PSYCHOLOGY: Ph.D. Kenneth F. Ley, Jr.

ADRENOCORTICOTROPHIC HORMONE: STUDIES OF BEHAVIORAL EFFECTS

The two-way shuttlebox was used to test the effect of exogenous adrenocorticotrophic hormone (ACTH) on the formation and extinction of conditioned avoidance responding (CAR) of Long-Evans Hooded male and female and Wistar albino male rats at several parameters of footshock. It was found that, depending upon the sex, stock and emotionality of the subject, and upon the footshock parameters, exogenous ACTH might retard, accelerate or have no effect upon the early stages of CAR formation and might retard, accelerate or have no effect on CAR extinction. It was also found that the zinc phosphate hydroxide vehicle in which some ACTH preparations are suspended for repository action seems to effect conditioned avoidance responding under some conditions of acquisition and extinction. Furthermore, ACTH was found to reduce VI:35 bar pressing in the Skinner box for food at 24 and 72, but not 48 hours of deprivation.

ACTH: Studies of Behavioral Effects K. F. Ley, Jr.

ADRENOCORTICOTROPHIC HORMONE: STUDIES OF BEHAVIORAL EFFECTS

by

Kenneth F. Ley, Jr.

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Department of Psychology
McGill University
Montreal - Canada

July, 1970

ACKNOWLEDGEMENTS

Aside from the invaluable advice and support of my research advisor, Dr. J.A. Corson, the contributions of the following persons are gratefully acknowledged: Mrs. S. Kertesz for help in data collection, Mrs. B.A. Ley for help in data collection, reduction and presentation, Mr. Herb Lee for help in electrical programming and Miss L. Parent for patient preparation of the typescript.

TABLE OF CONTENTS

INTRODUCTION

In recent decades there has been a growing appreciation of the range and importance of the behavioral effects mediated by endocrine function in general and by the pituitary-adrenal axis in particular. This has largely followed upon an increased understanding of the complex function of the tiny pituitary gland at the base of the mammalian brain.

The mammalian pituitary is divided into three parts: the anterior portion or pars distalis, the intermediate lobe, and the posterior portion, the pars nervosa. The pars distalis is the source of the endocrine trophic hormones. This discussion will be concerned with one of the more important of these, the adrenocorticotrophic hormone or ACTH. This hormone is released in response to physical and psychological trauma to act upon the adrenal cortices. The various products of these in turn alter the metabolic state of the organism. The adrenocortical secretions include the 11-oxygenated corticosteroids or glucocorticoids which function primarily in carbohydrate and protein metabolism, the 11-deoxycorticoids and aldosterone, which control water and electrolyte balance, and the various sex hormones, androgens, progesterone and the estrogens (Turner, 1966). The adrenal medulla which is sympathetically

enervated is directly subject to adrenocortical function through its dependence upon glucocorticoids in the manufacture of its catecholamine products, the chief of which is the sympathetic amine, epinephrine (Turner, 1966). The pars distalis is thought to lack neural innervation, the secretion of its various trophic hormones, including ACTH, being mediated by the hypophyseal portal blood flow from the hypothalamus.

The pars intermedia, a thin sliver of tissue between the pars distalis and the pars nervosa, secretes melanocyte stimulating hormone, MSH, which acts in the skin darkening process. This hormone has two forms, the alpha and the beta, which are structurally similar to portions of the ACTH molecule. The alpha form, in fact, contains the identical sequence of amino acid subunits found in the first thirteen positions on the ACTH molecule (Li, 1963).

The neural portion of the mammalian pituitary, the pars nervosa, secretes vasopressin, which has pressor and antidiuretic action, and oxytocin, which causes smooth muscle contraction and milk ejection (White, Handler & Smith, 1964).

Recent studies in several fields have implicated the endocrine system and its neural connections (collectively

known as the neuroendocrine system) in behavioral adaptations ranging from individual affective state to population dynamics. In the first category, numerous studies have elaborated some of the neural interconnections of the pituitary-endocrine system with subcortical limbic structures which may be concerned with motivation and emotional behavior (de Groot, 1966). This evidence is quite consistent with the clinical reports that replacement therapy with factors from the pituitary-endocrine system produces changes in the affective state of humans (Cleghorn, 1952; Ronziger & Kindwall, 1948; Seward & Seward, 1937; Werner, 1935). These findings, among others, have led several authors to formulate positions implicating the biogenic amines, epinephrine, norepinephrine, dopamine and serotonin in the affective disorders (Schildkraut & Kety, 1967; Rubin, 1962); and Sourkes (1964) has emphasized the relevance of neuroendocrine variables, in general, to mental disease.

In the second category are the investigations of ethologists, ecologists and endocrinologists into the role of neuroendocrine function in the regulation of mammalian populations. In a study of rodent populations inhabiting the essentially isolated niches of Baltimore City block areas, Christian and Davis (1956) concluded that adrenocortical function as measured by weight and histological

changes in these glands was directly related to population density. Reproductive function as measured by litter size and frequency and gonadal size and morphology, on the other hand, showed a strong negative relation to population density. Christian, Lloyd and Davis (1965) have recently shown that this phenomenon has great generality in mammalian populations. The critical factor in producing these endocrine imbalances seemed to be an undefined social pressure which was thought to be related to agonistic behavior, especially the physical trauma of fighting. However, since the number of interanimal fights has been shown to be a direct negative function of population density (Welch & Welch, 1966), this straight-forward hypothesis based on physical stressors alone has been called into question.

An alternative hypothesis has been presented that psychological factors are important in activating the endocrine mechanisms responsible for the maintenance of stable density in rodent populations (Friedman & Derks, 1966; Bronson & Eleftheriou, 1965; Ader, 1964; Sawrey, Conger & Turrell, 1956). The report that psychological phenomena powerfully influence neuroendocrine function in higher primates and humans (Mason, 1959) supports the generality of this position.

Thus far, two areas of endocrine-behavioral interaction have been described, the individual case in which neuroendocrine imbalances have been implicated in affective disorders, and the population studies which have shown that a readjustment of endocrine balances may follow upon increased population density as a result of psychological factors. That these two lines of evidence are not unrelated may be concluded from some reports of altered behavior patterns associated with artificially maintained high population density and its concomitant endocrine changes.

Calhoun (1962) has investigated the aberrant behavioral patterns that can become manifest in situations of artificially maintained high population density, and reports such as the Manhattan Study (Srole, Langner, Michael, Opler & Rennie, 1962) indicate that such phenomena may extend to the human level. Just how high population density and the concomitant endocrine upset act to generate aberrant behavior is not yet clear although an early study by Young and Rundlett (1939) on hormonal induction of homosexual behavior in guinea pigs and the recent work of Fisher (1964) with rats showing that artificially produced hormone excesses in the central nervous system can produce abnormal sexual behavior, are at least suggestive.

Further evidence that behavior may be modified by altered neuroendocrine states comes from the study of pituitary-adrenal function in avoidance learning.

Several behavioral techniques have been used to study the extent of pituitary-adrenal involvement in avoidance learning. Some investigators (Lissák and Endröczi, 1961; Levine & Jones, 1965) have used the Conditioned Emotional Response, or CER, technique, which involves examination of the effect of nociceptive stimulation on responses which had been previously established for appetitive reward. They report that the degree of appetitive response suppression after establishment of CER is larger in subjects with greater pituitary-adrenal response as measured by plasma levels of corticosterone (PC) and in subjects treated with ACTH to raise PC levels prior to the nociceptive stimulation.

This evidence suggests that ACTH may act by elevation of PC levels to increase response suppression in aversive situations.

An alternative, but not necessarily incompatible, suggestion arises, however, from studies on the role of pituitary-adrenal activation in active avoidance tasks. It has been reported (Murphy & Miller, 1955; de Wied, 1966) that exogenous ACTH prolongs extinction of shuttlebox conditioned avoidance responding (CAR). This suggests that elevation

of pituitary-adrenal function is related to response activation in aversive situations.

Levine (1968) has recently attempted a synthesis of these seemingly divergent observations by suggesting two complimentary postulates (which will be labelled 'A' and 'B' for convenient reference). He has suggested that (A) elevated PC levels are associated with the development of inhibitory processes which lead to response suppression, whereas (B) ACTH may counteract inhibitory processes and thus prevent shuttlebox CAR extinction.

A possible difficulty with this dual hypothesis is that elevated PC levels rapidly follow ACTH administration or release and most studies have tested performance at least 90 minutes after injection of long-acting ACTH (12 hours duration of action or more). By this time, both PC and ACTH levels should be quite high so that difficulty is encountered in explaining the differential behavioral effects in the two situations on the basis of differential effects of the two hormones.

However, it may be possible to account for prolongation of shuttlebox CAR extinction by exogenous ACTH solely on the basis of (A) the elevation of inhibitory processes by increased PC after ACTH.

Arousal theory (Malmo, 1959; Hebb, 1966) suggests that the level of arousal optimal for performance of a given task is a function not only of the subject but also of the task complexity. The general shape of these functions has been shown to take the shape of an inverted-U as in figure I:1. It may be proposed from such a relationship that, depending on the arousal level of the control subjects (Ss), acceleration of shuttlebox CAR extinction may just as well result from decreased inhibition (leading to hyper-arousal and possibly immobility) as from increased inhibition. In this context, the prolongation of shuttlebox CAR extinction by ACTH could conceivably arise either from the elevation of inhibitory processes by PC or from an opposite effect produced by ACTH.

The power of extending the hypothesis (A) relating the behavioral effects of ACTH to elevation of PC levels to include all the reported behavioral effects of ACTH will be explored in the following review of the literature. Considerable attention will be paid to the technical details reported in the research literature such as sex, stock and age of Ss, unconditioned stimulus (UCS) intensity, and type of task as these are widely recognized to influence the shape of the relationship pictured in figure I:1. (Whenever, in the following discussion, these details are not

presented, this will mean that they did not appear in the report cited.)

The studies reviewed will be organized around the three major subject preparations in which pituitary-adrenal function has been studied: the intact animal, the adrenalectomized animal, and the hypophysectomized animal. A further section is added to include some recent studies of hormone analogues, which are behaviorally active synthetic fractions of hormone molecules.

Studies of the intact animal make up the greater bulk of the behavioral studies of pituitary-adrenal function and, of these, studies of the effects of ACTH injections make up at least half the number. However, the type of ACTH preparation, dose level and time of administration relative to behavioral testing vary widely and render comparison difficult.

Three major types of ACTH preparation have been studied: ACTHAR is a commercial preparation (Armour, Co.) of highly purified pork ACTH suspended in a gelatin vehicle for repository action. Like other gelatin preparations it produces a large adrenocortical response in the first hour after administration and maintains an elevated adrenocortical secretory response for several (12 to 15) hours when given

intramuscularly. Cortrophin Zinc (Organon, Oss, Holland) is another commercial preparation of highly purified pork ACTH suspended in a zinc phosphate hydroxide complex (see de Wied, 1966 for the formula). This preparation has a longer time course of action (24 - 72 hours) than does the gelatin preparation, and de Wied (1966) has reported dissimilar behavioral results using ACTH suspended in the two vehicles. Some workers have also used ACTH in physiological saline suspensions; this preparation exerts immediate effects on adrenocortical secretion which are not significantly prolonged beyond the first hour after administration (depending on the dose).

Dose levels of ACTH have varied from roughly five International Units (I.U.) per kilogram of body weight (5 I.U./kg.bw.) in the rat to 200 I.U./kg.bw. Although the stress secretion rate of ACTH has not been determined in the rat, the basal concentration has been estimated (Mangili, Motta & Martini, 1966) at less than .001 I.U./100 cc of rat blood (a rat has about 20 cc of blood). Consequently, it may be inferred that even the lowest levels investigated in behavioral studies represent plasma concentrations unlikely to be maintained for long periods of time in the normal animal.

The time of administration prior to testing might be a significant variable in studying the relative role of ACTH and PC since there may be a time lag between ACTH administration and PC elevation. However, this variable has not yet received careful study.

STUDIES ON THE INTACT ANIMAL

Mirsky, Miller and Stein (1953) reported a sharp decline in behavioral suppression by CER (i.e., a behavioral activation) in monkeys given ACTH (15 mg. ACTHAR) during the CER training. Monkeys were trained to bar press (B-P) for a tone followed by a piece of grape; they were then exposed in the same apparatus to unavoidable severe footshock signalled by the same tone. During this latter phase experimental Subjects (Ss) were given ACTH daily and control Ss received gelatin vehicle. At the end of 10 days of tone-shock pairing (10 presentations per day), the reward situation was reintroduced, and the B-P again produced the tone and the grape. Subjects receiving ACTH during 'fear training' responded consistently higher during subsequent testing over a five day period.

In a similar testing situation, Ss were trained to press a bar to avoid signalled footshock. After reaching an acquisition criterion, they were given ACTH (15 mg. ACTHAR) or control injections of gelatin vehicle and placed on an extinction schedule. ACTH Ss extinguished considerably more quickly than control Ss.

In the same report the authors describe the performance of rats in an apparatus used by Miller (1948)

in his demonstrations of fear as an acquired drive. The rats were first trained to avoid a distinctively marked white compartment by running into an adjoining black chamber in response to footshock. After the avoidance behavior was established, a door operated by a manipulandum (ratchet wheel) was inserted between the two compartments. Rats were injected with ACTH (5 mg. ACTHAR) or gelatin vehicle, and their performance in the new situation was studied. During the early stages of learning the new task, ACTH Ss made fewer ratchet-turning responses and had higher median escape latencies than control Ss. These results were discussed in terms of the ability of ACTH to reduce the effects of traumatic memory on the behavior of the Ss.

However, it is noteworthy that in two of the studies presented the Ss were tested under extinction conditions following large doses of exogenous ACTH. In these studies ACTH was associated with considerable behavioral slowing in both monkey and rat Ss. In the third study the effect of ACTH on CER was studied in the monkey. However, the ACTH was given daily for the 10 days prior to testing and not during the testing period itself. This is a significant departure from the procedure in the other two studies. It is difficult to infer the relative state of the endocrine system of the Ss under these conditions. If the prior

10 days of treatment with large doses of ACTH had resulted in adrenal depletion, then it is possible that endogenous levels of ACTH might have been quite high in the absence of feedback suppression by the depleted adrenal. Alternatively, it is also possible that endogenous PC levels might have been high following prolonged ACTH treatment, and, that as a consequence, endogenous suppression of ACTH release might have resulted, at least for the first few days of testing.

On the other hand, the increase in responding may represent an interaction of prior ACTH treatment with an appetitive drive state or with incentive motivation. However, at present there is no evidence which permits unequivocal selection of an explanation for these effects.

It is of considerable interest that both the rat and monkey responded to exogenous ACTH with behavioral slowing in an aversive situation in view of the studies by Woodbury (1954) suggesting that the principal adrenal steroids of these two species tend to have opposite effects on central nervous system (CNS) excitability. Using several measures based on the electro- and chemoconvulsive thresholds, cortisol, the major adrenal glucocorticoid in the cat, monkey and man, was found to exert considerable effects on electrolyte distribution and general irritability or excitability in the CNS. However,

corticosterone, the principal adrenal glucocorticoid in rat and mouse was found to have little effect on CNS seizure thresholds. This evidence suggests that other physiological mechanisms may participate in these apparent similarities between rat and monkey in response to exogenous ACTH.

Further complexities are introduced by a later report from the same laboratory (Murphy & Miller, 1955). The effect of exogenous ACTH on CAR acquisition as well as extinction was studied in the rat. There were four groups of Ss: one received ACTH (5 mg. ACTHAR) during both acquisition and extinction of shuttlebox CAR; one received ACTH during acquisition and gelatin vehicle during extinction; one received vehicle during acquisition and ACTH during extinction; and one received vehicle during both acquisition and extinction. No significant effect of ACTH was noted during acquisition of the CAR nor was any significant effect noted when ACTH was given only during extinction. However, ACTH given during acquisition significantly prolonged CAR extinction regardless of whether treatment was maintained or discontinued during extinction.

