Effects of ACTH and zinc phosphate vehicle on shuttlebox CAR*

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Conditioned avoidance responding of Ss receiving subcutaneous injections of adrenocorticotrophic hormone, zinc phosphate vehicle, and physiological saline was studied in the two-way shuttlebox at three UCS intensity levels. In no case did ACTH Ss differ significantly from saline control Ss, but the zinc phosphate vehicle was found to alter performance both during acquisition and extinction.

Many investigators have reported behavioral effects of exogenous ACTH. Animals studied include the rat (Murphy & Miller, 1955), cat (Endröczi & Lissák, 1962), mouse (Koranyi et al, 1967), rabbit (Bertolini et al, 1969), dog (Ferreri et al, 1963), monkey (Mirsky et al, 1953), and man (Cleghorn, 1952). Some of the behaviors include: sexual behavior (Bertolini et al, 1969), the "stretching syndrome" (Ferrari et al, 1963), active avoidance (Murphy & Miller, 1955), passive avoidance (Weiss et al, 1969), and CER (Levine & Jones, 1965).

The effect on active avoidance is particularly interesting and well documented. Murphy and Miller (1955) reported that exogenous ACTH served to prolong the conditioned avoidance responding (CAR) of rats in the two-way shuttlebox during extinction. Later, essentially the same finding was reported with adrenalectomized rats (Miller & Ogawa, 1962). This raised the possibility that CAR prolongation by exogenous ACTH was mediated by direct action of the hormone at the level of the CNS. This possibility has been actively explored in a series of reports by De Wied and several colleagues, and the work has been well summarized elsewhere (De Wied et al, 1968). The purpose of this paper is to report two studies that demonstrate a significant behavioral effect of the zinc phosphate vehicle in which some ACTH preparations are suspended for repository action (De Wied, 1966).

EXPERIMENT 1

A total of 48 Wistar male rats was used. Sixteen received subcutaneous injections of ACTH (Cortrophin Zinc, Organon, Montreal), 16 received zinc phosphate vehicle prepared by the method of De Wied (1966), and 16 received physiological saline, equal volumes throughout.

These heterogeneously bred animals were supplied by Canadian Breeding Laboratories, St. Eustache, Quebec, and weighed from 140 to 180 g when received. They arrived on Wednesday, were injected on Sunday, and were tested beginning the immediately following Monday.

Apparatus

The Ss were tested in two-way shuttleboxes similar in design to those used by Murphy & Miller (1955) and Bohus & De Wied (1966); they were 18 x 6 x 10 in. with a floor of grid bars spaced ½ in. apart. A small barrier (¾ in. high) across the center served to add definition to the Ss' responses which were signaled by microswitch relays. Perching on the barrier was eliminated by electrification of the barrier. The CS consisted of the simultaneous activation of a centrally mounted buzzer speaker (Foringer Multiple Stimulus Panel No. 1166-4) and of the overhead light (6 W) in the chamber to be avoided. CS-UCS interval was 5 sec, and both CS and UCS were terminated together. In the case of failure to respond during extinction, an upper limit of 20 sec was set for the CS duration. The intertrial interval ranged from 15 to 45 sec and averaged 30 sec. Each shuttlebox was encased in an individual sound-attenuating chamber and operated by independent electromechanical programming and recording equipment. The UCS was supplied by a specially constructed power source that allowed the duplication of the UCS parameters used by De Wied (1966). This source delivered 25-V ac 5.0-mA short-circuit values to the terminals of a Grason-Stadler grid scrambler.

Procedure

Ss were given 10 trials per day in the described apparatus. The first trial each day began an average of 30 sec after S was placed in the apparatus, and S was removed approximately 30 sec after the end of the last trial each day. Daily acquisition sessions contined to the criterion of 80% averaged over 3 days, with no less than 80% CAR on the last day. Ss not reaching criterion after 150 trials were discontinued. As mentioned above, injections began the day before the first day of training. Ss were injected subcutaneously with 10 I.U./kg Cortrophin Zinc (Organon) 40 I.U./cc every second day, or with an equal volume of physiological saline or zinc phosphate vehicle (De Wied, 1966). Extinction testing was conducted as was acquisition, but without presentation of the UCS. Extinction was terminated when S reached the criterion of less than 50% CAR on 2 consecutive days.

Each S was run at the same time of day each day and in the same apparatus, and all cells in the design were counterbalanced as to apparatus and time of day.

