ACTH: Differential Effects on Avoidance and Discrimination

A diversity of behavioral effects following various pituitary-adrenal interventions has been reported[1-7] and is usually attributed to one of two variables: 1. the level of fear or arousal induced by the testing situation[6,8] and 2. the reactivity of the subject sample[7,8].

This paper describes a study of the effect of these 2 variables on the influence of exogenous ACTH on acquisition of a Y-maze brightness discrimination by the albino male rat. We used the Organon preparation of ACTH in zinc-phosphate vehicle; endocrine and behavioral effects of similar preparations have been documented in other publications[2,9]. To examine the effect of differences in level of fear or arousal we used 2 levels of footshock, with the subjects in each group being divided equally between the shock levels; and to examine the effect of differences in individual reactivity to shock we further divided each group on the basis of latencies on the first trial in the Y-maze. Subjects scoring above the median latency were placed in group A (hypo-reactive), those below the median, in group B (hyper-reactive). This index of subject reactivity offers several advantages over some of the others commonly used: 1. it is an index of in situ activitydes irable because of the low intercorrelation of various indices of behavioral reactivity[10], and their low correlation with performance in aversive testing situations[11]; 2. since the Ss were injected prior to testing it was necessary to have an index not affected by drug treatment; pilot experiments in a pole climb apparatus and a shuttlebox, as well as subsequent analysis of these data (Table I) revealed that this index satisfies this criterion; and 3. it was necessary to use an index unconfounded by acquisition performance, and this consideration limited in situ measures to the first or early trials.

Materials and method. 72 Wistar male albino rats (160 ± 15 g) were tested in a varnished plywood Y-maze, the arms of which were 12 inches long, 6 inches wide, and 18 inches high. Each arm was equipped with a 15 watt house light at the far end, and footshock (650 Vac; 0.2 or 0.4 mA) was independently provided to each of the 3 arms through a Grason-Stadler grid scrambler.

The subjects were given 2 s.c. injections (behind the neck) of ACTH, vehicle or saline. The first injection was given 3 days before testing and the second was given 1 day before testing. The ACTH was in the form of long-acting Cortrophin Zinc (Organon, Oss, Holland), 40 IU/cm³, and the dose was 10 IU/kg body weight. This dose was used by DE WIED, BOHUS and GREVEN[9], as well as in previous studies in this laboratory[12]. The control vehicle, a zinc-phosphate complex, was prepared according to DE WIED[2], and the saline group was included in response to the finding[5] that the control vehicle has behavioral effects under some conditions.

30 sec after placing S in arm 1 of the maze, the arm in which S was located and another arm to the right or left was illuminated. 5 sec later electric shock was applied to the grid bars of the 2 illuminated arms until S entered the darkened arm. The illumination and footshock were then terminated simultaneously. This procedure was followed for 100 trials in a single session or until S made 16 correct choices out of 20 consecutive trails. The inter-trial interval was 30 sec, and the position of the darkened arm relative to S was determined by a Gellermann series[13]. All training took place in a darkened room.

In order to examine the effect of these interventions on different aspects of behaviour we took measures of both latency and errors. They were manually recorded by an observer who was unaware of the injection treatment of the subjects. Only the first error on each trial was scored. An error was defined as extension of head and forepaws under the partition marking the incorrect arm.

Table I. Latencies on trial one

UCS	ACTH	Saline	Vehicle
0.2 mA	6.08	5.08	6.70
0.4 mA	6.04	6.06	6.50

Values presented represent the $\sqrt{x} + \sqrt{x+1}$ transformation used in the statistical analysis.

Table II. Mean number of Y-maze responses in less than 5 sec (avoidance) made by subjects of low (A) and high (B) behavioral reactivity treated with ACTH (A), vehicle (V) or physiological saline (S), and tested at 2 levels of footshock, 0.2 mA or 0.4 mA.

	Group *A*			Group *B*		
UCS	ACTH	Saline	Vehicle	ACTH	Saline	Vehicle
0.2 mA	8.5	13.6	12.5	26.7	12.5	11.3
0.4 mA	26.0	12.3	15.6	16.0	21.7	21.1

Brackets indicate significant mean comparisons at $p < 0.05$.

Table III. Mean errors to acquisition of two-choice brightness discrimination in the Y-maze

	Group *A*			Group *B*		
UCS	ACTH	Saline	Vehicle	ACTH	Saline	Vehicle
0.2 mA	11.0	16.3	14.3	19.0	18.2	16.0
0.4 mA	35.6	17.0	16.3	16.9	26.8	32.5

Analysis of variance 14; triple interaction significant at $p < 0.01$ (df 2,60; F 6.73). Brackets indicate mean comparisons significant at $p < 0.05$.

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Results. First-trial latency: Analysis of variance of the first trial latencies (corrected for non-homogeneity of variance by the $\sqrt{x}$ and $\sqrt{x+1}$ transformation; WINER[14]) revealed no effect of injection or unconditioned stimulus (UCS) intensity (Table I).