A second experiment reported in the same paper studied the effect of ACTH given only during CAR acquisition.

Using a more stringent acquisition criterion the authors found an even larger difference between the sharply prolonged CAR extinction of the ACTH Ss and the rapid CAR extinction of the gelatin control Ss. The authors conceded that these results contradicted the earlier report of Mirsky et al. (1953) in which ACTH during acquisition seemed to speed CAR extinction. Several possibilities may serve to explain this contradiction in results.

Murphy and Miller suggested the possibility of subject and procedural differences. And, in fact, a detailed analysis of the procedures employed in the two sets of studies raises the very real suggestion that the ACTH given during the establishment of the original CAR in the Mirsky et al. experiment may have prolonged the behavior and resulted in competing response tendencies which slowed acquisition of the new (ratchet-turning) behavior.

However, the data of Murphy and Miller (1955) may also be subject to reinterpretation. These data showed a strong, but non-significant trend for Ss receiving ACTH to make fewer CAR during acquisition even though they reached criterion in about the same number of trials as the controls. Furthermore, this report gave sufficient detail so that it was possible to do a statistical comparison of extinction CAR of Ss receiving gelatin vehicle during acquisition and

ACTH during extinction with Ss receiving ACTH during acquisition and either ACTH or gelatin during extinction; the comparison reveals a significant ($p<.01$, Festinger test, Festinger, 1943) reduction in CAR performance of the Ss receiving ACTH for the first time during extinction. Both of these observations suggest that ACTH may effect behavioral slowing in the shuttlebox under certain conditions, and are in accord with postulate A. However, the prolonged CAR extinction by Ss receiving ACTH during acquisition may be cited as support for postulate B. But, it is important to note that the effect of ACTH was not altered if the injections were discontinued during extinction. This suggests that the effect of ACTH given during acquisition may have been to prevent the development of internal inhibition during extinction by depletion of the adranal cortex during acquisition. It is important to note that the possibility of behavioral influences of adrenal depletion effects is not explicitly raised by either postulate A or postulate B. However, these effects can easily be incorporated into postulate A and for the purposes of this discussion will be dealt with as such.

In contrast to the study (Murphy & Miller, 1955) reporting that ACTH given only during extinction has no effect on shuttlebox CAR, de Wied, Bohus and Greven (1968)

reported that ACTH (long-acting zinc phosphate preparation) injections given only after acquisition serve to prolong shuttlebox CAR extinction in the intact rat. Using low voltage (25 V), high amperage (1.8 mA) footshock, Wistar male rats were given 10 trials per day CAR acquisition training in the shuttlebox. After reaching and maintaining an 80% CAR performance level for three consecutive days, acquisition was terminated and Ss were injected with ACTH (0.75, 1.5, or 3.0 I.U. per rat), or with zinc phosphate vehicle complex, every other day. There was a graded effect of the dose levels of ACTH on CAR performance during extinction: the highest level of ACTH maintained avoidance levels near 100% over the two weeks of testing; Ss receiving 1.5 I.U. every other day during extinction had dropped to about 85% CAR performance by the end of two weeks, whereas Ss receiving only 0.75 I.U. dropped quickly to about 30% CAR over the two week period. The vehicle control group had reached complete CAR extinction within 11 days.

The authors also reported that the increasing effect of the higher ACTH doses in prolonging CAR extinction parallels the increasing corticotrophic activity of the injections; they proceeded to demonstrate that corticosterone facilitates CAR extinction and that the effect is greater at higher doses of corticosterone. They also

suggested that the opposite behavioral effects of ACTH and corticosterone provide further evidence for an extra-adrenal role of ACTH in behavioral processes. They did not, however, offer any suggestion as to how ACTH might act to counteract the effect of its target organ secretion, nor why the CAR prolongation by ACTH should be dose dependent in a fashion paralleling increased release of the target gland hormone (corticosterone) which they have shown to have opposite behavioral effects.

It has further been shown by the same authors that the suppression of CAR during extinction by exogenous corticosterone occurs in the hypophysectomized (hypox) rat. This implies that the behavioral effect of corticosterone is not merely a reflection of its function in feedback suppression of ACTH release in the intact animal, since the hypox animal releases no ACTH.

It seems that these results are open to several interpretations. The one suggested by de Wied et al. (1968) and Levine (1968) is that ACTH may act independently of its corticotrophic activity, and perhaps at the level of the CNS, to prolong shuttlebox CAR extinction (postulate B). An alternative interpretation in terms of postulate A can also be offered. Such an interpretation might suggest that

the low intensity UCS parameters used by de Wied et al. (1968) did not induce the behavioral inhibition, or passive avoidance tendencies often associated with high UCS levels in the shuttlebox (e.g., Levine, 1966); from this it might follow that the control animals rapidly extinguished due to high levels of arousal and that the long-acting zinc-based ACTH preparation released small, but sufficient levels of adrenal corticosterone to increase internal inhibition to an optimal level for shuttlebox CAR performance. The only evidence that poses serious difficulty for this hypothesis is the report of de Wied et al. (1968) that exogenous corticosterone facilitates CAR extinction. But this relationship is confused by the use of different vehicles; de Wied (1966) has reported that the prolongation of shuttlebox CAR extinction is dependent upon the zinc-based long-acting ACTH preparation, and the corticosterone control study was done with an acute steroid preparation in saline suspension for the good reason that long-acting corticosterone preparations are not available.

Another possible interpretation involves the issue of the time lapse between injection and testing. In their studies of the long-acting ACTH preparation these investigators injected Ss 18 to 24 hours prior to testing. Although the time course of the adrenal secretory response

to zinc-based ACTH is not known in such detail, most long-acting ACTH preparations induce a vigorous adrenal secretory response over the first few hours following administration. This response soon tapers off, however, due both to the inability of the adrenal to maintain elevated secretion rates for an indefinite period of time, and to the inactivation of the administered ACTH. Thus, after some as yet undetermined period of time, the endogenous pituitary-adrenal response to an acute stressor such as shuttlebox extinction testing would be considerably weakened. Pursuing this argument further, the endogenous pituitary-adrenal response would be larger in the control Ss, resulting in the response suppression associated with PC elevation in the testing situation (postulate A).

"CER" STUDIES

Further evidence supporting the possibility that the primary behavioral activity of ACTH is exerted by its peripheral corticotrophic activity is supplied by consideration of a number of studies of appetitive response suppression resulting from footshock in the goal situation. As mentioned earlier, this technique is often referred to as the Conditioned Emotional Response technique, or simply "CER".

Endröczi, Telegdy and Lissák (1957) reported that injecting rats with 2 I.U./kg.bw. ACTH (Cortrophin Zinc) facilitated the acquisition of an appetitive (food) conditioning task in which food was obtained only after the presentation of the conditioned stimulus (CS). They also reported that the CER which developed after the presentation of footshock (unspecified intensity) in the appetitive situation was markedly increased (i.e., increased behavioral inhibition) in animals receiving ACTH, and that the degree of appetitive response inhibition was monotonically related in both experimental and control Ss to the degree of adrenal ascorbic acid depletion after the footshock; maximum depletion, an index of high adrenocortical activity was found in Ss with the longest CER durations. This is in accord with postulate A, and supports the suggestion that the

behavioral effects of ACTH may be understood in terms of its adrenal corticotrophic activity. However, these conclusions may not be warranted since level of deprivation was not specified in this study, and control Ss were not injected.

A similar study by Levine and Jones (1965) affirmed that chronic injections of ACTH (Cortrophin gel; 25 I.U./kg.bw.) prolong the suppression of an appetitive response following response contingent footshock (2 seconds, 1.0 mA; once on each of 2 consecutive days). These investigators used 5 experimental groups: 1) an ACTH-injected, non-CER group to assess the effect of ACTH on continuous reinforcement (CRF) B-P for water at 23 hours deprivation; 2) a group injected with ACTH 3 days prior to, during and 7 days after CER acquisition; 3) a group injected with ACTH 3 days prior to and during the 2 days of CER acquisition, after which injections were terminated; 4) a group injected with vehicle in the same manner as group 2, and 5) a non-injected CER group. They reported that the total average B-P for the 7 days of testing following CER acquisition was, as follows, in the groups described above: group 2, 0.6; group 3, 174.4; group 4, 92.4. There was no effect of ACTH on CRF responding (group 1), nor were control groups 4 and 5 significantly different from one another.

The authors also reported a sharp bimodality in the degree of response suppression following CER training in groups 3, 4 and 5. Approximately half the Ss in groups 4 and 5 made an average total of 166.2 responses over the 7 days, while the other half responded on the average only 2.2 times. This bimodality was associated with a significant difference in adrenal weight, with the higher responding group having the lighter average adrenal weight. A similar bimodality in responding (318.8 Vs. 1.0 responses) between two classes of Ss in group 3 was not associated with significantly altered adrenal weights.

It seems that the presence of a relationship between adrenal weight and performance in groups 4 and 5 and absence in group 3 may reflect the effect of the three days of ACTH injections in group 3. Discontinuing ACTH after CER training would allow endogenous idiosyncratic levels of function to determine behavioral responsiveness whereas the weight changes wrought by ACTH injections would not immediately disappear.

Further evidence for the importance of pituitary-adrenal function in aversive behavior was provided by Wertheim, Conner and Levine (1969). These authors trained Sprague-Dawley albino male rats to avoid unsignalled footshock (1.3 mA) by B-P in the Skinner box. Each B-P

postponed the subsequent appearance of shock for 22.5 seconds. It is probably significant that in the absence of successful avoidance the UCS was delivered not only through the grid floor but also through the B-P lever. Six rats were given 180 one-hour daily training sessions. The difficulty of the task can be appreciated from the range in subject performance from 46% to 93% successful avoidance responses during the 180th session (after approximately 32,400 possible UCS presentations).

Prior to training in the Skinner box, two plasma corticosterone samples were determined for each subject: a basal sample taken by venipuncture immediately after etherization and a stress sample taken 15 minutes later when the secretory response to the ether stress was presumed at maximum. The authors reported a strong relationship between the basal levels of plasma corticosterone and early avoidance performance and a perfect rank order correlation between stress level of P.C. in a given subject and his subsequent level of avoidance performance after 180 daily sessions.

Lissák and Endröczi (1961) have also reported comparative studies of pituitary-adrenal function in the maintenance of CER in the dog and cat. As mentioned earlier, the adrenal secretory response to stress of these animals (along with primates and man) is different in several

important respects from that of the rat. In the dog, cat, monkey and man, the principal adrenal glucocorticoid is cortisol or hydrocortisone, although a considerable amount of corticosterone may also be secreted. Woodbury (1954) has shown that injection of cortisol into the rat markedly increases cortical excitability as reflected in convulsive thresholds, whereas corticosterone does not have this effect and, in fact, will serve to counteract the effect of cortisol.

Lissák and Endröczi (1961) reported that individual differences in the duration of CER suppression of appetitive behavior seen in dogs after ACTH is related to the ratio of these two adrenal steroids: the higher the ratio of these two endogenous steroids in favour of cortisol (hydrocortisone) the longer the duration of behavioral inhibition. Furthermore, this balance may change with time after administration of ACTH or a stress experience.

These observations represent comparative evidence for postulate A, since the balance between behavior activation and inhibition following pituitary-adrenal activation may be understood in terms of the interaction of adrenal steroids, rather than an interaction of adrenal steroids and the trophic hormone, as would be suggested by postulate B.

ACUTE STUDIES

The studies reported on the previous pages have concerned the long-term behavioral adjustments made to chronic injections of long-acting ACTH. The three studies reviewed below report the effects of acute treatment with quick-acting ACTH preparations immediately prior to behavioral testing at the time of the presumed maximum of PC elevation.

Wertheim, Conner and Levine (1967) studied the effect on free operant shock avoidance in the Skinner box (Sidman Avoidance) of acute injections of ACTH (8, 10, or 12 I.U. per 350 gram rat). Control injections included physiological saline (in which the ACTH was suspended) and dexamethasone, a powerful synthetic steroid which mimics and suppresses secretion of adrenal glucocorticoids and suppresses secretion of ACTH as well. The authors reported that both ACTH and dexamethasone reduced response frequency and yet also resulted in fewer UCS presentations in the unsignalled Sidman Avoidance design. They suggested that this was strong evidence that the behavioral effects of ACTH on performance in aversive situations resulted from trophic activity on adrenocortical secretion.

Further evidence on this point was reported by Kasper-Pandi, Hansing and Usher (1970) in a study on the incubation of shuttlebox avoidance. They gave Holtzman male albino rats 25 training trials in a two-way shuttlebox to avoid footshock (1 mA). Half of the Ss had been injected with dexamethasone prior to training, and half with saline. After 25 training trials one-third of each injection group was given another 25 trials immediately, one-third was given the extra trials one hour later, and the final third was tested four hours later. In no case did dexamethasone influence shuttlebox avoidance although it did block the stress-induced elevation of PC levels and presumably ACTH also. The authors reported, however, that both experimental and control Ss tested immediately after the original 25 trials performed significantly better than those tested at the one or four hour interval. They concluded that this performance difference which was not reflected in differential steroid levels argues against the effective participation of the pituitary-adrenal axis in avoidance behavior. They did suggest, however, that their high UCS intensity might have masked any differential effect of pituitary-adrenal function. This may well be true since other investigators (e.g., Levine, 1966) have also suggested that high levels of footshock intensity may act to inhibit CAR performance during acquisition. The best level of

performance Kasper-Pandi et al. were able to report in the second 25 trials was in the range of two to five avoidances. If the primary effect of dexamethasone, as suggested by Wertheim et al. (1969), is inhibitory, then the additional inhibition imposed by dexamethasone may have gone unnoticed because of the already impressive amount of response inhibition induced by the high levels of footshock.

Korányi, Endröczi, Lissák and Szepes (1967) added a significant dimension to the studies of pituitary-adrenal function and behavior by dividing their Ss (albino male mice) into hyperactive and hypoactive groups on the basis of open-field performance. Some of the animals were then given passive avoidance training and others were trained in the shuttlebox active avoidance task. The passive avoidance Ss were given two pre-training trials (one per day) in which the latency to enter a darkened chamber from a brightly lit open field was recorded. Hyperactive Ss given an acute injection of ACTH (10 I.U./kg.bw. saline suspended) prior to the first experience (trial one) showed a significant increase in latency to enter the darkened chamber on trial one (there was no hypoactive control). However, both hyperactive and hypoactive Ss receiving ACTH prior to trial two showed significantly reduced latencies to enter the darkened chamber on trial two when compared to saline

injected controls. On trial two those Ss entering the darkened chamber were confined there by a door and given 'short' (duration was not specified) electric shocks (3.0 mA). Following this the Ss were given seven retest trials (six at daily intervals, the seventh a week after the sixth), and the percentage of Ss entering the darkened chamber with latencies of less than one minute was recorded. A significantly greater percentage of saline-treated hypoactive than ACTH-treated hypoactive Ss reentered the chamber over the course of retesting; on the other hand, a significantly greater percentage of ACTH-treated hyperactive than saline-treated hyperactive Ss reentered. It is important to note that the performance of both hypoactive and hyperactive ACTH Ss was nearly identical while the saline hypoactive Ss performed significantly above and the saline hyperactive Ss performed significantly below the level of ACTH-treated Ss. The authors concluded that the effect of ACTH on performance may depend upon the task difficulty and the behavioral reactivity of the subject population.

