*5*) and that the inhibitory effect of steroids on ACTH release does not occur until more than 1 hour after stress (*6*), fit well with the view that adrenal steroids could be acting to restore normal excitability after the release of an excitatory hormone.

Male albino rats (20 hypophysectomized by the vendors, 20 bilaterally adrenalectomized in our laboratory, and 20 untreated) (*7*) were used. Hypophysectomized and adrenalectomized animals were used so that there was no opportunity for release of ACTH or corticosterone to influence the secretion of the other hormone. According to the above conception, hypophysectomized animals, which are not able to release ACTH, should therefore become less afraid in a fear situation than normal rats. Adrenalectomized rats, in contrast, can release ACTH but lack steroids and, thus, should become more fearful than normal rats. The latter effect would arise from the lack of certain steroids themselves or from the lack of their normal negative feedback control over ACTH release, or both. Circulating ACTH is high in adrenalectomized female rats 1 week or more after adrenalectomy (*8, 9*); and ACTH release, which occurs within 10 seconds of stress onset, is also greater in adrenalectomized than in normal rats (*9, 10*). Our adrenalectomies were performed 7 to 10 days before experiment.

Fear was measured in a standard passive avoidance situation. The apparatus consisted of a small chamber (17.5 by 9 cm, Plexiglas walls with grid floors, bars 2.5 cm apart) adjoining a large compartment (36 by 36 cm, same construction as small chamber), with a Plexiglas door between them. On each day of the experiment, each animal was weighed and placed into the small chamber so that the animal faced away from the large chamber. When the door was withdrawn, an electronic timer was activated. On day 1 when the rat stepped into the large compartment, the door was closed, and a 1.0-ma shock was delivered through the grid floor for 1.5 seconds, after which the animal was immediately returned to its home cage.

The next day, the animal was returned to the small chamber and the time that the animal delayed (latency) before reentering the large compartment where it had been shocked was measured. No shock was given on this or on subsequent fear tests. It should be noted that, in the passive avoidance test, an animal must step out of the small chamber rather than remain stationary in order to show loss of fear, so that any debilitation among hypophysectomized animals (they are smaller and less robust than normal animals) would work against their being judged less fearful. After the initial test, animals were rested for 2 days and then tested again on each of the 2 succeeding days. If, on any trial, a subject did not step out of the small chamber within 5 minutes, the trial was terminated. Throughout the experiment, ani-

mals were maintained in group cages and given free access to food and water, except that adrenalectomized animals received 0.15*M* saline solution.

Results are shown in Fig. 1 (left of dotted line). On the initial treatment day (before shock), normal adrenalectomized, and hypophysectomized groups showed similar short latencies to leave the small chamber (median latencies, respectively: 6.6, 6.5, and 7.3 seconds) and did not differ in defecation during weighing or in the apparatus (mean number of boluses excreted, respectively: .25, .55, and .30), an indication that there was no apparent effect of the particular gland removed or of the surgical procedure on the tendency of the animals to walk out of the small chamber or on emotional elimination under these conditions. After shock, all groups were reluctant to enter the shock compartment, but hypophysectomized animals were less reluctant than other groups. More hypophysectomized than normal animals entered the shock compartment on the first test ($P < .05$), and more hypophysectomized than adrenalectomized animals came out on the first two fear tests (both, $P < .05$). Defecation in the hypophysectomized group was also significantly less than in the adrenalectomized group on the first two tests ($P < .001$ and .05, respectively). Normal and adrenalectomized groups did not differ significantly in entering the shock compartment, but normal rats defecated less than adrenalectomized animals on the first two tests ($P < .02$ and .05, respectively). By the third test, the groups did not significantly differ on either measure.

