ADRENOCORTICAL INFLUENCES ON FREE-OPERANT AVOIDANCE BEHAVIOR¹

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Rats were conditioned to avoid shock on a free-operant avoidance schedule in which no exteroceptive stimulus signaled impending shock. Injections of adrenocorticotrophic hormone (ACTH) or dexamethasone raised blood levels of glucocorticoids. These increases were accompanied by changes in avoidance performance: there was a higher frequency of long-duration interresponse times, a greater stability among them, and fewer short interresponse times, total responses, and shocks.

This paper describes some of the effects on rats' behavior of administering either adrenocorticotrophic hormone (ACTH) or dexamethasone (a synthetic corticosteroid) in a free-operant avoidance schedule in which no exteroceptive "warning" stimulus signaled impending shock (*c.f.*, Sidman, 1953). ACTH and corticosteroids are chemical substances characteristic of the organ system known as the hypothalamo-hypophyseal-adrenocortical (HHA) axis. ACTH stimulates the adrenal cortex to release steroid hormones into the blood; dexamethasone simulates the effects of some of the naturally occurring corticosteroids, principally the glucocorticoids. The organs of the HHA axis constitute the principal components of a negative feedback loop system. That is, the release of steroids from the adrenal cortex is modulated by circulating levels of ACTH; in turn, ACTH release from the anterior pituitary (adenohypophysis) is influenced by circulating levels of adrenocorticoids, which modulate the transport of corticotropin releasing factor from the hypo-

thalamus to the adenohypophysis (Tepperman, 1962).

In the present experiment, HHA activity in rats was altered by injections of ACTH and dexamethasone. These two compounds were selected in order to determine if subsequent behavioral changes could be primarily attributed to ACTH or to glucocorticoid activity. Because of the negative feedback properties of the HHA system, relatively small doses of a highly potent glucocorticoid such as dexamethasone cause an almost complete block of ACTH release; this action of dexamethasone is in contrast to that of, *e.g.*, cortisol or corticosterone, which must be administered in very large doses before ACTH release is markedly suppressed (Hedner and Rerup, 1962). It was for this reason that dexamethasone was used instead of naturally occurring compounds such as corticosterone.

One component of the physiological stress response is an increase in HHA activity (Tepperman, 1962). Recent work has shown that such increases, as reflected in urine or plasma corticosteroid levels, occur in situations in which behavior is controlled by aversive stimuli, *e.g.*, in the conditioned emotional response (CER) situation and during free-operant avoidance (Mason, Brady, and Sidman, 1957). A converse effect, that administration of ACTH modifies aversively-controlled behavior, has also been demonstrated. For example, ACTH injection increases the duration of punishment-induced suppression of positively reinforced responding (Levine and Jones, 1965); moreover, ACTH increases response frequency during both acquisition and

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extinction on discriminated avoidance schedules (Bohus and Endroczi, 1964; Murphy and Miller, 1955; Bohus and de Wied, 1966; de Wied, 1966).

From the above findings, it is apparent that there is considerable interaction between adrenocortical and behavioral responses in environments in which the behavior is under aversive stimulus control. The present study attempted to elucidate some aspects of this relationship by addressing the following questions. Do changes in the concentrations of hormones of the HHA axis alter steady-state free-operant avoidance performance? What is the nature of any such changes, e.g., does avoidance performance improve or deteriorate? Can these behavior changes be related to extra-adrenal effects of ACTH (c.f., Engel, Fredericks, Lopez, and Albertson, 1958), or to adrenocortical activity?

METHOD

Subjects and apparatus

Six albino male rats (S1 through S6) of the Sprague-Dawley strain, 90 to 120 days old at the start of experimentation, were used. The aversive stimulus was an electric shock, 1.3 ma in intensity, delivered from a Grason-Stadler shock source and scrambler (model E1064GS) through the grid floor, walls, and lever of a standard Foringer rat chamber. The sequence of experimental events was programmed by electromechanical switching circuits and recorded on decade counters, a cumulative recorder, and a paper-tape punch.