Such a relationship may best be understood on the basis of the previously mentioned curvilinear relationship that has been shown to exist between arousal and performance in numerous behavioral situations (e.g., Malmo & Belanger, 1968). The shape of these curvilinear

relationships has also been shown (e.g., Broadhurst, 1957) to vary widely along the dimension of task complexity. Figure I:2 depicts two hypothetical tasks differing in complexity. The simple task (A) has a broad and flat arousal-performance curve, while the difficult one (B) is relatively leptokurtic. Assuming that ACTH acts to reduce arousal, the performance of hypoactive (O) and hyperactive (□) Ss in the untreated (open figures) and the ACTH-treated conditions (solid figures) is shown. It is clear that a simple task such as runway escape will require a considerable drop or elevation in arousal before a detectable change in performance will occur. However, the complex task can also result in considerable misinterpretation since the treated Ss on opposite limbs of the curve could show the same performance on the task. Figure I:2 B may represent a situation similar to that reported by Korányi et al. (1967) in the passive avoidance situation. The hypo- and hyper- active Ss treated with ACTH performed similarly, whereas the respective control groups differed considerably.

APPETITIVE BEHAVIOR

The effect of pituitary-adrenal function on appetitive behavior in the intact animal has rarely been studied. There was a suggestion in the previously cited work of Endröczi *et al.* (1957) that ACTH (Cortrophin Zinc) facilitated the acquisition of a simple food approach situation, but no control injections were reported. Levine and Jones (1965) commented on unpublished results that ACTH did not alter extinction of B-P response for food which had been established under conditions of continuous reinforcement in the Skinner box.

The only other reported study of the effects of chronic injections of ACTH on appetitive behavior in the rat involves two-choice discrimination in the 'Y' maze (Beatty, Stolle & Beatty, 1969). After 5 days of pre-training to find water in the maze, Ss were injected with ACTH (40 I.U./kg.bw.) or gelatin vehicle and trained 15 trials per day to acquire a spatial preference reinforced by .10 cc water. Performance was studied during original learning and reversal with the Ss 22½ hours water deprived. No significant differences between the two groups were reported. The Ss in this study were albino rats and were housed under conditions of continuous illumination.

In cats, Endröczi and Lissák (1962) reported a strong inhibition by ACTH of 'spontaneous goal directed motor activity' in an appetitive situation. But Corson, Kirkpatrick and Ley (1970) have criticized the experimental design as containing strong aversive components. Cats were trained to jump to a ledge during the CS following which food would be available from the ledge. If the cat had not voluntarily left the ledge 15 seconds after the food was presented he was pushed from the ledge by a mechanical device. It is this pushing procedure which may be interpreted as introducing a strong aversive component into the reported experimental design.

ADRENALECTOMIZED SUBJECTS

Since adrenalectomized animals have tonically elevated levels of ACTH secretion due to the absence of feedback suppression by the products of the target gland (Cox, Hodges & Vernikos, 1958), and since they also lack the behavioral suppression associated with elevated adrenocortical activity one would expect high levels of CAR performance. However, any behavioral change would be difficult to explain since it would be unclear whether the primary influence was produced by elevated ACTH release or by the absence of PC elevation of behavioral inhibition. Such a preparation is also subject to the numerous (and largely unexplored) possibilities that some other aspects of the metabolic upset resulting from adrenalectomy may exert significant behavioral effects. It is interesting in this regard that Woodbury (1954) observed no alteration in CNS excitability following adrenalectomy in rats maintained on 0.9% saline.

One of the most important of the studies with adrenalectomized Ss was reported by Miller and Ogawa (1962). This research followed the essential aspects of the paradigm used by Murphy and Miller (1955), and was done to determine whether the prolonging effect on extinction found

in the earlier paper was due to the effect of ACTH per se, or its trophic effect on the adrenal cortex. Shuttlebox CAR extinction was studied in 100 to 120 day old Wistar male rats that had been adrenalectomized prior to acquisition training. Ten acquisition trials were given per day using 1.0 mA UCS intensity. Subjects were injected only during acquisition with either gelatin vehicle or ACTH (5 mg/kg.bw., ACTHAR). Subjects receiving ACTH during acquisition exhibited significantly prolonged CAR extinction when compared with Ss receiving vehicle. This is a striking and paradoxical finding as it suggests that a pharmacological dose of ACTH given during acquisition at intense UCS levels when endogenous ACTH release should have been maximal (Hodges & Jones, 1964) produced a further behavioral activation during extinction when neither UCS nor exogenous ACTH was present.

It is of interest to compare the performance levels of the adrenalectomized rats with the performance of the intact Ss of the previously mentioned experiment (Murphy & Miller, 1955). If elevated levels of ACTH are associated with CAR enhancement it could be argued that the adrenalectomized Ss should show higher CAR than the intact Ss. However, their CAR performance was sharply reduced compared to intact Ss. Injections of ACTH did not abolish the disparity between intact and adrenalectomized subjects.

Adrenalectomized Ss given ACTH avoided an average of 31.5 times in 53 trials to extinction, while adrenalectomized Ss given vehicle made only 12 CAR in 34 extinction trials. However, the intact Ss given ACTH averaged 113 CAR in 197.5 trials compared with intact vehicle Ss performing 27 CAR in 84.5 extinction trials. These results suggest that the use of adrenalectomized Ss to assess pituitary-adrenal function in the intact animal is a questionable procedure at best, since the profound metabolic effects of adrenalectomy seem to produce effects which are not clearly related to normal function. However, these data do suggest that ACTH can have some behavioral effects which are not mediated by the adrenals. Furthermore, it is interesting to note that the large performance decrement noted with the adrenalectomized rats is not at all consistent with Woodbury's (1954) report that adrenalectomy had no noticeable effect on CNS excitability in Ss maintained on 0.9% saline.

Finally, several reports (de Wied et al, 1968; Weiss, McEwen, Silva & Kalkut, 1969) have suggested that adrenalectomy prolongs shuttlebox CAR extinction. These results are in clear contrast with the report cited above.

HYPOPHYSECTOMY

Many investigators have attempted to alter pituitary-adrenal function by surgical removal of the pituitary (hypophysis). Although the procedure certainly succeeds in eliminating functional levels of ACTH and circulating corticosteroids, the concomitant elimination of thyrotrophic hormone, somatotrophic hormone, and numerous gonadotrophins seriously limits the relevance of such an approach to the study of the intact system. The animals are, in general, considerably debilitated (Appley, 1964), although some psychologists have risen to the challenge of devising measures upon which they do not differ from control or sham operated animals (Weiss et al., 1969). Gaito, Mottin, Davison and Rigler (1966) have studied the performance of hypophysectomized (hypox) rats in a one-way avoidance task (no parameters given). Immediately after 15 trials of acquisition the Ss were sacrificed by immersion in liquid nitrogen and several indices of CNS protein synthesis were taken. These authors found no difference in CAR performance and little difference in CNS protein synthesis between hypox and sham operated Ss.

De Wied (1964) has reported that removal of only the anterior pituitary, leaving the posterior pituitary

(which partially regulates fluid retention) intact, seriously impairs performance of rats in active runway escape and shuttlebox avoidance (1.8 mA, 40 V. UCS) tasks and that replacement therapy with ACTH (Cortrophin Zinc) or with a cocktail of ACTH, thyroxine and testosterone improves performance considerably over non-injected adenohypophysectomized controls, but still leaves the Ss significantly lower in performance than sham-operated non-injected controls. However, serious difficulty is encountered in attempting an interpretation of the directional effects of ACTH in this preparation. Although ACTH treatment of hypox animals has been reported to result in apparent behavioral activation (prolongation of shuttlebox CAR extinction, normalization of runway escape speeds), hypox Ss have been reported to be hypersensitive to environmental stimulation (e.g., Weiss et al., 1969). In this context, ACTH could be seen as acting via the elevation of PC to reduce behavioral hyperreactivity and thus to normalize CAR performance and runway speed (postulate B). Belanger (1958) has, in fact, suggested such an explanation to account for improved performance of ACTH-treated hypox Ss.

Both incubation and passive avoidance studies have been performed with hypox Ss in an attempt to assess the role of pituitary-adrenal function in these behavioral

situations. Anderson, Winn and Tam (1968) trained hypox Ss to B-P for a sucrose solution under mild deprivation (6 hours). They then established CER under conditions similar to the procedure of Levine and Jones (1965) and studied the rate of B-P recovery over 7 days, of 4 groups of Ss: those shocked and maintained on injections of (1) gelatin vehicle, (2) ACTH (8 I.U. per rat), (3) hydrocortisone (Cortisol, .05 mg/ rat) and (4) ACTH combined with hydrocortisone. They found that Ss receiving vehicle or hydrocortisone gradually recovered to within 75 - 80 per cent of their operant B-P level after 7 days while Ss receiving ACTH or ACTH combined with hydrocortisone did not move significantly from the zero B-P level over the week of daily test periods. The authors suggested that this was evidence for a behavioral effect of ACTH opposite in direction to the effect of glucocorticoids. They seem to have overlooked the fact that hydrocortisone is not the naturally occurring steroid in the rat and that it has been shown to have effects on CNS excitability (Woodbury, 1954) which are very different from the effects of corticosterone, the naturally occurring steroid in the rat. However, the finding that ACTH counteracts the effects of hydrocortisone in their preparation gives some behavioral support to the data of Woodbury suggesting that corticosterone and hydrocortisone are in fact competitive in function; this study also offers some

further support to the theoretical suggestion of Lissák and Endróczi (1961) that the ratio of these two steroids in species in which they both occur naturally may be related to behavioral reactivity in aversive situations.

Marquis and Suboski (1969) have reported a study of hypox Ss in the incubation of active shuttlebox avoidance and in passive avoidance. Prior to training, half of the hypox Ss received an injection of a very high dose of long-acting ACTH (32 I.U. Cortrophin Zinc). The remaining hypox Ss received vehicle, and sham animals were not injected. The authors reported the appearance in all groups of a significant incubation of response in both testing situations, although hypox Ss had lower baseline response rates in all tests. This result suggests that although pituitary-adrenal function in the intact animal has considerable influence on response magnitude, the shape of the incubation curve is not altered by interruption of pituitary-adrenal function by hypophysectomy. (However, it must be noted that no information was provided as to the reliability of the hypophysectomy, which was performed by the supplier.)

Several studies of appetitive behavior in hypox Ss are quite contradictory of one another. For example, Ivanov (1961) reported complete inability to condition appetitive responses in hypox Ss, whereas Marquis and

Suboski (1969) successfully trained hypox rats to B-P for a sucrose solution. Furthermore, Appley (1964) reported that hypox Ss showed deficits in appetitive tasks, but could learn these tasks.

However, since the effects of the other possible deficiency states introduced by hypophysectomy have not been studied as yet, it is difficult to determine the relative role of the interruption of pituitary-adrenal function in the behavioral deficits shown by the hypox animal.

STUDIES WITH STEROID ANALOGUES

Further evidence for an extra-adrenal behavioral function of ACTH (postulate B) is reported in several studies showing that small molecular fragments (or analogues) of the intact ACTH molecule exert similar behavioral effects to those of the intact molecule. These fragments, however, exert little or no trophic activity at the level of the adrenal. De Wied (1966) gave Wistar male rats from an inbred strain (140-180 grams) CAR training in the shuttle-box (UCS: 40 V., 1.8 mA). Subsequent to training Ss were given injections of highly purified pork ACTH, alpha-MSH or beta-MSH in long-acting zinc phosphate suspension, or control injections of the vehicle alone. He found that alpha-MSH, beta-MSH and 10 I.U./kg.bw. ACTH were of equal potency in producing significant prolongation of CAR extinction. This suggested that the active portion of the hormones involved might be the peptide fraction they held in common.

This possibility was tested in the pole climb apparatus (UCS: 25 V., 5.0 mA). After training Ss received injections of synthetic ACTH 1-24, ACTH 1-10, ACTH 11-24, ACTH 5-10 or zinc phosphate vehicle. The ACTH molecule contains 39 amino acid subunits. ACTH 1-24 refers to ACTH from which the

last 15 amino acid units in the molecular chain have been removed by methods of biochemical fractionation. ACTH 5-10 would be that part remaining when the first 5 and the last 29 amino acids have been removed.

The results indicated that only the ACTH 1-10 or 1-24 increased the number of CAR to extinction. However, no statistical assessment of the difference was given, and no Ss were injected with the highly purified pork ACTH preparation for comparison.

Another study compared the performance of Ss receiving highly purified ACTH in the zinc phosphate vehicle with that of Ss receiving highly purified ACTH in gelatin vehicle. As mentioned earlier, it was found that the ACTH in the zinc vehicle was far more potent in prolonging pole climb CAR extinction than was the gelatin ACTH preparation. De Wied suggested that this may be due to the greater repository action of the zinc preparation. He also noted that the zinc preparation has greater corticotrophic activity than the gelatin preparation, but as mentioned before, he has shown that corticosterone accelerates rather than prolongs CAR extinction.

In further studies of the biological activity of the ACTH 1-10 fraction (Bohus & de Wied, 1966), it was found that the behavioral response to the fraction was

eliminated by altering the molecular structure. Polypeptides such as ACTH 1-10 consist of amino acids chained together by structural bonds. All of these amino acids may assume a biologically active or a biologically inactive form depending upon the temperature, $_{p}H$, humidity, etc., under which they exist (e.g., see Doty, 1957). Bohus and de Wied (1966) prepared ACTH 1-10 with one of the amino acids (phenylalinine, in the 7th position along the molecular chain) in the non-active form. They found that CAR extinction of Ss receiving the altered polypeptide was more rapid, even than Ss receiving control vehicle, while CAR extinction of Ss receiving the natural form was prolonged. The authors further demonstrated that the effect of the altered polypeptide was not eliminated by prior hypophysectomy. This suggests that the altered molecule was not merely interfering with natural pituitary function by competitive biochemical inhibition.

While these data may be consistent either with postulate A or postulate B, de Wied (1966) and Bohus and de Wied (1966) suggested that these substances acted at the level of the CNS to alter behavior (postulate B). However, other alternatives can be offered which are consistent with postulate A and with the published data.

For instance, it has been shown (Schwyzer, 1964) that ACTH 1-10 has little corticotrophic activity. It may be that this non-functional fraction competes with endogenous ACTH at the level of the adrenal, thus reducing the adrenocortical output, removing the feedback suppression of pituitary ACTH secretion, and leading ultimately to elevated endogenous pituitary-adrenal activity. In this context, the time course of testing relative to the time course of these effects can be quite important. In this context also, the behavioral effects of the ACTH 1-10 with the phenylalinine in the non-natural state could possibly be explained on the basis of considerably slower metabolic inactivation of the non-functional polypeptide due to its altered molecular conformation. This could lead, for instance, to the functional absence of adrenal glucocorticoids.

Alternatively, de Wied (1966) has reported that the zinc phosphate vehicle elevates both behavioral and adrenocortical activity of ACTH; it may do so also for the ACTH 1-10 fraction. However, no information is available concerning the comparative pituitary-adrenal function of ACTH 1-10 in various suspensions; this problem and a variety of others (including lack of information on the behavioral effect of the zinc phosphate vehicle relative to some reference point such as saline injections) prevent final acceptance of any of the suggested interpretations.

THE PRESENT INVESTIGATION

It is clear from this review that the dual hypothesis offered by Levine (1968) is consistent with most of the data reported. This hypothesis suggests that elevated PC levels are associated with behavioral inhibition (postulate A), but that, in certain circumstances, ACTH may cause behavioral disinhibition which would lead, for example, to prolongation of CAR extinction (postulate B).

Throughout this presentation an attempt has been made to interpret all behavioral effects in terms of postulate A. This was done because the primary evidence (with intact animals) for postulate B occurs in the study of CAR extinction, and it became clear that these results could be explained on the basis of inhibitory effects (postulate A) which lead to a level of behavioral activation consonant with prolonged extinction in this situation. However, such an explanation attributes an important role to subject and task variables which must be further examined in order to permit advancement of our understanding of the behavioral role of the pituitary-adrenal axis.