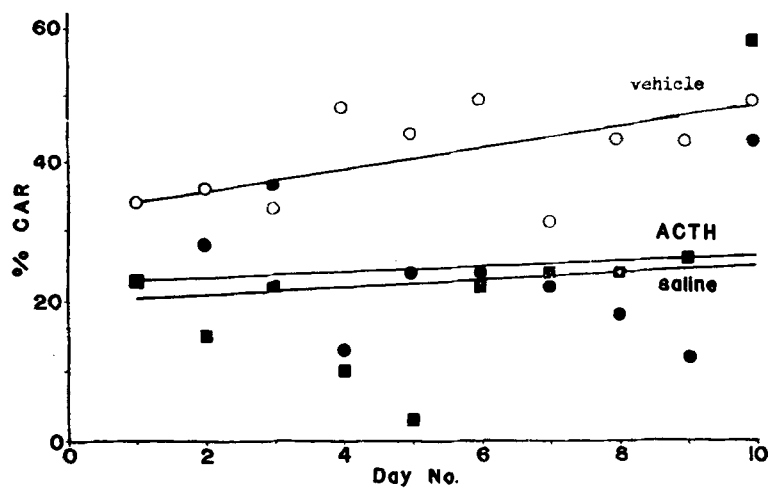

Fig. 1. Mean percent daily CAR for Ss receiving ACTH (●), Saline (■), and zinc phosphate vehicle (0) during shuttlebox acquisition.

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Table 1
Acquisition and Extinction Performance of Vehicle and NaCl Control Ss (Mean ± SE)

	0.2 mA		0.5 mA	
	Vehicle	Saline	Vehicle	Saline
CAR to Criterion	30.8 ± 1.52	33.2 ± 1.52	40.2 ± 2.11	35.5 ± 1.98
Trials to Criterion	40 ± 0	50 ± 6.12	68 ± 7.16	55 ± 7.46
CAR in 40 Trials	30.8 ± 1.29	24.4 ± 5.14	18 ± 3.10	18 ± 5.82
CAR to Extinction	85.6 ± 39.73	47.8 ± 6.84	94.0 ± 33.39	30.5 ± 13.15

Latencies and UCS presentations were independently and automatically recorded.

Statistical analyses employed the appropriate models of the parametric analysis of variance suggested in Winer (1962). Where the homogeneity of variance assumption was significantly violated, the test designed by Festinger (1943) for exponential distributions was used. All probabilities given are two-tailed.

Acquisition performance will be reported and discussed in terms of trials and number of CARs to acquisition and number of avoidances in the first "X" trials ("X" being set at the minimum number of trials required by any S in an experiment to reach criterion). This last index can be extremely sensitive to progress during the early, and probably the most important, stages of acquisition. Its sensitivity accrues from the lack of necessity to discard nonlearners and, more important, from its relative resistance to performance fluctuation often seen in endocrine studies employing spaced practice over days (e.g., Mason, 1959). The major disadvantage of this index is that an unusually quick learner may set "X" so low that many Ss have not left baseline by that trial. This possible weakness, however, did not manifest itself in these studies.

Extinction performance will be reported in terms of the number of CAR to criterion.

Results

Figure 1 records the percent daily CAR for each of the three groups: ACTH-, NaCl-, and zinc phosphate-injected, during the first 100 trials of acquisition. Analysis of variance revealed that the injection ($p < .01$), training sequence ($p < .01$), and interaction effects ($p < .05$) were all statistically significant. Individual comparisons among the means revealed that the zinc phosphate vehicle group differed significantly from both the ACTH ($p < .05$) and the saline ($p < .05$) groups, which did not differ significantly from one another.

Extinction performance was not assessed since the small number of Ss (about 35%) reaching acquisition criterion would render the conclusions equivocal.

EXPERIMENT 2

Experiment 2 was designed as a test of the generality of the finding reported in Experiment 1 over a larger range of UCS parameters.

Apparatus and procedure are the same as in Experiment 1, with the exception that a Grason-Stadler operant conditioning apparatus (No. E 1064GS) supplied the UCS. This apparatus delivered a working current of 350 V ac into a multiple resistor bank with which E could control the milliamperage delivered to S through a range of 0.05 mA to 4.0 mA. Preliminary studies revealed that 0.2 mA was the lower limit consonant with consistent motivation of our S population, while above 0.5 mA there were sharp reductions in the percentage of Ss reaching acquisition criterion within our training limits (150 trials).

Twenty Ss similar to those employed in Experiment 1 were divided into four groups: (1) saline, 0.2 mA, (2) zinc phosphate vehicle, 0.2 mA, (3) saline, 0.5 mA, and (4) zinc phosphate vehicle, 0.5 mA.

Results

Table 1 records the mean trials and CAR to acquisition, mean CAR in 40 trials of learning, and mean CAR to extinction for the four groups. In the case of all indicies of acquisition there was no significant main effect (of injection), but in all cases there was a significant effect of UCS intensity ($p < .01$) and a significant UCS by Injection interaction ($p < .01$). Close inspection of Table 1 reveals why this is so: Zinc phosphate vehicle slightly facilitated performance at 0.2 mA and had a slightly detrimental effect at 0.5 mA. In no case did the comparison of individual mean differences yield statistical significance.