Procedure

Conditioning. The animals were conditioned to depress a lever to postpone or terminate the shock on a free-operant avoidance schedule in which no exteroceptive stimulus signaled impending shock; each avoidance response postponed shock for 21.5 sec, the response-shock (R-S) interval. If shock occurred, a response was required to terminate it; this escape response also began the next R-S interval. Only the initial downward press on the lever terminated or postponed shock; holding the lever down had no effect on shock occurrence.

Experimental sessions were conducted five to seven days per week, for approximately 50 weeks, at about the same hour each day. The

present experiment was begun after six initial weeks of avoidance conditioning, during which all the animals achieved stable performance levels. The results of the next 44-week period, which constituted the experiment proper, are described. During this period, most experimental sessions were 60 to 70 min in duration, and there were always at least five consecutive sessions in each seven-day period.

Hormone treatment. At the start of the experiment proper, the injection regimen was instituted. At regular seven-day intervals, for each animal, an avoidance conditioning session would be preceded by a subcutaneous injection of 0.3 ml of either normal saline, saline plus ACTH (8, 10, or 12 units), or saline plus dexamethasone (200 μ g).² Each such "injection session" was preceded and followed by at least two consecutive "no treatment" sessions of avoidance conditioning.

Injections of saline alone were administered to all subjects 15 min before the appropriate conditioning session of weeks 1, 3, and 5 of the experiment proper, and 120 min before the injection session of weeks 2, 4, and 6. During weeks 7 through 38, the once-weekly injection regimen continued; in this latter 32-week period, each animal received a total of six injections each of the 8- and 12-unit doses of ACTH and of the 200- μ g dose of dexamethasone, and four injections of the 10-unit dose of ACTH. Interspersed among these treatments were 10 injection sessions during which only saline was administered. There were three different sequences of injections, each of which was applied to two of the six animals; the sequences are indicated in Fig. 1. When hormone administration was begun, the animals' average weight was 525 ± 30 g.

Throughout the experiment, saline continued to be administered alternately 15 and 120 min before an experimental session, while the ACTH was always administered 15 min before a session, and the dexamethasone 120 min. The particulars of the hormone administration procedures, viz., the time lag between hormone injection and the start of a

²ACTH: Corticotropin solution (Wilson), 40 USP units/ml, aqueous solution; one U.S.P. unit ACTH is approximately 0.88 mg. Dexamethasone 21-Phosphate: Decadron phosphate (Merck), 4 mg/ml, aqueous solution; this is a non-fluorescing compound.

session, the route of administration, and the doses employed, were chosen in order to achieve a marked effect on HHA activity throughout the avoidance session on a hormone injection day. The appropriate values of these parameters were based on preliminary experiments in this laboratory, as well as on the results of previous studies in which similar techniques were employed (*c.f.*, Hedner and Rerup, 1962; Glick, Nakane, Levine, and Jones, 1966; Jones and Stockham, 1966). These prior investigations indicated that, using these procedures, plasma corticosteroid levels reach an initial maximum approximately 10 to 15 min after ACTH administration, and that 2 hr after dexamethasone treatment there is minimal adrenocortical steroid release (due to the suppression of the release of endogenous ACTH). To ensure the effectiveness of these procedures, measurements were made of the adrenocortical response of the subjects under several of the conditions of the present experiment.

Adrenocortical activity measurement. Physiological effects of the hormone treatments were assessed during weeks 39 through 44 of the experiment proper; during this period, the avoidance conditioning regimen was maintained except on days when steroid activity was measured. This measurement was carried out by determining the concentration of corticosteroids in the plasma fraction of the blood. Each blood sampling was preceded by at least 22 hr during which the animal rested in the home cage. At least four days intervened between the collection of successive samples.

On a sampling day, the animal would be picked up and handled, and then replaced in the home cage either immediately (*i.e.*, without receiving any injection), or after receiving a single subcutaneous injection of 0.3 ml of either normal saline, or saline plus 8 or 12 units ACTH, or saline plus 200 μ g dexamethasone. A blood sample was obtained from each animal under each of the following conditions: 0.5 min, 15 min, and 120 min after handling alone, 15 min and 120 min after saline injection, 15 min after ACTH injection, and 120 min after dexamethasone injection. A non-systematic treatment sequence was employed in the collection of the eight samples from each animal.