STUDY OF UCS AND INJECTION PARAMETERS

The two sets of published reports of shuttle-box CAR extinction prolongation by exogenous ACTH differ in many methodological details. Murphy and Miller (1955), as described earlier, gave all possible combinations of injections of ACTH and vehicle during both learning and extinction and found that ACTH prolonged CAR extinction only when given during acquisition and regardless of treatment during extinction. They used a constant-current shock source at a high UCS intensity level (1.3 mA), and tested the performance of 100-120-day old rats. Such rats would weigh about 350±50 grams (age-growth data courtesy of Carworth Farms). De Wied (1966) reports that ACTH given only after acquisition, and every second day during extinction prolonged CAR extinction in 150±20 gram rats (about 50 days of age). He also used low voltage, high amperage footshock (25-40 Volts, 1.8 to 5.0 mA in various studies). The dose levels of ACTH given by the two investigators also differ considerably. The level used by de Wied might possibly constitute an approximation of physiological release levels under stress. As mentioned earlier, the basal plasma level of ACTH in the rat has been estimated at less than .001 International Unit per 100 ml of blood (Mangili, Motta & Martini, 1966). De Wied injected 3 I.U.

every second day in a long acting repository preparation. Exactly how close his approximation comes to a physiological level depends on the release rate of the zinc phosphate preparation (which is not known in sufficient detail to permit a decision) and the range of endogenous levels of ACTH released under stressful conditions (which also must be investigated further).

However, one point is quite clear; the levels used by Murphy and Miller (1955) represent a pharmacological dose with a much more rapid release rate than the preparation used by de Wied. Murphy and Miller (1955) used five milligrams of ACTHAR per rat per day. Although the potency of the preparation in I.U. was not stated, five milligrams probably represents more than 200 I.U.

The difference in UCS intensity is also of considerable importance since it has been shown that higher UCS levels reduce shuttlebox CAR acquisition (Levine, 1966), although the relationship between UCS intensity and CAR extinction is not completely clear. Furthermore, the pituitary-adrenal response to inescapable shock has been shown to vary directly with current intensity (Friedman, Ader, Grota & Larson, 1967).

In order to investigate some of these testing and treatment parameters, three experiments were done using the subject sample specified by de Wied (1966): Wistar male rats 160±20 grams.

Experiment one deals with the effect of high voltage, low amperage (constant current) UCS intensity and three doses of ACTH (Cortrophin Zinc) on shuttlebox CAR acquisition; a comparison is made of the effects on CAR extinction of the ACTH dose used by de Wied (1966) when given during acquisition and extinction (as was done by Murphy & Miller, 1955), compared with administration only during extinction (as was done by de Wied, 1966).

Experiment two deals with the effect of the ACTH dose used by de Wied (1966) when given during acquisition at low voltage, high amperage UCS intensity.

Experiment three deals with the effects of ACTH given only during extinction in Ss acquiring shuttlebox CAR under conditions of low voltage, high amperage UCS.

GENERAL METHOD AND PROCEDURE

Shuttlebox Conditioned Avoidance Responding:

Since several studies were undertaken using the same procedure, the general methodology will be described here. Departures from this shuttlebox technique will be indicated wherever they occur.

Subjects:

Unless otherwise noted all Ss were acquired from the Canadian Breeding Laboratories (CBL), St. Eustache, Quebec. They weighed between 140 and 160 grams when they arrived, and were housed two per cage (8" X 8" X 10"), in a temperature controlled vivarium. The lighting in the vivarium was entirely artificial (flourescent) and was present on a 12 hour on-off cycle.

Apparatus:

The Ss were tested in two-way shuttleboxes similar in design to those used by Murphy and Miller (1955) and Bohus and de Wied (1966): 18 inches long by 6 inches wide by 10 inches high with a floor of grid bars spaced ½ inch apart. A small barrier (3/4 inches high) across the center served to add definition to the Ss responses which were signalled by micro-switch 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 watts) in the chamber to be avoided. CS-UCS interval was 5 seconds, and both CS and UCS were terminated together. In the case of failure to respond during extinction, an upper limit of 20 seconds was set for the CS duration. The intertrial interval ranged from 15 to 45 seconds and averaged 30 seconds. Each shuttlebox was encased in an individual sound-attenuating chamber, and operated by independent electromechanical programming and recording equipment. Two types of power source were used to provide the UCS in the various studies. A Grason-Stadler Operant Conditioning Apparatus (No. E 1064GS) supplied the high voltage, or constant current, UCS. This apparatus delivered a working current of 350 Volts, A.C. into a multiple resistor bank with which E could control the milliamperage delivered to the subject through a range of 0.05 mA to 4.0 mA. The low voltage UCS was supplied through a variable transformer and a variable resistor which allowed the duplication of the UCS parameters used by de Wied (1966) in his several studies. The low voltage power source was connected to the input terminals of a Grason-Stadler grid scrambler which delivered shock to the grid floor of the shuttlebox.

Procedure:

Subjects were given 10 trials per day in the described apparatus. The first trial each day commenced an average of 30 seconds after subject was placed in the apparatus, and subject was removed approximately 30 seconds after the cessation of the last trial each day. Daily acquisition sessions continued to the criterion of 80% averaged over 3 days, with no less than 80% CAR on the last day. Subjects not reaching criterion after 100 trials were discontinued. Injections began the day before the first day of training. Subjects were injected subcutaneously (behind the neck) every second day, with 10 I.U./kg ACTH (Cortrophin Zinc, Organon) 40 I.U./cc, 20 I.U./kg.bw. ACTH: 80 I.U./cc, or 2 I.U./kg.bw. ACTH: 8 I.U./cc, 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 subject reached the criterion of less than 50% CAR on two consecutive days.

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

Latencies and UCS presentations were independently and automatically recorded.

Results:

The performance of Ss in this series of studies will be reported and discussed in terms of number of UCS to acquisition and CAR to extinction. In addition, a third index, CAR in 'X' trials will be used. 'X' will be defined as the minimum number of trials required by any subject in a given experiment to reach acquisition criterion. This 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 Ss failing to reach acquisition and, more importantly, from its relative resistance to performance variations often seen in endocrine studies employing spaced practice at or near asymptotic levels of performance (e.g., Mason, 1959). The major disadvantage of this index is that an unusually quick learner may set 'X' so low that most Ss are still performing at basal level. A further advantage of the CAR in 'X' trials index of performance is its direct relationship to acquisition. It was, however, necessary to use the inverse measure, UCS, to acquisition criterion since the number of trials was free to vary, and 30 CAR to

criterion in 95 trials is quite different to 30 CAR to criterion in 35 trials. UCS to criterion contains an assessment of both CAR and trials to criterion in a single index.

All statistical analyses employed the appropriate model of the parametric analysis of variance described by Winer (1962), and all probability statements are in terms of the two-tailed distribution. In cases wherein the data were found to violate the homogeneity of variance assumption they were transformed by the $\sqrt{x} + \sqrt{x+1}$ transformation suggested by Winer (1962) for data in which the variance is proportional to the mean.

EXPERIMENT I

One hundred forty-four Wistar male rats were trained in the two-way shuttlebox. Four independent samples were taken from the available commercial population over a period of 7 months. These samples contained 24, 36, 48 and 36 rats, respectively. Within each sample, Ss were equally distributed among the 6 injection groups and two UCS levels in the design. The injection groups included: (1) Ss receiving twenty International Units of ACTH per kilogram of body weight (20 I.U./kg.bw.), (2) Ss receiving 10 I.U./kg.bw. ACTH, (3) Ss receiving 2 I.U./kg.bw. ACTH, (4) Ss receiving zinc phosphate vehicle in equal volume to that administered to groups 1, 2 and 3 and (5) Ss receiving equal volume of physiological saline. Group number (6) consisted of Ss receiving saline during acquisition (as a control for injection trauma) that were given ACTH (10 I.U./kg.bw.) during extinction as in the procedure of de Wied (1966). These Ss were selected from the acquisition saline group in counterbalanced order. For statistical analysis of the acquisition data the performance of each counterbalanced pair of Ss was averaged to compensate for the double sample size of saline Ss.

The UCS levels were 0.2 mA and 0.5 mA, constant current. Preliminary studies had revealed that 0.2 mA was

the minimum UCS intensity leading to reliable shuttling behaviour in the subject population under study and that levels higher than 0.5 mA were associated with large percentages of Ss failing to reach acquisition within 100 training trials.

Results and Discussion:

Figures 1 and 2 respectively, record the effects of chronic injection of ACTH during shuttlebox CAR acquisition and extinction. It is clear that injection did not significantly alter the number of UCS to acquisition. However, there was the expected effect of UCS intensity ($p < .05$), with the Ss at the higher shock level receiving consistently more UCS presentations during acquisition. Injection treatment did not interact with UCS intensity, nor were there any significant mean differences in UCS to acquisition. This is in accord with the report of Murphy and Miller (1955) that exogenous ACTH did not influence acquisition of shuttlebox CAR.

During the early stages of acquisition, however, both UCS intensity ($p < .01$) and injection treatment ($p < .05$) exerted significant effects on conditioned avoidance responding. During the first 30 trials of acquisition Ss at

the .5 mA UCS level made consistently fewer CAR than did those at .2 mA. However, it is important to note the effect of the hormone treatment on the magnitude of the footshock-induced CAR depression. CAR was significantly reduced at the higher UCS level in both the saline (10.4 Vs. 15.9, $p < .001$) and the vehicle groups (10.7 Vs. 20.1, $p < .001$), whereas the 2 I.U./kg.bw. dose of ACTH significantly reduced CAR at the .2 mA level ($p < .05$, compared with vehicle) but had no noticable effect at the .5 mA level. The net result, combining data from both mA levels, was that the magnitude of the UCS-induced CAR suppression was reduced by 2 I.U./kg.bw. ACTH (11.0 Vs. 14.6, $p < .10$). Moreover, the 10 I.U./kg.bw. dose of ACTH considerably reduced CAR at the .2 mA level ($p < .01$, compared with vehicle) and marginally reduced CAR at the .5 mA level ($p < .10$, compared with vehicle) with the net result of restoring the UCS-induced response difference (7.3 Vs. 11.8, $p < .05$). The results were somewhat different, though, at the 20 I.U./kg.bw. dose level of ACTH. At .2 mA CAR of these Ss was slightly (but non-significantly) reduced compared with vehicle Ss, whereas at .5 mA, responding was slightly elevated over the vehicle group. The net result was that the response reduction associated with the higher UCS level in the other injection and control groups did not occur with the Ss receiving

20 I.U./kg.bw. ACTH (13.2 Vs. 15.0, p=ns).

It is important to reiterate the effects of injection treatment on the early stages of shuttlebox CAR acquisition. The zinc phosphate vehicle in which ACTH is suspended for repository action is associated with a slight, but not significant, elevation of CAR acquisition performance at both UCS levels studied, when compared with Ss injected with physiological saline. The 2.I.U./kg.bw. dose of ACTH, however, reduces CAR performance at the .2 mA level and has no effect at the .5 mA level; the 10 I.U./kg.bw. ACTH dose reduces CAR at .2 and to a lesser extent at .5 mA, and the 20 I.U./kg.bw. ACTH dose slightly reduces CAR at .2 and slightly elevates CAR at .5 mA UCS. Moreover, the effect of the 20 I.U./kg.bw. dose at the .5 mA UCS intensity represents a significant ($p < .001$) elevation of CAR performance over the 10 I.U./kg.bw., and 2 I.U./kg.bw. dose levels. Thus, it would seem that there exists a considerable dose-response effect of exogenous ACTH on shuttlebox CAR acquisition, and that the magnitude and direction of the effect depend, in part, on UCS intensity level and the subject population studied.

During shuttlebox extinction, however, there was no effect of UCS intensity on CAR, and no injection-by-UCS interaction, but there was a significant ($p < .01$)

effect of injection treatment. In view of the absence of significant UCS mean effects and interaction effects, the CAR to extinction data were combined over UCS levels for clarity. The resulting relationship appears in figure 3.

When given during both acquisition and extinction, zinc phosphate vehicle significantly prolongs shuttlebox CAR extinction performance compared with correspondingly-injected saline controls (73 Vs. 31 CAR, $p < .001$). Furthermore, the effect of ACTH when injected during both acquisition and extinction, is to accelerate (or facilitate) shuttlebox CAR extinction in an inverse dose-response relationship: the smallest dose has the largest effect. Two I.U./kg.bw. ACTH significantly ($p < .01$) accelerated shuttlebox extinction when compared with vehicle (39 Vs. 73), while 10 I.U./kg.bw. ACTH exhibited an intermediate effect (50 Vs. 73, $p < .05$) and 20 I.U./kg.bw. did not significantly alter shuttlebox CAR extinction (60 Vs. 73) when compared with vehicle. It is important to note that ACTH (10 I.U./kg.bw., same dose as used by de Wied, 1966) given only during extinction also significantly accelerates CAR extinction (26 Vs. 73, $p < .001$) in the population studied.

These results contrast sharply with those of de Wied (1966) and of Murphy and Miller (1955). However, these latter investigators gave daily injections of a tenfold greater dose of ACTH than the maximum dose in the present study (which was given every second day). The observation that the maximum dose in the present study had no effect on shuttlebox CAR extinction, while the smaller doses caused CAR reduction may suggest that at at the very high dose (200 I.U./rat) used by Murphy and Miller (1955), some sort of pharmacological effect of ACTH, per se, may have been observed. However, the comparison given in the present introduction between the effect of ACTH in intact and adrenalectomized rats suggests that such an effect is rather small considering the dose levels of ACTH required to produce it. Furthermore, in the intact animal, the confounding effect of pituitary-adrenal depletion has not been adequately assessed either in the former (Murphy & Miller, 1955) or the present studies.

The results of the present study suggest the following interpretation: in the present acquisition and extinction situations, the vehicle was acting as a behavioral stimulant and ACTH, by way of the inhibitory action of adrenal glucocorticoids, counteracted the CAR elevation produced by the vehicle. This hypothesis runs

afoul of the observation that minimum doses of ACTH produced the maximal behavioral effects unless account is taken of either; a) the fact that the Ss were injected during acquisition as well as extinction; or b) the possibility that the doses of 10 and 20 I.U. are pharmacological and may thus be producing effects by altogether different means. The first of these observations would allow for the possibility of differential pituitary-adrenal depletion or resetting of CNS behavioral thresholds by the higher doses of ACTH. The differential effect of hormone treatment when given during acquisition as opposed to extinction is apparent from a comparison of CAR extinction of those Ss receiving ACTH (10 I.U./kg.bw.) only during extinction with that of Ss receiving the same dose of ACTH during both acquisition and extinction. There was a strong (but not statistically significant) trend for ACTH given only during extinction to accelerate CAR extinction (31 Vs. 49 CAR, $p < .10$).

The further observation that, when compared to Ss injected with vehicle, those receiving ACTH only during extinction showed a very large acceleration of extinction (73 Vs. 26 responses, $p < .001$) clearly contradicts the report of de Wied (1966), that ACTH given only during extinction prolongs shuttlebox CAR extinction. However,

the present data are compatible with the conclusion that, compared with vehicle, ACTH does produce a large behavioral effect; only the direction of the effect is at issue.

EXPERIMENT II

Several investigators have suggested that UCS intensity is a critical consideration in studies of shuttle-box acquisition and extinction (Levine, 1966; Moyer & Korn, 1964), and the pituitary-adrenal response has been shown to vary directly with increasing UCS intensity (Friedman et al, 1967). These observations suggest that the reverse direction of the behavioral effect of ACTH shown in Experiment I compared to the studies of de Wied (1966) may be the result of the difference in UCS parameters used.

Experiment II represents a replication of the technique of Experiment I using the lowest voltage, highest amperage UCS parameters (25 V., 5.0 mA) reported in the several studies by de Wied (1966). In the interest of economy, only the dose of ACTH used by de Wied (1966) was studied.