The picture changes somewhat during extinction, however. Zinc phosphate vehicle appears to prolong CAR performance at both 0.2 and 0.5 mA during extinction. These data were analyzed with the Festinger test when preliminary analysis suggested extreme heterogeneity of variance. Due to the small sample sizes involved, the large mean differences at 0.2 mA were not significantly different and those at 0.5 mA were only marginally so ($p < .1$).

Table 2 compares the mean latency scores during acquisition for the saline Ss in both experiments. By means of this comparison, it can be appreciated that the UCS parameters used in Experiment 1 represent conditions of minimal motivation for our S population. This probably accounts for the small percentage of Ss reaching the acquisition criterion in Experiment 1.

DISCUSSION

Earlier reports by De Wied (1966) and his colleagues have shown that ACTH, in low doses (10 I.U./kg), injected during extinction markedly prolongs shuttlebox CAR; that this behavior is completely dependent upon the structural integrity of a well-defined segment of the molecule (Bohus & De Wied, 1966); and that lesions in the parafascicular nucleus of the thalamus abolish the effect (Bohus & De Wied, 1967). Also, a series of studies by Weijnen & Slangen (1970) seems to suggest a remarkable behavioral specificity for this effect.

On the other hand, a large literature has recently been surveyed by Levine (1968), suggesting that increased levels of pituitary-adrenal activity, usually associated with increased ACTH secretion, have a slowing effect on performance in numerous behavioral situations.

The present data suggest a possible reinterpretation of De Wied's work that is consistent with both behavioral slowing produced by elevated levels of adrenal corticosteroids and prolonged CAR extinction resulting from injection of zinc-based ACTH.

Activation theory (e.g., Malmo, 1959) suggests that optimal performance on any given task is obtained within a restricted range of behavioral arousal, increase or decrease in which leads to performance deterioration. It is conceivable on the basis of the present evidence that the stimulant action of the zinc phosphate vehicle led to detrimentally high levels of arousal in De Wied's (1966) control group, while the corticotrophic activity of the ACTH given the experimental group counteracted the stimulant effect of the vehicle, normalizing arousal and prolonging extinction.[1]

This interpretation would be consistent

Table 2
Latency During the First 4 Days of Acquisition at Three UCS Intensity Levels (Mean ± SE) for Saline Groups in Both Experiments

	Day 1	Day 2	Day 3	Day 4
25 Vac. 5.0 mA	11.5 ± 0.76	11.6 ± 1.11	10.7 ± 1.15	13.5 ± 1.36
350 Vac. 0.2 mA	5.1 ± 0.37	2.7 ± 0.64	1.7 ± 0.42	1.7 ± 0.41
350 Vac. 0.5 mA	5.4 ± 0.96	4.7 ± 0.98	4.9 ± 1.54	3.9 ± 1.47

with De Wied's (1966) report that CAR prolongation could only be obtained with the zinc phosphate preparation.

Another important fact apparent from these data is the high degree of variance in S behavior. This is a common finding in research on the pituitary-adrenal system. The human clinical literature (Cleghorn, 1952) reports opposite behavioral effects of ACTH in different patients, and the research literature refers to bimodal or J-shaped distributions (e.g., Murphy & Miller, 1955) often extreme enough to warrant the application of statistical methods developed for exponential distributions. Several strategies have been employed to circumvent some of these difficulties. These include discarding slow learners (or "nonlearners"), discarding fast learners (or "hyperreactive" Ss), discarding fast (or zero avoidance) extinguishers, discarding (or more often discontinuing) Ss slow to extinguish, and the use of highly inbred S populations. Information regarding the effects of some of these procedures is only gradually becoming available (Joffe, 1964; Brush, 1966).

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NOTE

1. However, preliminary results from our laboratory (using other strains of Ss in the shuttlebox, as well as Ss of the strain used here but tested in other behavioral situations) show that the effect of the vehicle must be determined separately for each new combination of S and task variables.

Gerbil's pinnae movement as related to stimulus frequency and intensity*

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Pinnae responses to onset of tones, which ranged in frequency from 0.5 to 10 KHz and in intensity from 51 to 101 dB, are reported. The quality and frequency of these responses varied directly with intensity, but were not systematically related to frequency. The extent to which these responses index auditory sensitivity or serve a protective function are considered.

In a recent study (Lippman & Galosy, 1969) gerbil's pinnae movement (RSO) in response to the onset of a pure tone stimulus was described and reported to vary with stimulus intensity. It was hypothesized that this response could serve a protective function, preventing damage to middle or inner ear structures from high-intensity auditory stimulation by attenuating the amplitude of auditory input. If the RSO does serve to modulate

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