To obtain a blood sample, an animal

would be removed from the home cage and quickly anesthetized with ether. The jugular vein would then be surgically exposed and a small quantity of blood (approximately 0.3 ml) drawn into a heparinized syringe. When all surgical procedures were completed, the incision was sutured. As a precaution against infection, an injection of approximately 150,000 units of an aqueous suspension of benzathine penicillin G was administered.³ All animals readily recovered after surgery, and no pronounced or prolonged ill effects were noted.

The entire blood sample collection procedure was completed in less than 90 sec, as measured from the time the animal was removed from the home cage until the blood began to flow into the syringe. Since the peripheral steroid response lags at least 2 min behind initial exposure to a stressor, (such as the anesthetization and surgery) (*c.f.*, Hodges and Jones, 1963), each blood sample provided an accurate measure of the animal's corticosteroid response to the treatment condition preceding the sample. Plasma corticosterone levels were measured by a highly reliable small sample fluorometric assay (Glick, von Redlich, and Levine, 1964).

RESULTS

The injections of ACTH and dexamethasone proved to have both physiological and behavioral consequences. The results of the plasma steroid measurements, presented in Table 1, are quite similar to those observed under comparable conditions, both in our preliminary work and in many prior experiments (*e.g.*, Hedner and Rerup, 1962; Smelik, 1963; Stockham, 1964; Glick, *et al.*, 1966; Jones and Stockham, 1966). The data in Table 1 serve principally to indicate that the hormone preparations modified endogenous steroid production in the present subjects: ACTH stimulated corticosteroid release from the adrenal cortex, and dexamethasone acted as a synthetic corticosteroid. Table 1 also reveals that adrenocortical activity was not significantly altered either by handling or by saline injection.

Hormone treatment consistently resulted in a marked and (in the case of ACTH) dose-

³Bicillin (Wyeth).

Table 1

<i>Treatment</i>	<i>H-0.5</i>	<i>H-15</i>	<i>H-120</i>	<i>S-15</i>	<i>S-120</i>	<i>A8</i>	<i>A12</i>	<i>D</i>
Mean Plasma Steroid level ($\mu\text{g}\%$ $\pm$ 1 s.d.)	18.7 $\pm$ 1.4	20.0 $\pm$ 0.9	20.5 $\pm$ 1.2	20.3 $\pm$ 1.9	19.9 $\pm$ 1.6	43.0 $\pm$ 1.8	52.0 $\pm$ 1.6	8.0 $\pm$ 1.6

Mean plasma corticosteroid levels (μg per 100 ml plasma, $\pm$ 1 s.d.) after exposure to each of the following treatments: 0.5 min after handling (H-0.5), 15 min after handling (H-15), 120 min after handling (H-120), 15 min after saline injection (S-15), 120 min after saline injection (S-120), 15 min after injection of eight units ACTH (A8), 15 min after injection of 12 units ACTH (A12), and 120 min after injection of 200 μg dexamethasone (D). One steroid determination was obtained from each of the six animals subsequent to each treatment.

dependent decrement in shock rate during free-operant avoidance (Fig. 1). Except on hormone injection days, there were no large day-to-day changes in the shock rates, and no systematic effects on days after hormone treatment. Because performance differed among and within animals, all the shock rate data of the hormone treatment period are included in Fig. 1. They clearly demonstrate the effect of the several treatments on free-operant avoidance shock rate.

A summary depiction of the effect of the several testing conditions on shock rate, for each of the six animals, appears in Fig. 2. Although there are inter-animal differences in the average magnitude of the effect of each treatment, the same trend appears in the data of all the animals. Dexamethasone produced the largest decrease in shock rate; higher doses of ACTH resulted in greater shock rate decrements, and the shock rate after saline injection did not consistently differ from that on days when no injection preceded the avoidance conditioning session.

Mean shock rates, averaged over all animals, are presented in Table 2. This tabulation summarizes the effects of ACTH, dexamethasone, and the "control" conditions on

the average shock rate, and indicates the degree of variability when the data of the several animals are grouped.