Method:

Seventy-two Wistar male rats were divided into 3 groups matched for average weight. Eighteen rats received 10 I.U./kg.bw. ACTH (Cortrophin Zinc), 18 received an equal volume of zinc phosphate vehicle, and 36 received an equal volume of physiological saline. As in the previous

experiment, the double saline group was divided in counterbalanced order of reaching acquisition criterion into two injection groups. Half continued receiving saline during extinction and half were switched to 10 I.U./kg.bw. ACTH during extinction. All Ss were trained at one UCS intensity level: 25 Volts, 5.0 mA.

Results and Discussion:

Learning performance corroborates the results of Experiment I in showing that vehicle has significant behavioral effects. Analysis of CAR in 'X' trials (figure 4), which, in this instance extends over 100 trials, revealed a significant ($p < .01$) elevation of the per cent CAR of the vehicle group over the ACTH and saline groups. This provides support for the suggestion that ACTH was acting to counteract the effect of the vehicle during shuttlebox CAR acquisition and extinction.

The reason for the poor acquisition performance of all Ss seems to be inadequate motivation. Many Ss took long applications of the footshock and seemed to be exploring the apparatus while doing so. Figure 5 compares the mean latencies of Ss from the earlier and the present study to support this suggestion.

The CAR to extinction for the Ss reaching acquisition criterion in this study (5 ACTH, 5 vehicle and 9 saline; 5 of which were shifted to ACTH during extinction) are presented in figure 6. The vehicle Ss made significantly fewer extinction CAR than did the ACTH ($p < .05$) or the saline ($p < .01$) Ss, but these latter groups did not differ significantly from one another. The overall treatment effect was significant at the .01 level.

It is important to point out that, in agreement with de Wied, ACTH injected only during extinction significantly prolonged shuttlebox CAR ($p < .05$) compared with vehicle in this highly selected group (26.4% of the total acquisition sample). However, the interpretation of such a result is considerably altered by the observation that when compared with saline Ss no such difference appears: neither ACTH given both during acquisition and extinction nor ACTH given only during extinction produced results significantly different from the extinction performance of saline-injected Ss.

It is interesting to compare the CAR extinction of Ss receiving similar injections from this study and the former one employing high voltage UCS. In terms of CAR to extinction the ACTH Ss from the two studies are not dissimilar (59.0 in the present, 49.25 in the former), whereas

vehicle Ss in the present study are sharply depressed (24.8 Vs. 78.9) and saline Ss are elevated (87.0 Vs. 38.8).

EXPERIMENT III

In a final attempt to produce the results reported by de Wied, 27 Ss were trained at a UCS intensity designed to match exactly that used by de Wied (1966): 40 V., 1.8 mA. The Ss were not injected during acquisition. Twenty-two reached acquisition, 4 were discarded due to ill health, leaving 6 Ss in each of the three injection groups: ACTH (10 I.U./kg.bw.); vehicle; saline.

Results and Discussion:

Figure 7 records that the mean CAR to extinction for the three groups are in the following order from high to low V > A > S. However, neither the mean differences nor the overall treatment effect are statistically significant. The data strongly suggest that it is impossible to replicate the result of de Wied (1966) using the Wistar population available to us; this suggestion is consistent with the reports of large differences in biochemical processes between laboratory stocks of the original Wistar strain (Eggleston & Krebs, 1969).

It is, however, interesting to note the qualitative shift in the response to ACTH as the UCS parameters changed in the three experiments reported above. At both

the high voltage UCS intensities, .2 and .5 mA (Experiment I), most Ss rapidly acquired shuttlebox CAR and showed facilitated CAR extinction in response to exogenous ACTH; at the 40 V., 1.8 mA level (Experiment III) most Ss acquired shuttlebox CAR, but no differential response was shown to ACTH during shuttlebox CAR extinction. However, at the 25 V., 5.0 mA level (Experiment II), few Ss acquired shuttlebox CAR, but those that did reacted to ACTH with prolonged CAR extinction relative to the Ss injected with vehicle.

It could be argued from these results that increased PC following ACTH may act to inhibit behavior (postulate A) only if the Ss are already experiencing an approach-avoidance conflict as might occur at the higher UCS intensities in the shuttlebox, and as must occur in the CER suppression of appetitive responding.

Such a position could be seen as being in accord with the report (Levine & Jones, 1965) that ACTH injections had no effect on CRF B-P for food, but produced significant B-P reduction after footshock in the B-P situation. The report by Korányi et al. (1967) that ACTH reduced latency of mice to enter a darkened chamber from an open field before footshock, but increased latency after footshock in the chamber also is in accord with this view.

However, the data of the present report suggest that the performance of Ss receiving ACTH remains relatively stable over the wide qualitative range of UCS intensities used in the three studies, whereas the CAR of the saline and vehicle groups varies with the testing conditions (see Figure 8). This is difficult to reconcile with the above speculations, and suggests that any inhibition following ACTH injections may develop independently of testing conditions. However, this is not to say that the direction and magnitude of the behavioral response will be independent of the testing situation, but suggests that the direction and magnitude of the apparent behavioral response to ACTH will be greatly influenced by the baseline of the control group.

EXPERIMENT IV

Preliminary studies (see appendix A) had suggested that the reactivity of the Long-Evans Hooded male rats commercially available was considerably different in the shuttlebox situation from that of the Wistar male animals used in the first three experiments. This observation combined with the difficulty of interpreting the prolongation of shuttlebox CAR extinction in the Wistar male using the same procedures used by de Wied (1966) led to the examination of CAR acquisition and extinction in the Long-Evans Hooded strain.

Numerous other investigators have suggested that large differences occur between the sexes in performance of behavioral tasks (e.g., Broverman, Klaiber, Kobayashi & Vogel, 1968), and in endocrine function (Saroff & Wexler, 1969; Thoman & Levine, 1969). This study was also conducted to investigate the possibility of sex differences in the magnitude and direction of the behavioral response to exogenous ACTH.

Method:

Seventy-two Long-Evans Hooded male and 72 Long-Evans Hooded female rats (150±10 grams) were trained in

the two-way shuttlebox described earlier. However, in an attempt to reduce the variance associated with spaced testing, Ss were given massed training; they were trained and given CAR extinction in a single session. The acquisition and extinction criteria were changed slightly from the spaced procedure used earlier. Criterion for acquisition was reduced to 80% CAR in 20 consecutive trials and extinction testing was terminated after 5 consecutive failures to avoid. The upper limits were arbitrarily set at 100 acquisition and 100 extinction trials.

Subjects were injected twice prior to testing, with either 10 I.U./kg.bw. ACTH (Cortrophin Zinc), an equal volume of zinc phosphate vehicle, or an equal volume of physiological saline. The first injection was given three days prior to testing and the second injection was given one day prior to testing; as in Experiment I, two UCS intensity levels were used: .2 mA and .5 mA, and groups were evenly distributed with regard to the order in which injection and testing took place.

Results and Discussion:

As figure 9 shows, there was a significant ($p < .01$) effect of UCS intensity on shuttlebox CAR acquisition as indexed by both CAR in 20 trials and UCS to learning,

but no effect of either injection treatment or sex of subject on these measures. There were also no interactions among the treatments during acquisition. Subjects at the .5 mA UCS level tended to acquire shuttlebox CAR more slowly than at .2 mA regardless of sex or injection treatment.

During extinction, however, females tended ($p < .01$) to make more CAR than did males, and Ss learning at .5 mA to make more CAR than those at .2 mA ($p < .05$). There was no effect of injection treatment nor were there any interactions among the treatments on CAR to extinction. The failure to find a significant effect of hormone treatment on shuttlebox CAR extinction led to an attempt to explore the suggestion raised by Korányi et al. (1967) that ACTH might in some situations effect hypoactive and hyperactive Ss differently. It seemed reasonable that a high degree of error variance in a heterogenously bred subject population could be a reflection of just such a differential effect of treatment on statistically identifiable subsets of the subject sample. In the study of Korányi et al. (1967) hypo- and hyper- activity were defined by high and low open field ambulation, respectively. In this study CAR in the first 20 acquisition trials ('X' trials as defined above) was used as a selection index because shuttlebox CAR acquisition has been shown to vary inversely with emotional

reactivity (Joffe, 1964), and because analysis revealed that the injection groups did not differ on this index. In Experiment I the injection groups did, in fact, differ on CAR in 'X' trials so that the hypo- and hyper- active breakdown could not be made without having it confounded by prior drug effect.

Subjects scoring below or equal to the median number of CAR in the first 20 trials of acquisition were placed in one group (L) and those scoring above this number were placed in another group (H). The median was used to insure numerical equality in the two artificial groups.

Figures 10 and 11 reveal that those Ss (L) making less than the median number of CAR during the early phase (first 20 trials) of acquisition were greatly influenced by injection treatment during CAR extinction ($p < .01$), whereas those (H) making more than the median CAR were not effected during extinction by injection treatment. Furthermore, among the L Ss, ACTH injection elevated ($p < .05$) extinction CAR in the males trained at .2 mA and suppressed ($p < .05$) extinction CAR in males trained at .5 mA, compared with Ss injected with control vehicle. On the other hand, extinction CAR of L-females receiving ACTH was not different from that of L-females receiving vehicle at .2 mA, but extinction CAR of L-females receiving ACTH at .5 mA was elevated ($p < .05$)

over corresponding vehicle injected Ss.

In order to adequately interpret these data it is helpful to consider the comparison of the acquisition performance of the Long-Evans Hooded males given massed training in the present study with that of Wistar males (N=10) and Long-Evans Hooded males (N=10) given spaced training in a pilot study. This comparison (appendix A) shows that the Wistar males given spaced practice learned more quickly at the .2 mA UCS level, whereas the Long-Evans Hooded males given spaced practice learned more quickly at the .5 mA UCS level. However, the Long-Evans Hooded males given massed training in the present experiment learned more quickly at the .2 mA UCS level.

In the context provided by the earlier discussion of arousal performance relationships, this overall pattern of results suggests that the Wistar males may be seen as emotionally hypo-reactive while the Long-Evans Hooded males may be hyper-reactive. This suggestion is supported by work from other laboratories (e.g., Joffe, 1964), which shows that Ss of high emotionality, as defined by low open field ambulation, are slow to acquire shuttle-box CAR when spaced practice is used.

These observations are of help in the interpretation of the present data because they suggest an

explanation for the similarity of the performance of the L-males receiving saline to those receiving vehicle. The results of Experiment I suggest that vehicle functioned as a stimulant for the Wistar males, but in the present experiment the performance of the L-males (hooded) was unaffected by vehicle injections. If, however, it is assumed that the hooded males are hyperactive, then the failure of the vehicle to further activate their performance may be understood. This explanation also has the advantage of placing the vehicle and saline groups on the same limb of the arousal-performance curve (fig.I: 1) so that the elevation of CAR by ACTH at .2 mA and the suppression of CAR by ACTH at .5 mA in the L-males can be seen as a shift in arousal level of the entire .5 mA sample as in figure 12, rather than a complicated shift in the relative position of the ACTH, vehicle and saline groups with respect to one another.

This opposite effect of ACTH (with hooded male Ss) at the two UCS intensity levels may reflect differential buildup of footshock inhibition at the two UCS levels or may reflect an interaction of the selection criterion with UCS intensity. It may well be that those Ss making less than the median CAR during early acquisition (first 20 trials) at .2 mA may be of quite different behavioral reactivity than those scoring similarly at .5 mA. The data contain little

helpful information on this point, but appendix A suggests that UCS intensity is of considerable importance in making behavioral reactivity judgments from CAR performance.

The opposite effects of ACTH in the two sexes at both UCS levels is intriguing as it suggests either that males and females of the same behavioral reactivity in the shuttlebox may be of opposite emotional reactivity or that ACTH exerts opposite effects on males and females of the same emotional reactivity. Unfortunately, no information is available to suggest which of the two alternatives may best fit the data.

Regardless of the precise directional interpretation of the data, this study makes several major points: (1) that Ss varying in behavioral reactivity may react quite differently to exogenous ACTH in aversive testing situations; (2) that the behavior of the reactive Ss may vary in direction and magnitude in response to changes in UCS parameters during acquisition; (3) that sex of subject is an important consideration in defining the behavioral response to ACTH and UCS intensity; (4) that under some circumstances, as suggested by de Wied (1966), exogenous ACTH may prolong CAR extinction compared with vehicle, (5) and that under some conditions, vehicle and saline may not differ in behavioral effects. This last point, however,

is not inconsistent with the interpretation of the effects of ACTH in terms of elevated inhibition following PC release (postulate A) offered for the L-males above.

EXPERIMENT V

The results of these experiments do not support the suggestion of a uni-directional behavioral effect of exogenous ACTH on the extinction of conditioned avoidance responding in the rat. They suggest instead that subject strain, stock, sex and behavioral reactivity interact with task variables such as UCS intensity to determine the direction and magnitude of the behavioral effects of exogenous ACTH on the acquisition and extinction of CAR. It could be argued that the subject variables tested thus far represent genetic (rather than environmental) influences, on the behavioral responsiveness to ACTH. Such a position would be supported by the following facts: (1) the emotionality seems to vary with stock (differences between the Wistar males used here and those used by de Wied, 1966); (2) emotionality varies with sex (differences between Long-Evans Hooded males and females); (3) emotionality varies with differences in behavioral reactivity in animals treated in identical fashion (Long-Evans Hooded "L" and "H").

Method:

In a test of this hypothesis 24 Long-Evans Hooded male rats, 90 days of age, were kept in the laboratory vivarium until they were 120 days of age at which

time they were tested in the shuttlebox. During the 30 days in the vivarium all Ss were housed two per cage. Half of the cages were not opened during those 30 days (non-handled, or NH Ss). The other half of the Ss (handled, or H Ss) were picked up and placed on a nearby feeding stand in a congested vivarium daily for 5 days a week. They were also subjected to 23 hour cyclic water deprivation (days 97-111), but were otherwise untrained. Three days prior to shuttlebox training all Ss were injected with either 10 I.U./kg.bw. ACTH, an equal volume of zinc phosphate vehicle, or sterile physiological saline. They were reinjected one day prior to testing, and were all trained under massed conditions at 1.0 mA UCS intensity. The age of the Ss and the UCS intensity were designed to follow Murphy and Miller (1955) in order to study the effect of ACTH on shuttlebox CAR acquisition.

Results and Discussion:

Figure 13 records that there was no performance difference between the H and NH Ss injected with saline. However, both ACTH and vehicle injections improved the acquisition performance of H Ss, but had no effect on the performance of NH Ss. Within the H group, only the Ss receiving injections of vehicle received significantly fewer UCS during acquisition than did the H Ss receiving

saline injections. This suggests that the emotionality variable may be extended to include both genetically and environmentally induced differences in behavioral reactivity.

EXPERIMENT VI

Further evidence concerning the behavioral mode of action of ACTH in producing CAR acquisition effects is provided by a study of another sample of handled Ss injected with either high (H) ACTH (20 I.U./kg.bw.), low (L) ACTH (2 I.U./kg.bw.) or zinc phosphate vehicle. These Ss were given 10 trials in the shuttlebox without UCS prior to CAR training. During these 10 trials the Ss receiving high ACTH made significantly ($p < .05$) fewer responses within 5 seconds of CS onset (avoidances) and tended to make fewer ($p < .10$) inter-trial responses during the first 10 trials than the Ss injected with vehicle or low ACTH which did not differ between themselves. The relationship was in the following order in terms of increasing responsiveness $H < L < V$ (figure 14). During massed acquisition trials half the Ss were trained at .5 and half at 1.0 mA UCS. As figure 15 records, injection did not influence performance at the 1.0 mA UCS level, but the low dose of ACTH significantly increased the number of CAR during the first 20 trials of acquisition among the Ss trained at .5 mA compared both to the high dose of ACTH ($p < .01$) and to vehicle ($p < .05$). There was no overall effect of injection on UCS to acquisition.