Conditioned avoidance responding (CAR): The design of the present testing situation was such that the subject could avoid footshock in the starting arm by leaving it during the 5-sec period between conditioned stimulus (CS) onset and UCS onset. Analysis of variance of such avoidance responses (Table II) revealed significant (df 2, 60; F 6.65; $p < 0.01$) interaction of injection, behavioral reactivity and UCS intensity. Using the error MS in the denominator of a t-test, individual comparisons of mean values showed that injections of ACTH significantly ($p < 0.05$) increased the number of CARs of group B subjects at 0.2 mA UCS intensity over those of saline or vehicle control subjects; the 2 control treatments did not differ in effects. Moreover, at the 0.4 mA UCS level ACTH significantly ($p < 0.05$) increased total CAR of group A subjects over those of saline or vehicle treatments, which did not differ in effect.

Errors: The number of errors during acquisition of the two-choice discrimination by groups A and B was differentially influenced by ACTH at the 0.4 mA UCS intensity, but not at the 0.2 mA level. At the higher UCS level injection of ACTH significantly ($p < 0.01$) increased the errors to acquisition of group A subjects and significantly ($p < 0.01$) decreased the errors of group B subjects (Table III).

It might be suggested that the large differences in error scores represent a selective bias introduced by dividing the treatment groups according to first trial latency. Such a bias could conceivably have been introduced if group A included a larger proportion of subjects making errors on trial one. However, examination of the data shows that most subjects (72%) made a correct choice (i.e. entered the non-electrified arm) on trial one, and eliminating the effect of the subjects making an incorrect response on trail one did not alter the relationships or statistical probabilities reported here.

Discussion. It could be argued that the number of conditioned avoidance responses a subject would score in leaving the starting arm of the Y-maze might be a function of the stage of learning of the 2-choice discrimination, and thus that the CAR (Table II) and error (Table III) data are not independent. Comparison of Tables II and III reveals a more complex situation. At the low level of footshock ACTH had no effect upon errors, but did affect CARs of group B, while at the higher level of footshock ACTH affected the errors of both groups A and B, but only influenced the CARs of group A.

The disjunction in this task between the effect on the avoidance component and the discrimination component has considerable theoretical significance. It has been suggested that the behavioral influence of pituitary-adrenal interventions may be maximal under conditions of low fear-arousal[6]. The tasks on which this notion is based include one-way active avoidance extinction, passive avoidance and CER[6]. In each of these tasks there is some form of response suppression under latency-sensitive conditions. The results in the present study of the latency-sensitive (avoidance) component of the Y-maze task support and extend this suggestion in that the behavioral effect of ACTH is present in the hyper-reactive subjects at the lower footshock level, and is obscured in these subjects by the more intense footshock. Moreover, these data also show that at the higher footshock level the effects of ACTH on CAR are revealed in subjects of a different activation level. However, the discrimination (error) measure presents a different pattern of effects altogether. The maximum effect of ACTH appears at the higher footshock level and is obscured at the lower level of intensity.

In more general terms these results clearly show that ACTH effects will be seen as varying with the type of behavioral measure used as well as with the type of situation. The theoretical and experimental literature provides neither explanation nor precedent for these results; our conclusion is that exploration of the behavioral and physiological effects of ACTH using a wide range of behavioral situations and indices will be essential before more robust theoretical statements can be justified.

Zusammenfassung. Andauernde ACTH-Behandlung beeinflusst sowohl Vermeidungs- als auch Fehlerkomponenten im Zweiwahl-Y-Labyrinthtest. Die Einwirkungen waren von der Intensität des Elektroschocks wie auch von der Reaktionsfähigkeit der Tiere abhängig.

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Modifikationen der Nebennierenrindenfunktion in vitro durch 3- und 4-Pyridinderivate[1]

In unseren beiden letzten Mitteilungen über dieses Thema haben wir die Bedeutung von Steroiden als Hemmstoffe bzw. Regulatoren diskutiert[2] und über verschiedene N-Heterocyclen wie Glutarimide und Benzylimidazole als Blocker der Desmolase bzw. der 11β-Hydroxylase und der 17-Hydroxylase berichtet[3].

In der vorliegenden Arbeit wird eine Reihe von Pyridin-Derivaten, die die Nebennierenrindenfunktion in vitro in differenzierter Weise modifizieren, beschrieben. Auf die Spezifität der Hemmwirkung einiger bereits länger bekannter Derivate, wie Metopiron®, Su-9055, Su-10603, haben wir vor einigen Jahren hingewiesen.[4]

Wir benützten die gleichen Nebennierengewebepräparationen und Inkubationstechniken und bedienten uns der gleichen Analytik und Interpretation, wie wir sie an anderer Stelle ausführlich beschrieben haben[3,5]. Als Mass

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