Examination of the effects of the several injection treatments on mean shock rate during successive 12-min segments of avoidance conditioning sessions reveals similar functional relations for all animals (Fig. 3). The largest hormone effects occurred early in a session, when shock rate was highest. (This initial high "warm-up" shock rate, consistently observed in all the subjects, has also been reported to occur reliably during rats' performance on free-operant avoidance schedules similar to that of the present experiment (Wertheim, 1965).) Since previous findings (*e.g.*, Hedner and Rerup, 1962; Glick, *et al.*, 1966) suggest that our procedures cause the hormone effects to be relatively constant for the entire duration of the avoidance conditioning sessions, we interpret the data of Fig. 3 to indicate that the hormones differentially reduce the frequency of occurrence of patterns of responding that result in a high shock rate.

Hormone effects on patterns of avoidance interresponse time (IRT) frequencies are illustrated in Fig. 4. This set of histograms is

Table 2

	<i>Shocks per hour, $\pm$ 1 s.d.</i>			<i>Responses per min, $\pm$ 1 s.d.</i>			<i>d²/s²</i>		
	Pre	Inj	Post	Pre	Inj	Post	Pre	Inj	Post
S	45.1 $\pm$ 1.3	45.3 $\pm$ 1.4	45.3 $\pm$ 1.5	5.4 $\pm$ 0.4	5.5 $\pm$ 0.3	5.5 $\pm$ 0.4	1.86	1.83	1.85
A8	45.8 $\pm$ 1.6	40.6 $\pm$ 1.9	45.7 $\pm$ 1.6	5.3 $\pm$ 0.3	5.0 $\pm$ 0.3	5.3 $\pm$ 0.3	1.83	1.55	1.79
A10	45.9 $\pm$ 1.7	34.8 $\pm$ 1.9	45.2 $\pm$ 1.2	5.4 $\pm$ 0.4	4.9 $\pm$ 0.3	5.4 $\pm$ 0.3	1.84	1.48	1.82
A12	45.9 $\pm$ 1.7	29.7 $\pm$ 1.8	45.4 $\pm$ 1.6	5.4 $\pm$ 0.3	4.7 $\pm$ 0.2	5.3 $\pm$ 0.3	1.81	1.34	1.80
D	45.1 $\pm$ 1.9	25.6 $\pm$ 1.5	45.8 $\pm$ 1.6	5.5 $\pm$ 0.4	4.6 $\pm$ 0.2	5.3 $\pm$ 0.3	1.86	1.30	1.78

Response and shock rates of entire avoidance sessions, and d^2/s^2 ratios of warm-up periods. Each table entry is the mean of all the determinations from each of the six animals, on days before ("Pre"), during ("Inj"), and after ("Post") injection of saline (S), ACTH (8 units (A8), 10 units (A10), or 12 units (A12)), or 200 μg dexamethasone (D). Each animal received 16 injections of saline, six injections each of 8 and 12 units ACTH, four injections of 10 units ACTH, and six injections of the dexamethasone. The significance levels for d^2/s^2 , indicating the probability of non-random trends among the successive IRTs ($N = 59$) are: $P_{.05} = 1.60$; $P_{.01} = 1.43$; $P_{.001} = 1.25$ (Hart, 1942).

Fig. 1. Shock rates for each animal during successive five-day periods of week 1, and weeks 6 to 38. The sequences of injection treatments are indicated on the abscissae; one treatment sequence was applied to animals S1 and S2, another to S3 and S4, and a third to S5 and S6. The treatments, always applied on the third day of each five-day period, are indicated as follows: saline (S), ACTH 8, 10, or 12 units (A8, A10, A12), or dexamethasone 200 μ g (D).

Fig. 2. Mean shock rates (± 1 s.d.) for each animal under each testing condition. The column labeled C represents the mean shock rate on "control" days, arbitrarily selected as the days preceding any injection during weeks 1 to 38. The other labels are as in Fig. 1.