However, the interesting data occurs when the Ss were given massed extinction trials one week later (figure 16). They had not been injected since training a week before, but at the 1.0 mA level, Ss trained with high dose of ACTH made significantly ($p < .01$) more extinction CAR than did those receiving low ACTH, but neither high nor low ACTH were different from vehicle, whereas at .5 mA the Ss trained under high dose of ACTH scored significantly ($p < .01$) less extinction CAR than did either vehicle or low ACTH Ss which did not differ from each other.

This suggests that, although the effect of ACTH on the acquisition of shuttlebox CAR may not be independent of its effects on behavioral reactivity, the artificial elevation of pituitary-adrenal function during acquisition may have long-term effects on performance in the situation which are not directly dependant upon maintained artificial elevation of pituitary-adrenal function.

EXPERIMENT VII

Several reports have suggested that ACTH does not effect behavior in appetitive states (Levine & Jones, 1965; Beatty et al., 1969). However, no parametric consideration has been given to drive intensity in these appetitive studies.

Malmo and Belanger (1968) suggest that activation, as measured by heart rate in the goal situation, increases with increasing deprivation level, and the studies presented earlier using aversive motivation suggest that overall subject activation, as reflected in hyper- or hypo- reactivity in the CAR situation, is an important consideration in the study of the behavioral effects of ACTH. Consequently, this experiment was designed to assess the effect of deprivation level on the behavioral response to ACTH in an appetitive task.

Method:

Fifty-eight Long-Evans Hooded male rats were supplied by the Canadian Breeding Laboratory, St. Eustache, Quebec. They weighed 250±20 grams at arrival and were housed two per cage 8" X 8" X 10".

The Ss were trained in two Foringer Skinner boxes housed in ventilated isolation chambers. One 6 watt house light provided the illumination in each box. The single bar was located to the right of the pellet delivery tube, which was supplied by a Grason-Stadler pellet feeder. The bar travel required to activate the feeder was two millimeters.

Subjects were trained to B-P for 45 mg. Noyes food pellets in the Skinner box on a CRF schedule. For a week prior to training they were kept on a 23 hour deprivation schedule and fed Noyes pellets in the Skinner box for 15 minutes, supplemented by Laboratory Chow (Purina) in the home cage. On the first day of training each subject was given a 15 minute shaping session followed by a further 15 minutes in the apparatus. Every other day from this point the Ss were given 15 minute sessions in the Skinner box on a CRF schedule until they made over 50 presses in one session. (Four Ss were discarded for failing to reach this criterion in two weeks.) The following session they were switched to a variable interval reinforcement schedule with an average interval of 35 seconds (VI:35). From this point the B-P rate of all Ss was allowed to stabilize until the mean B-P rate for all Ss taken together did not vary more than 10% over three days of testing at the 24 hour deprivation level.

The Ss were then divided into three groups of 18 matched for mean B-P rate. One group received injections of 10 I.U./kg.bw. ACTH, one received an equal volume of zinc phosphate vehicle, and the other received an equal volume of sterile physiological saline. Injections were given every second day for a week prior to testing. Subjects were tested, for one 15 minute session, at each of three deprivation levels, 24 hour, 48 hour and 72 hour. The order in which a subject was tested at each deprivation level was chosen at random. Subjects were given a full day's access to food and three days cycling at 23 hours deprivation between the testing session at each deprivation level.

Results and Discussion:

Figure 17 records the B-P rate of each at the deprivation levels tested. Both the injection ($p < .01$) and deprivation ($p < .01$) effects were statistically significant as well as the interaction between deprivation and injection ($p < .05$). Subjects receiving ACTH tended to press less than Ss receiving saline or vehicle, which did not differ between themselves. This effect was significant ($p < .01$) at the 24 hour and 72 hour deprivation levels, but not at the 48 hour deprivation level. Figure 18 records

the performance difference between ACTH and vehicle Ss at each deprivation level.

The behavioral dimensions underlying the response reduction by ACTH are unclear. By way of interaction with the glucocorticoid hormones of the adrenal cortex (e.g., Turner, 1966) ACTH might well influence the metabolic response to food deprivation, but it is unclear why this would be behaviorally evident at the 24 and the 72 hour deprivation levels and not at the 48 hour level. Alternatively, the response reduction might reflect the enhanced reaction to those aversive aspects of the testing situation which Corson (1966) has suggested might play a large role in determining the behavioral response of laboratory Ss in almost any testing situation.

SUMMARY AND GENERAL CONCLUSIONS

The review of the literature contained in the introduction to this thesis suggested that exogenous ACTH has no effect on shuttlebox CAR acquisition (Murphy & Miller, 1955), that it prolongs shuttlebox CAR extinction when given during acquisition (Murphy & Miller, 1955) or extinction (de Wied, 1966), and that it has no effect on appetitive behavior in the Skinner box (Levine & Jones, 1965) and the 'Y'-maze (Beatty et al., 1969). However, the results of this thesis demonstrate: (a) that under certain circumstances ACTH may have no effect on shuttlebox CAR acquisition; (b) that ACTH may influence the early stages, or the total process of acquisition; (c) that ACTH may have no effect, may prolong or may inhibit shuttlebox CAR extinction; (d) that sex differences influence the direction and magnitude of the behavioral response to ACTH in the acquisition and extinction of shuttlebox CAR; (e) and that under certain circumstances, ACTH may influence appetitive behavior in the Skinner box. The evidence gathered here demonstrates the generality of the complex interaction of subject and task variables in determining the direction and magnitude of the behavioral response to ACTH.

These observations suggest certain further questions concerning the role of ACTH in behavioral processes.

The time course of the behavioral effects following administration of long-acting ACTH should be carefully studied, preferably including an assessment of the time course of changes in PC levels as well. This would provide direct information on the role of ACTH and PC in producing behavioral effects.

Another broad area of suggested investigation following from the results of these experiments concerns the neurophysiological basis of the task and subject interactions reported. Sex differences are one of the most interesting in this regard since the sexes are known to differ along several dimensions of neuroendocrine function.

Finally, further study of appetitive drive states is required to assess the relative contribution of drive state, drive intensity and possible aversive components of deprivation and appetitive testing, to alterations of performance by ACTH. Perhaps incentive motivation could avoid some of the interpretative difficulties associated with interactions of ACTH with physiological processes related to deprivation state and intensity as well as with

the aversive components of deprivation. It should also be helpful in this context to explore the interaction between prior behavioral reactivity and the behavioral response to ACTH in appetitive and incentive situations.

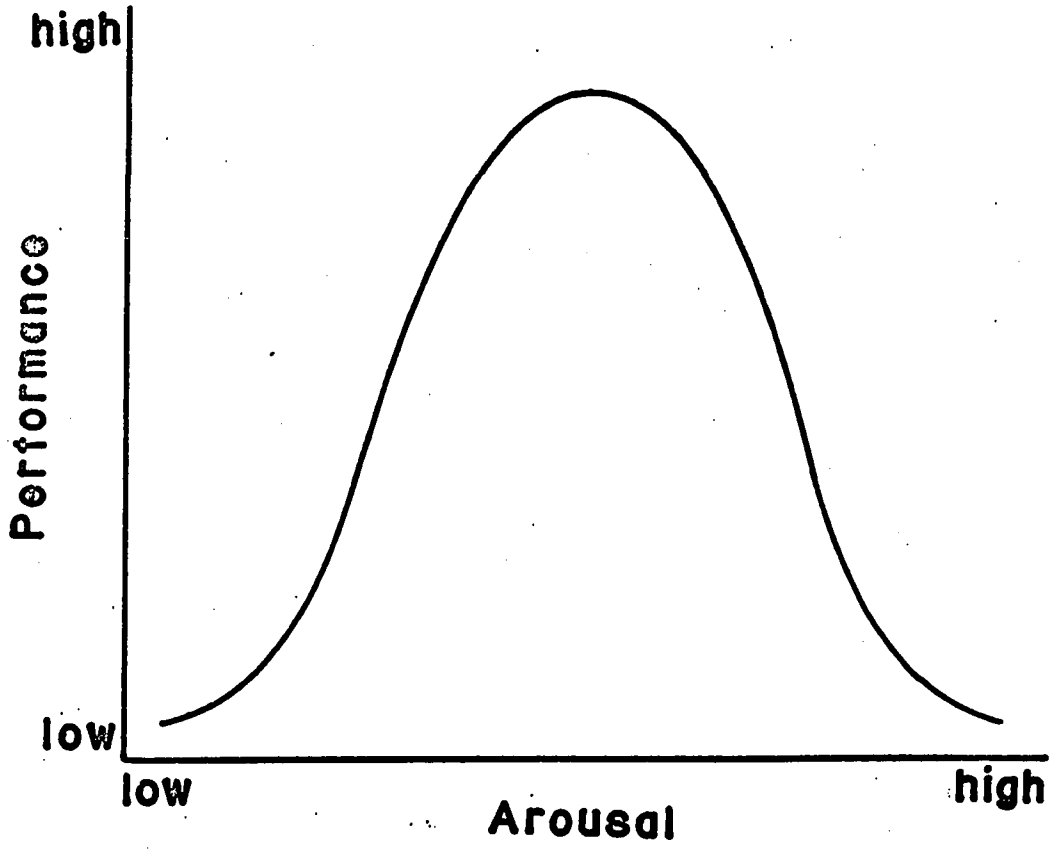


Figure I:1. Theoretical inverted-U function relating arousal to task performance.

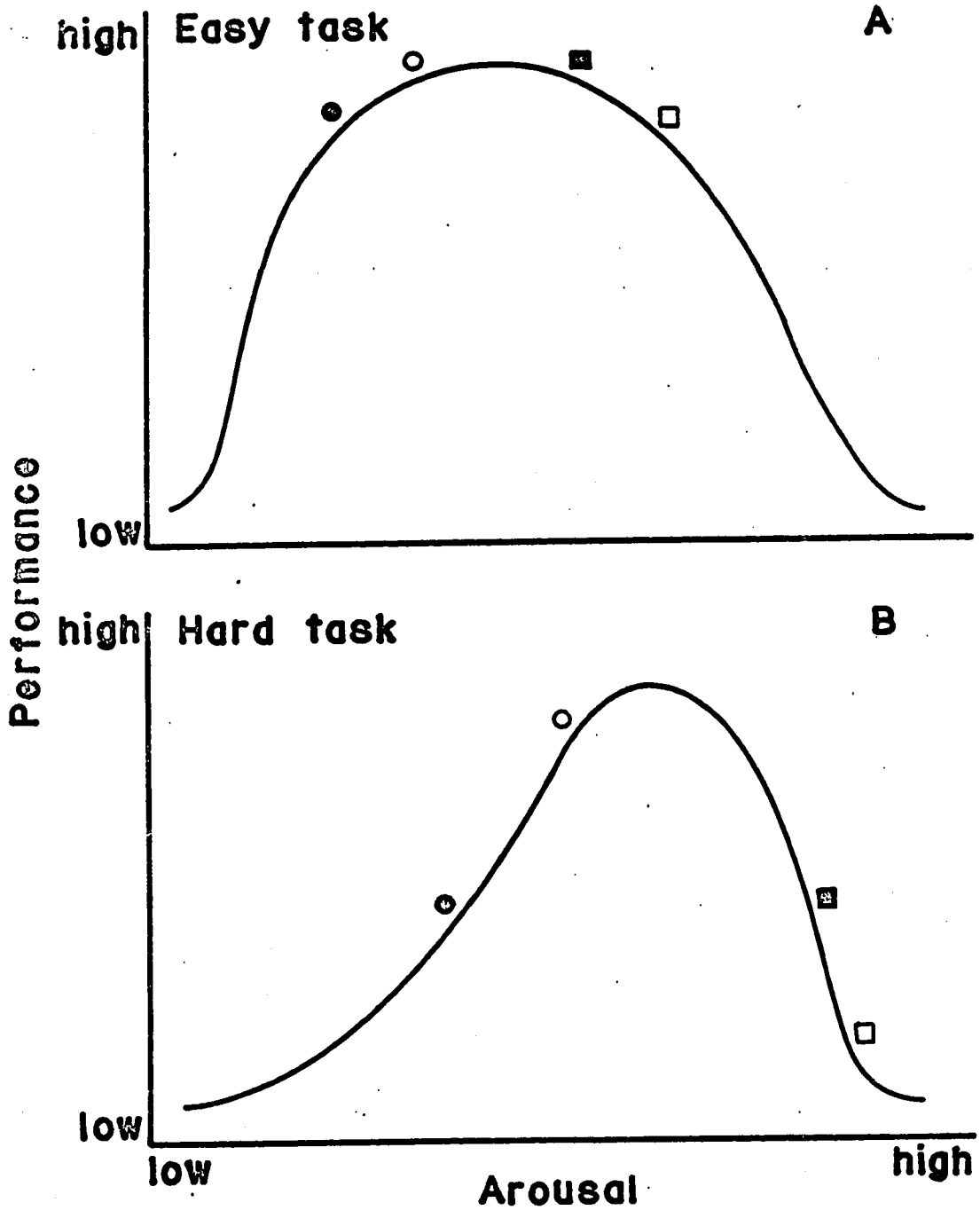


Figure I:2. Theoretical inverted-U function relating arousal to task performance; A: low task difficulty; B: high task difficulty. See text (page 31) for meaning of symbols.

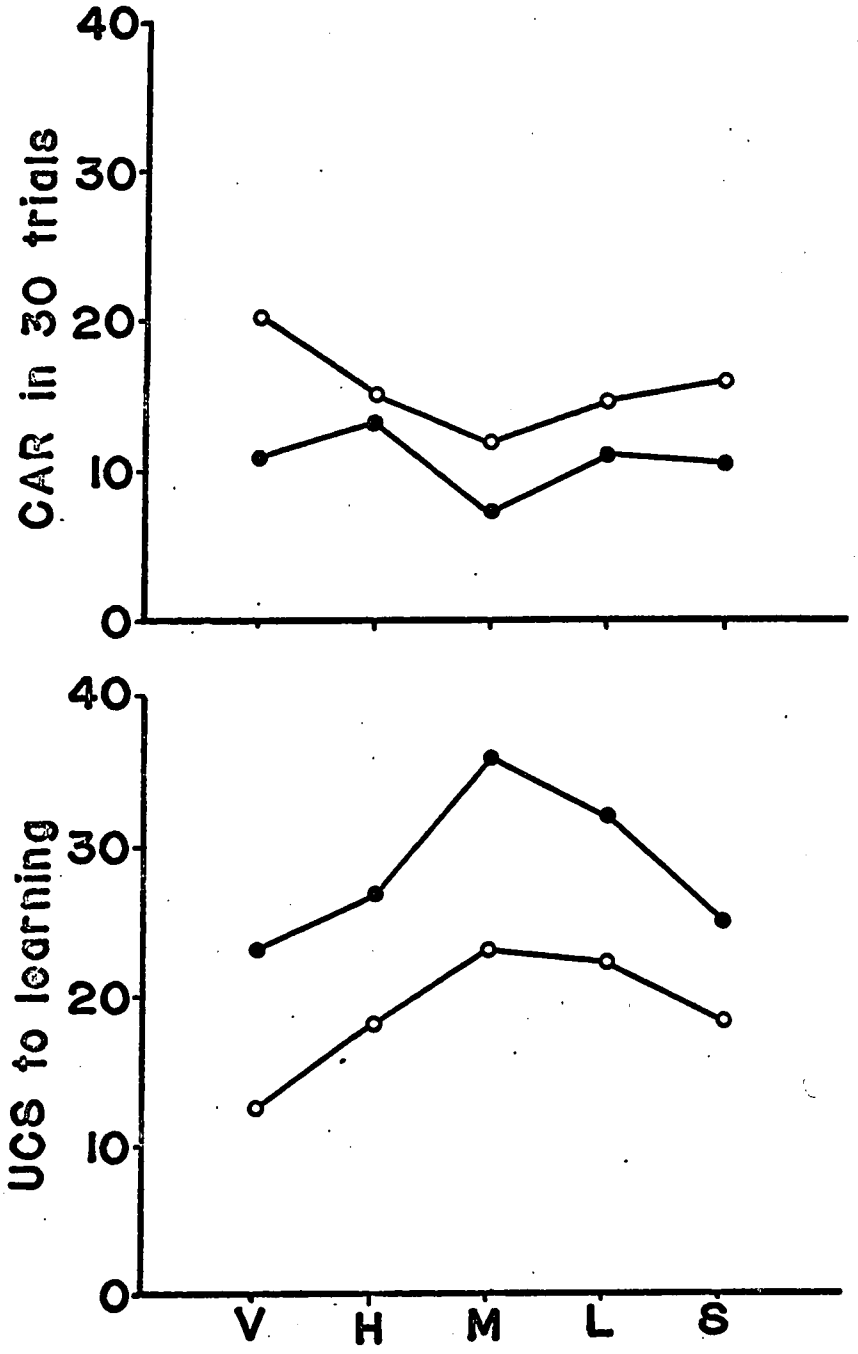


Figure 1. Shuttlebox CAR acquisition performance of Wistar male rats treated with vehicle (V), high (H), medium (M), or low (L) doses of ACTH or with physiological saline (S). See Experiment I for details.
○ 0.2 mA UCS; ● 0.5 mA UCS.