The second part of the experiment was then conducted; we reduced the general level of fear in the step-out situation by changing some of the environmental stimuli, which eliminated various fear cues. This fear-reduction procedure was suggested by results of other studies (*11*), which showed that when fear is very strong the influence of the pituitary-adrenal system on fear responding is much less than if fear is weak. For example, we have measured suppression of operant responding for food and water as an index of fear and found that suppression to a tone presented several seconds before a strong shock was marked and did not differentiate normal, hypophysectomized, or adrenalectomized animals. But the suppression we observed to the box alone, where the tone-shock pairings were given, differed among these groups,

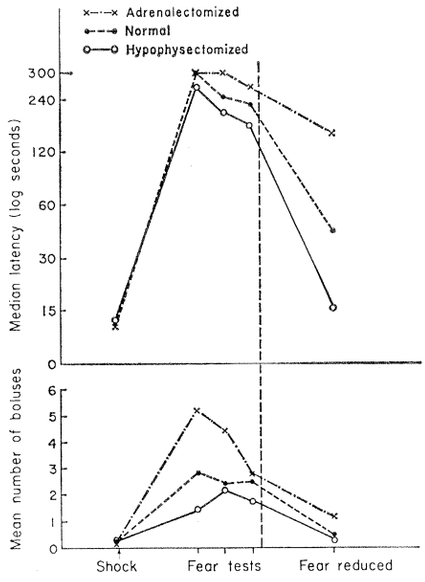

Fig. 1. Latency before entry into the shock compartment and defecation during tests for adrenalectomized, normal, and hypophysectomized groups.

showing that the pituitary-adrenal system was much more important in influencing this milder, more generalized fear response. In the present experiment, to examine effects under conditions of mild fear with less specific "warning" stimuli, we altered the step-out apparatus, covering the Plexiglas walls with white paper and the grid-bar floor with a white, solid floor.

As shown in Fig. 1 (right side of dotted line), all groups can be differentiated under these conditions. Hypophysectomized animals entered the shock compartment sooner than normal animals ($P < .001$), whereas adrenalectomized animals took longer than normals ($P < .01$). The difference between hypophysectomized and adrenalectomized groups was also highly significant ($P < .001$).

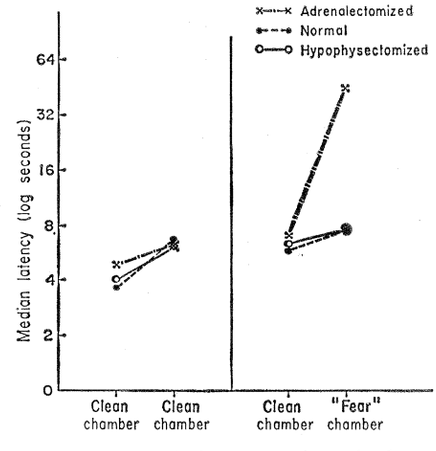

Fig. 2. Latency before entry into the large compartment for adrenalectomized, normal, and hypophysectomized groups tested twice in a clean apparatus (left side) or after shocked animals on the second test (right side).

The results of the foregoing experiment indicated that hypophysectomized animals were less fearful than normal animals, whereas adrenalectomized animals were more fearful, particularly under conditions of mild, generalized fear. In a second experiment, we found that adrenalectomized animals were so responsive to nonspecific fear stimuli that they would take longer to leave the small chamber when simply tested after other shocked animals had been in the apparatus. Sixteen hypophysectomized, 16 adrenalectomized, and 16 normal rats were tested in the step-out situation on two successive days without shock. Before half the animals in each group were tested on day 2, several animals that had received strong electric shock were allowed to defecate and urinate in the small chamber. This creates a distinctive odor that alerts other rats (*12*), but is a very weak and generalized fear stimulus to a normal, naive rat. Figure 2 shows that all groups came out rapidly except the adrenalectomized animals tested after shocked rats. This group came out more slowly than hypophysectomized ($P < .01$) or normal animals ($P < .01$) tested under the same conditions, and adrenalectomized animals tested in a clean apparatus ($P < .01$). Although one might suggest that adrenalectomized animals could be hypersensitive to any novel or strong environmental stimuli, such as odors, the important fact is that they respond to such stimuli by showing more pronounced attention or fear responses consisting of more freezing and less exploratory activity than normal rats.