"representative", in that similar effects on the IRT distribution always appeared under similar experimental conditions. As is most clear in the case of dexamethasone, on hormone treatment days the frequency of short IRTs decreased (this was most marked in the case

Fig. 3. Mean shock rates for each animal during each of the first five successive 12-min periods of all experimental sessions under each treatment condition. The treatment labels are as in Fig. 1.

of IRTs of less than 6-sec duration), the mode of the IRT distribution shifted to the right, and the frequency of IRTs greater than 12 sec increased. (The decreases in shock frequency are also apparent in this figure.) One concomitant of these changes in IRT distribution is that mean response rate decreased on hormone treatment days (*c.f.*, Table 2).

Effects of hormone treatment on patterns of IRT sequences were also examined; as described below, this analysis indicated that the hormones effected an increased stability and consistency in the animals' response patterns. Consistent trends have been shown among successive IRTs during free-operant avoidance (Wertheim, 1965). A measure of such trends is the ratio of the mean square successive difference (d^2) to the variance (s^2); this ratio is inversely related to the degree of correlation among adjacent (successive) events, and smaller values of d^2/s^2 indicate less variability (*i.e.*, greater stability) in the pattern of successive IRTs (von Neumann, 1941; Leiderman and Shapiro, 1962). We computed d^2/s^2 for IRTs of the warm-up period of each session before, during, and after hormone or saline injection. (The warm-up was arbitrarily defined as encompassing the first 60 responses in a session.) Average values of d^2/s^2 were consistently lower after hormone injection (*c.f.*, Table 2), indicating a greater clustering among successive IRTs, and sug-

Fig. 4. IRT frequency histograms, for animal S1, on days before ("Pre"), during ("Inj"), and after ("Post") the fifth injection of A8, the first injection of A10, the fourth of A12, and the fourth of D. (Treatments labeled as in Fig. 1). IRT categories are coded so that category 1 includes all IRTs of 0- to 2-sec duration, 2 includes IRTs of 2- to 4-sec duration, *etc.*, except that category 10 includes all IRTs greater than 21.5 sec (*i.e.*, escape responses).

gesting an increase in the influence of one IRT upon the next. These ratio changes alone do not specify which IRTs are modified; however, since hormone treatment markedly increased the relative frequency of long-duration avoidance IRTs, and decreased short IRTs and shocks, the decreases in d^2/s^2 probably reflect an increased stability of sequences of the long avoidance IRTs. As indicated in Table 2, the order of magnitude of the several hormone treatment effects on d^2/s^2 is the same as that on shock rate and response rate.

In a further exploration of the hormone effects on patterns of avoidance and escape responding, conditional IRT distributions were calculated. Two of these transition matrices, presented in Table 3, indicate typical first-order sequential dependencies during no-treatment and hormone-treatment sessions. There were marked hormone effects on several IRT transition sequences. For example, on the hormone treatment day there was a large reduction (from 53 to 10) in the frequency of two successive IRTs of 0 to 4-sec duration. There was also a systematic increase in the frequency of occurrence of two successive avoidance IRTs of greater than 8-sec duration, as well as more clustering within each of the conditional IRT distributions (as indicated by

the greater number of zero-frequency entries). Comparison of the bottom row of each of the matrices indicates that dexamethasone effected relatively little change in the post-shock IRT distribution other than a reduction in shock frequency: on both the day of dexamethasone injection ("D") and the preceding day ("Pre-D"), a shock was most often followed either by another shock or a response in the shortest IRT category. Moreover, examination of the right-most entry in the top row of each matrix reveals virtually no hormone effect on the frequency with which a short IRT was followed by a shock. These two findings suggest that the hormone had little effect on IRTs just after a shock.