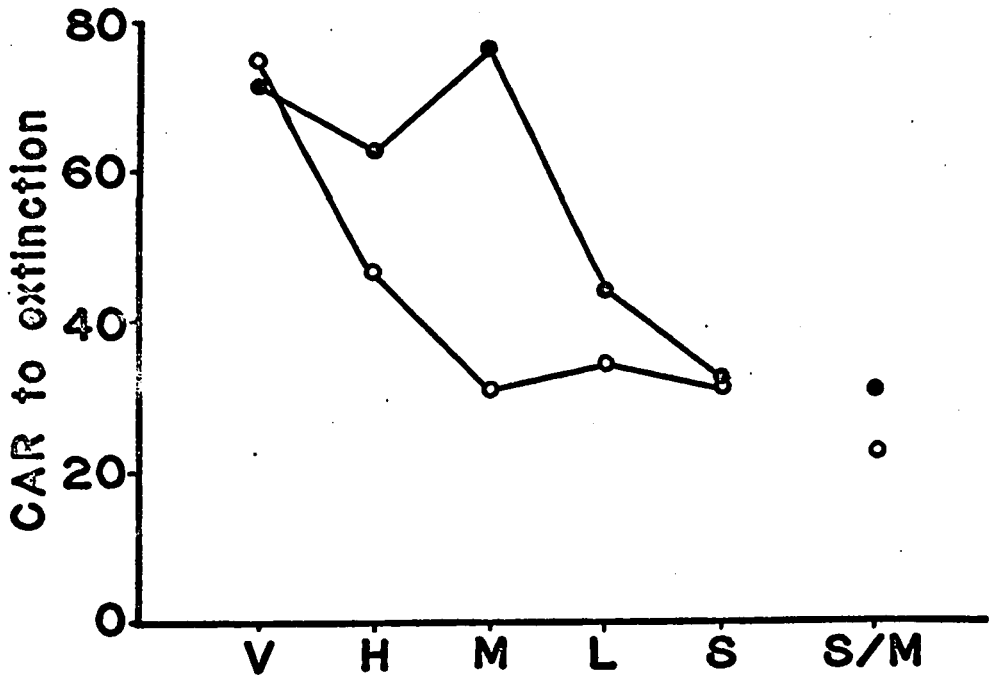


Figure 2. Shuttlebox CAR extinction performance of Wistar male rats treated with vehicle (V), high (H), medium (M), or low (L) doses of ACTH or with physiological saline (S). See Experiment I for details.

o 0.2 mA UCS; ● 0.5 mA UCS.

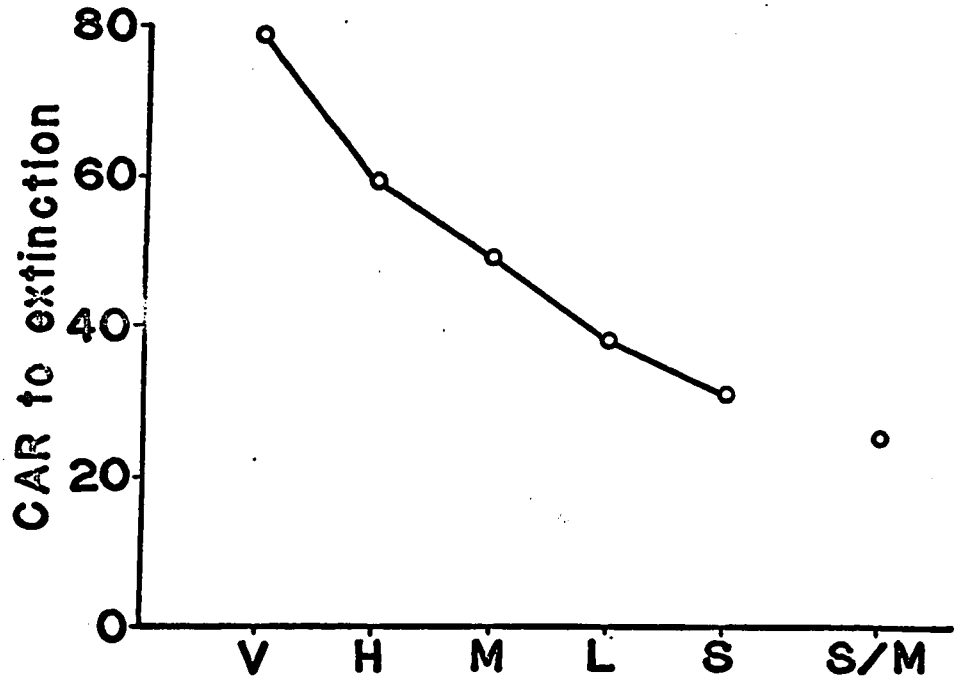


Figure 3. Averaged CAR extinction performance of Wistar male rats trained at 0.2 and 0.5 mA UCS intensity, and treated with vehicle (V), high (H), medium (M), or low (L) doses of ACTH or with physiological saline (S). Ss in the S/M group received saline during acquisition and the medium dose of ACTH during extinction.

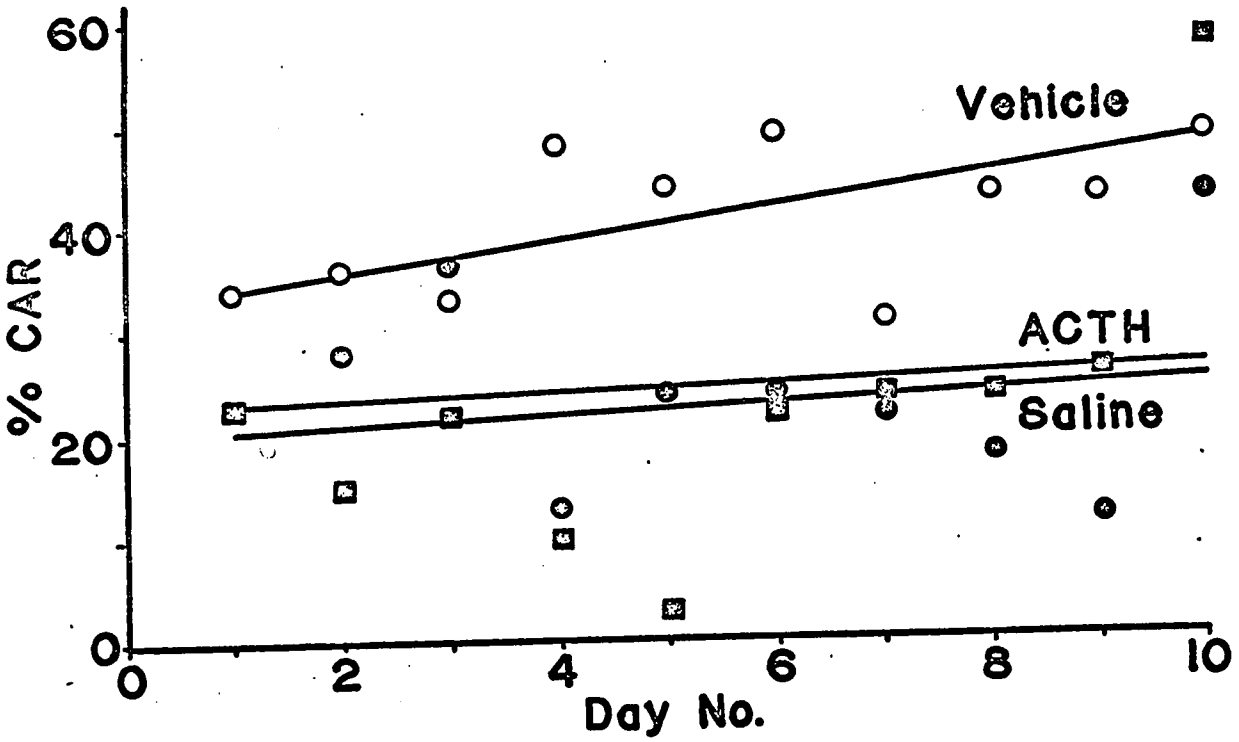


Figure 4. Shuttlebox CAR acquisition of Wistar male rats given low voltage footshock. See Experiment II for details. Subjects received ACTH (●), vehicle (O), or physiological saline (■).

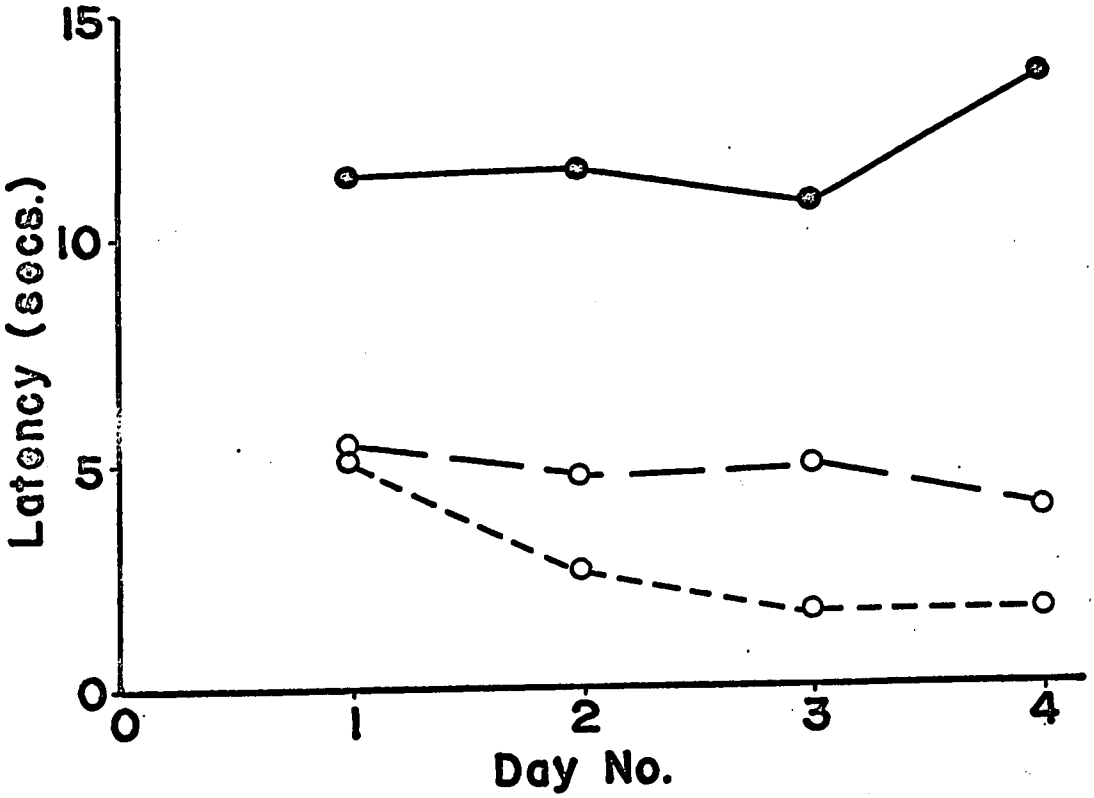


Figure 5. Mean shuttlebox response latency of the saline-treated Wistar male rats during the first four days of acquisition at three UCS intensity levels:
25 V., 5.0 mA, 350 V., 0.5 mA, 350 V., 0.2 mA.

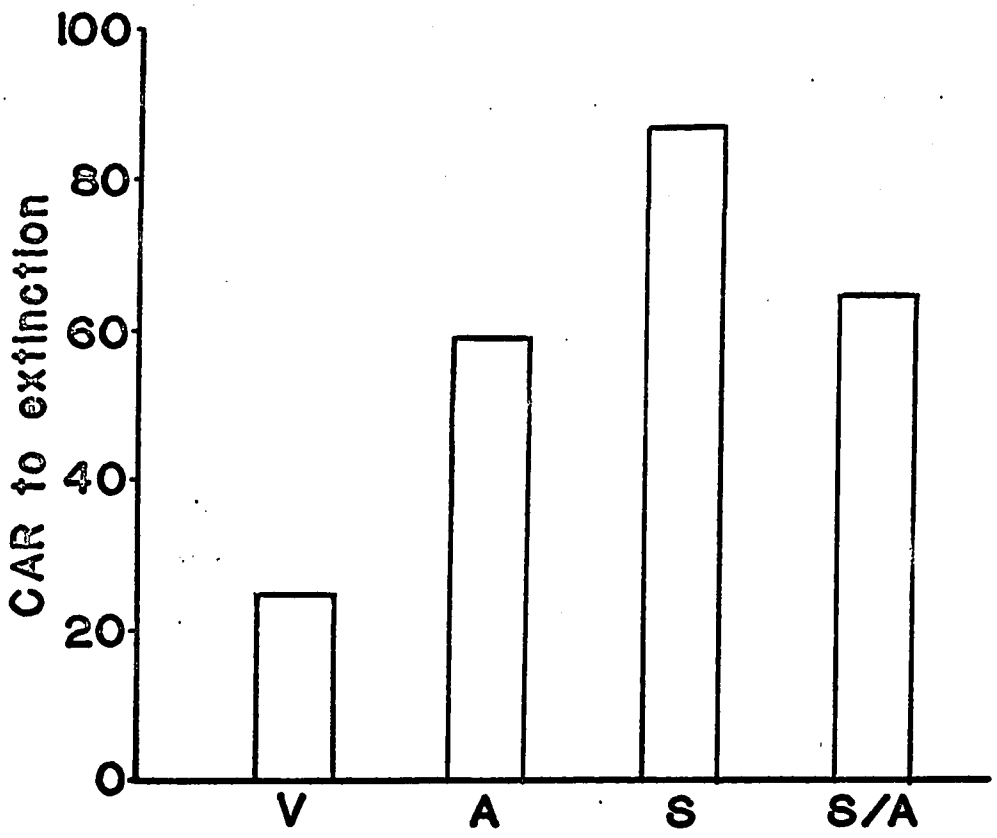


Figure 6. Shuttlebox extinction performance of Wistar male rats trained at 25 V., 5.0 mA UCS, and receiving vehicle (V), ACTH (A), or physiological saline (S) during acquisition and extinction, or saline during acquisition and ACTH during extinction (S/A).