One might suggest that, in the above experiments, adrenalectomized rats came out more slowly because they were debilitated rather than because they were more fearful, but this does not account either for the short latencies under nonfear conditions or for the differences in defecation. Another alternative is that hypophysectomized rats came out more rapidly because they were hyperactive or had a heightened exploratory drive as opposed to their being less fearful. Although latencies under nonshock conditions offered no suggestion of such a difference, we measured the activity of six hypophysectomized and six normal rats on an activity platform and found no

differences approaching significance in two 15-minute sessions 24 hours apart. Appley (*13*) has reported similar results. Thus, activity differences which might have influenced step-out performance seem unlikely.

We also used a one-way active avoidance task in which the animal is taught to run from one place to another to avoid shock. If adrenalectomy increases fear, adrenalectomized rats should maintain active avoidance responding for more trials than normal animals, whereas the opposite should occur if adrenalectomized rats are simply debilitated. Conversely, if hypophysectomy decreases fear, hypophysectomized animals should perform fewer avoidance responses than normals, which is the opposite of what should occur with hyperactivity. This experiment showed that adrenalectomized animals indeed maintained a learned one-way avoidance response for significantly more trials during extinction than did normal rats, while hypophysectomized rats made significantly fewer avoidance responses than did normals. This could not have been due to any group receiving more or fewer shocks during learning since the groups did not differ in shocks received. These results further indicate that, in our experiments, hypophysectomy and adrenalectomy affected fear.

Since we chose to eliminate ACTH and corticosterone completely by hypophysectomy and adrenalectomy, other hormones that might have played a role in producing the effects observed were also eliminated. Nevertheless, the evidence suggests that ACTH and certain adrenal steroids, particularly corticosterone, were the principal hormones involved. When we injected ACTH (25 I.U.; behavior test given 15 minutes later) into hypophysectomized animals trained in the active avoidance task, these animals showed a tenfold (highly significant) increase in avoidance responding, which reached up to and even exceeded normal levels; but other hypophysectomized animals, that were matched for avoidance performance and received control injections, continued to make characteristically few responses. Conversely, to determine if corticosterone would reduce avoidance responding, we injected corticosterone (0.75 mg daily for 3 days; test given 1 hour after last injection) into adrenalectomized animals trained in the active avoidance task. These animals showed a significant reduction toward normal in the number of avoidance responses made in comparison to matched adrenalectomized rats receiving control injections (*14–16*). The results of hormone injection thus suggest that ACTH enhances, and corticosterone reduces, fear-motivated responding.

Earlier investigators have not generally observed effects of the pituitary-adrenal system upon acquisition of fear-motivated behavior, where an animal learns to respond to an unconditioned stimulus, such as electric shock, that is clearly signaled and imminent. Rather, effects of ACTH administration, adrenalectomy, and so on, have been generally seen during extinction of behavior, where performance reflects how long declining fear responses are maintained.

Our results are consistent with these, showing that the influence of the pituitary-adrenal system was most obvious when fear was moderate or even weak, and rather generalized, while differences were less evident when specific fear stimuli elicited strong fear. This suggests that the pituitary-adrenal system plays a rather subtle role in fear responding, probably influencing the rat's general level of arousal or emotionality. Thus, effects of this system can be easily obscured by raising fear to a ceiling, which may account for a number of failures to obtain differences, particularly between adrenalectomized and normal rats (*13, 17*). It also seems logical to us that a hormonal system, which is relatively slow acting, would influence general level of arousal, while other systems, probably neural, would be mainly responsible for immediate reactions to clear and present dangers.

Behavioral effects of ACTH and adrenal steroids may not by any means be restricted to fear-motivated behavior; rather, if arousal or emotionality is indeed affected by these hormones, one would expect a wide range of behavior to be influenced by their secretion. The nature of the effect would depend upon the stimulus situation; the above experiments suggest only those effects under fear-provoking conditions.