DISCUSSION

The principal aim of this study was to determine how administration of HHA axis hormones would alter free-operant avoidance behavior. The results indicate a strong relation between blood glucocorticoid levels and avoidance behavior. In particular, after injection of both ACTH and dexamethasone, changes in the response pattern included both an increase in the frequency of long-duration avoidance IRTs and in a greater stability among these IRTs; there were also fewer

Table 3

<i>Pre-D</i> <i>IRT_n</i>	<i>0-4</i>	<i>4-8</i>	<i>IRT_{n+1}</i> <i>8-12</i>	<i>12-16</i>	<i>16-21.5</i>	<i>> 21.5</i>	<i>Sums</i>
0-4	53	13	8	4	1	4	83
4-8	4	16	4	8	2	3	37
8-12	6	7	65	14	8	1	101
12-16	1	0	19	12	6	10	48
16-21.5	1	0	4	8	3	6	22
> 21.5	18	1	1	2	2	21	45
							336
<i>D</i> <i>IRT_n</i>	<i>0-4</i>	<i>4-8</i>	<i>IRT_{n+1}</i> <i>8-12</i>	<i>12-16</i>	<i>16-21.5</i>	<i>> 21.5</i>	<i>Sums</i>
0-4	10	3	3	2	3	6	27
4-8	3	4	1	0	0	0	8
8-12	0	1	30	14	2	3	50
12-16	0	0	10	86	15	2	113
16-21.5	0	0	6	8	13	6	33
> 21.5	14	0	0	3	0	10	27
							258

First-order conditional IRT frequency distributions, in 4-sec class intervals, for animal S1, on the days before ("Pre-D") and during the fourth injection of dexamethasone ("D"). Each matrix indicates the frequency of transition from one IRT (n) to the next ($n+1$). IRTs greater than 21.5 sec represent shock escape responses. The row sums of the Pre-D and D matrices are graphically depicted (in 2-sec class intervals) in the top and middle histograms, respectively, of the right-hand column of Fig. 4.

short IRTs, total responses, and shocks. The similarity of the behavior modifications by both ACTH and dexamethasone suggests an interaction of avoidance behavior with effects of increased plasma corticosteroid levels, and not of ACTH *per se*. If the behavioral role of ACTH were principally other than in its glucocorticoid-releasing effect, then opposite behavior changes would have been expected after the two compounds were administered, since dexamethasone markedly suppresses endogenous ACTH levels. While the present results in no way suggest any behavior changes due to extra-adrenal effects of ACTH, it should be noted that such effects cannot be entirely ruled out, since ACTH has been found to increase response frequency during discriminated avoidance extinction in adrenalectomized rats (Miller and Ogawa, 1962).

In a previous study of the interaction of free-operant avoidance behavior and HHA axis activity (Sidman, Mason, Brady, and Thach, 1962), R-S interval decreases were generally accompanied by increases in shocks, responses, and steroid levels, while extinction of the avoidance response was accompanied by a decrease in steroid level. However, unavoidable shocks during extinction did not increase the steroids; moreover, re-institution of an avoidance schedule increased steroids, even though performance was so proficient that no shock occurred. The latter two findings suggest that shock does not always increase steroid levels and that, under some conditions, endogenous steroids and avoidance responding may correlate independently of shock frequency. One implication of such an interaction is that steroid activity itself might influence avoidance responding; the results of the present study lend some support to this hypothesis. Also consistent with this analysis is the report that animals with higher baseline (home cage) steroid levels receive fewer shocks during discriminated avoidance conditioning (Bohus, Endroczi, and Lissak, 1964).

The present findings do not completely specify the relation between steroid levels and avoidance behavior, since we did not exert independent control over the shock and response frequencies. However, the primary effect of the hormone treatment in the present subjects must have been upon avoidance behavior, since only a change in some pattern of avoidance responding could reduce shock

frequency. Some of the behavior changes could have resulted from the hormone-induced decrease in shock rate (*c.f.*, Verhave, 1961); however, such relations are not immediately apparent in the present data, since hormone treatment did not markedly alter the pattern of the post-shock distribution of avoidance IRTs (*c.f.*, Table 3).

Further information concerning particular avoidance patterns which interact with HHA hormones could be obtained by experimentally manipulating specific components of free-operant avoidance response patterns, such as was done by Clark and Steele (1963) in the analysis of differential effects of chlorpromazine upon post-shock bursts *vs* other categories of IRTs. However, since marked dependencies have been found among as many as seven post-shock IRTs, only the first two of which were bursts (Wertheim, 1965), the study of hormone effects on long chains of IRTs may prove important in specifying the degree and kind of stimulus control exerted by these hormones over particular components of avoidance responding.

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