JAY M. WEISS, BRUCE S. MCEWEN
M. TERESA A. SILVA
MARCIA F. KALKUT
Rockefeller University,
New York 10021

References and Notes

1. J. V. Murphy and R. E. Miller, *J. Comp. Physiol. Psychol.* **48**, 47 (1955); S. Levine and L. E. Jones, *ibid.* **59**, 357 (1965); D. de Wied, *Proc. Soc. Exp. Biol. Med.* **122**, 28 (1966); B. Bohus and D. de Wied, *Science* **153**, 318 (1966); D. de Wied and G. Pirie, *Physiol. Behav.* **3**, 355 (1968).
2. R. E. Miller and N. Ogawa, *J. Comp. Physiol. Psychol.* **55**, 211 (1962).
3. G. Sayers and M. A. Sayers, *Proc. Soc. Exp. Biol. Med.* **60**, 162 (1945); F. E. Yates, S. E. Leeman, D. W. Glenister, M. F. Dallman, *Endocrinology* **69**, 67 (1961); M. G. Dallman and F. E. Yates, *Mem. Soc. Endocrinol.* **17**, 39 (1968).
4. E. Endroczi, K. Lissak, M. Tekeres, *Acta Physiol. Hung.* **18**, 291 (1960); J. M. Davidson, L. E. Jones, S. Levine, *Endocrinology* **82**, 655 (1968).
5. J. R. Hodges and M. T. Jones, *J. Physiol.* **167**, 30 (1963); A. M. Barrett and M. A. Stockham, *J. Endocrinol.* **26**, 97 (1963).
6. A. Brodish and C. N. H. Long, *Endocrinology* **59**, 666 (1958); P. G. Smelik, *Proc. Soc. Exp. Biol. Med.* **113**, 616 (1963).
7. Hypophysectomies are routinely confirmed in our laboratory by visual inspection for absence of the pituitary. Concentration of corticosterone in plasma 30 minutes after ether stress is also determined for hypophysectomized, adenalectomized, and normal rats; the values found are 4.4 ± 1.3, 3.7 ± 0.9, and 32.2 ± 14.4 mg per 100 ml of plasma.
8. G. S. Cox, J. R. Hodges, J. Vernikos, *J. Endocrinol.* **17**, 177 (1958).
9. J. R. Hodges and M. T. Jones, *J. Physiol.* **173**, 190 (1964).
10. W. D. Gray and P. L. Munson, *Endocrinology* **48**, 471 (1951).
11. J. M. Weiss, B. S. McEwen, M. F. Kalkut, in preparation.
12. R. Runyon, thesis, Yale University (1953).
13. M. H. Appley, paper read at Canadian Psychological Association meetings, Halifax, N.S. (1964).
14. It is important that adrenalectomy removed the medulla as well as the cortex, causing loss of epinephrine and some norepinephrine. Several investigators (*15*), however, have reported that loss of the adrenal medulla alone does not alter various fear responses, and Moyer and Bunnell have suggested that increased elimination in the open field after adrenalectomy is due to loss of the adrenal cortex and not to demedullation. Nevertheless, negative results might be the result of the measures taken, as Levine and Soliday (*16*) point out, so that a possible effect of adrenal medullation in the above results cannot be ruled out.
15. K. E. Moyer, *J. Genet. Psychol.* **92**, 17 (1958); ——— and B. N. Bunnell, *ibid.* **96**, 375 (1960); *J. Comp. Physiol. Psychol.* **52**, 215 (1959).
16. S. Levine and S. Soliday, *J. Comp. Physiol. Psychol.* **55**, 214 (1962).
17. K. E. Moyer, *J. Genet. Psychol.* **92**, 11 (1958).
18. Supported by PHS grants MH 13189 and NM 07080.

7 October 1968