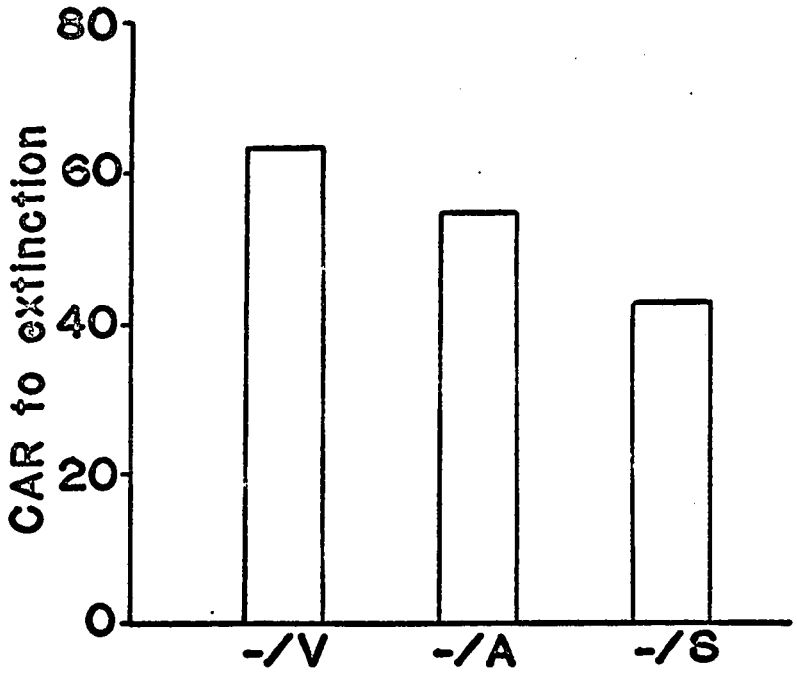


Figure 7. Shuttlebox extinction performance of Wistar male rats trained at 40 V., 1.8 mA UCS, and receiving vehicle (V), ACTH (A), or physiological saline (S) during extinction only.

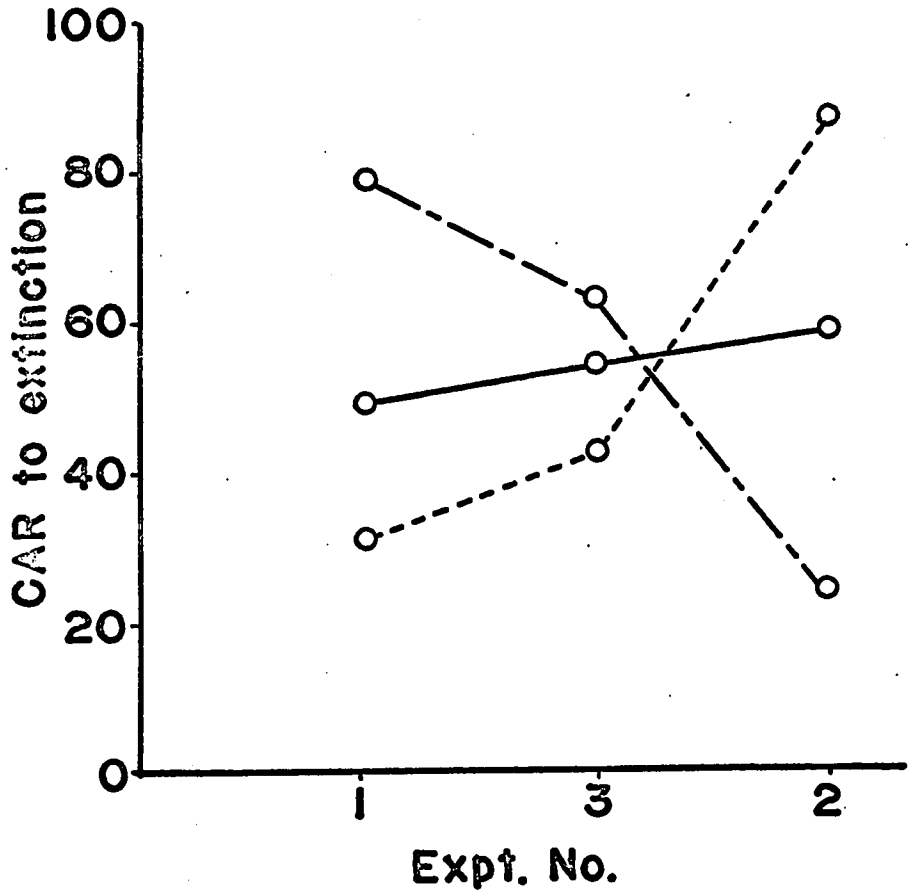


Figure 8. Shuttlebox extinction performance of Wistar male subjects trained with high voltage footshock and receiving ACTH (———), vehicle (—— — ——) or physiological saline (----------) during both acquisition and extinction (Experiment I) compared with subjects trained with medium voltage footshock and receiving injection treatment only during extinction (Experiment III) and with subjects receiving low voltage footshock and injection treatment during both acquisition and extinction (Experiment II).

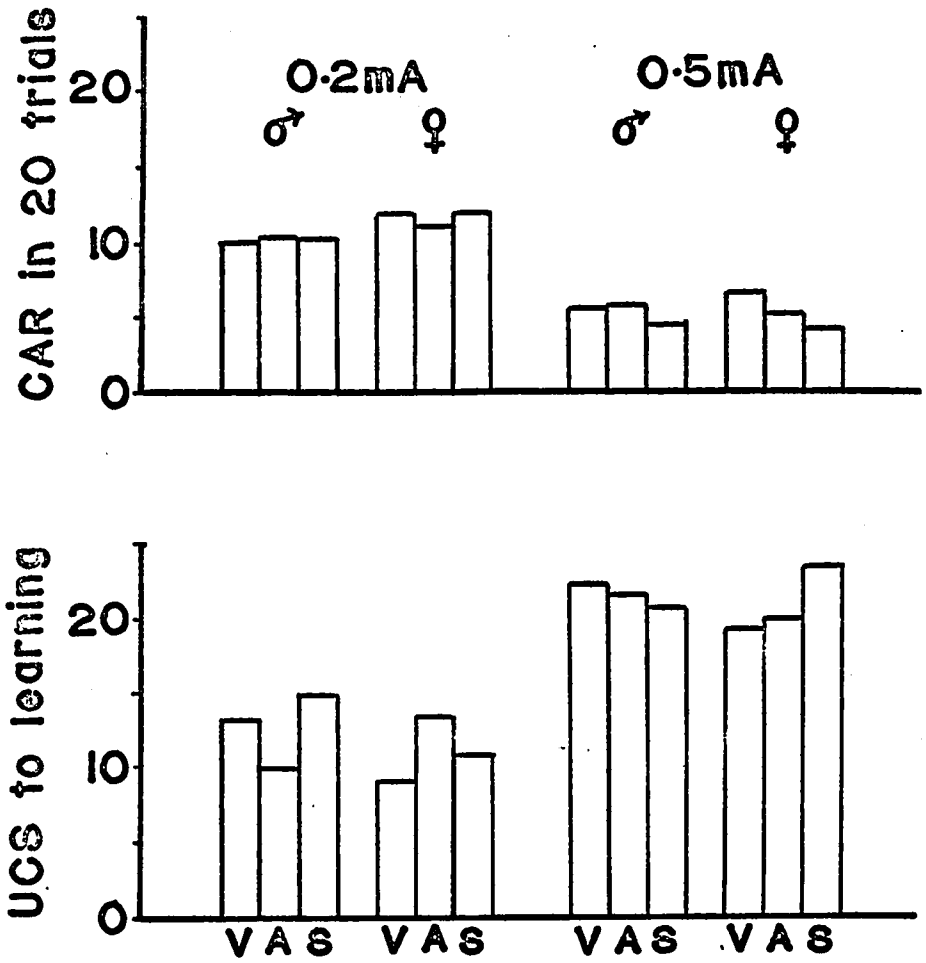


Figure 9. Shuttlebox acquisition performance of Long-Evans Hooded male and female subjects given vehicle (V), ACTH (A), or saline (S), and trained at 0.2 mA or 0.5 mA UCS intensity.

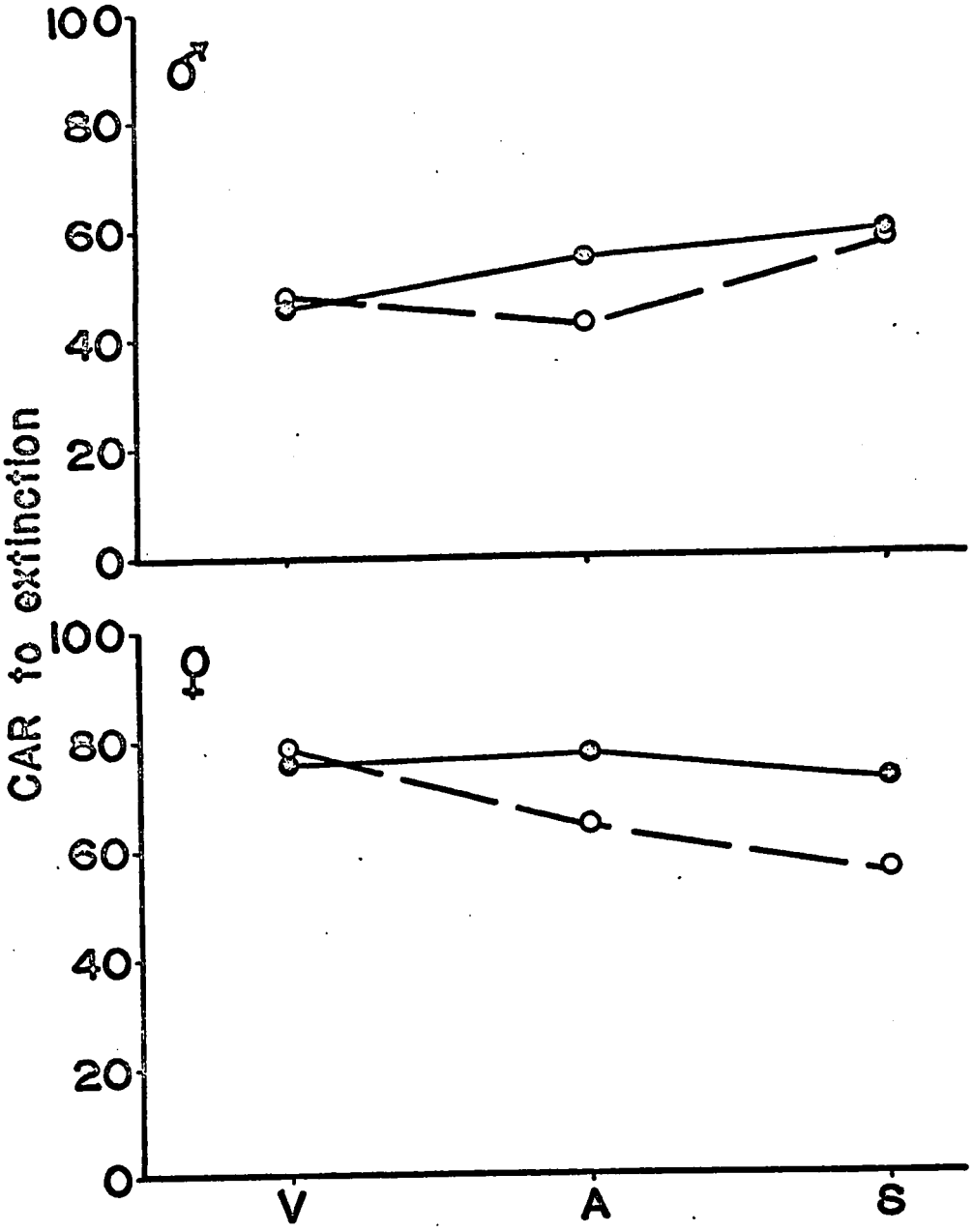


Figure 10. Shuttlebox extinction performance of Long-Evans Hooded male and female rats scoring high (H) on early acquisition performance. See Experiment IV for definition of group H. ●——● 0.5 mA UCS; ○— — ○ 0.2 mA UCS.

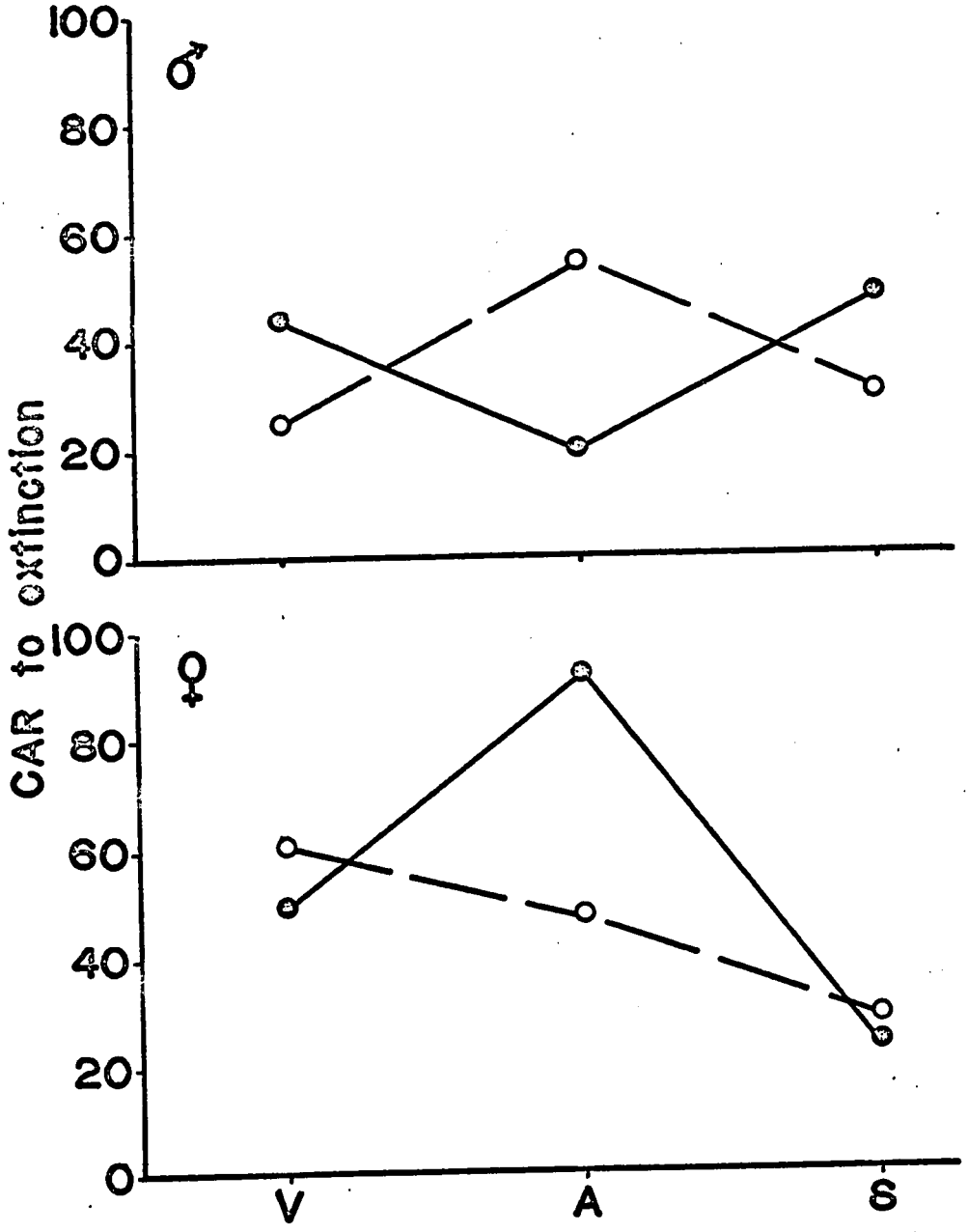


Figure 11. Shuttlebox extinction performance of Long-Evans Hooded male and female rats scoring low (L) on early acquisition performance. See Experiment IV for early definition of group L.
0——— 0.5 mA UCS, O——— 0.2 mA UCS.

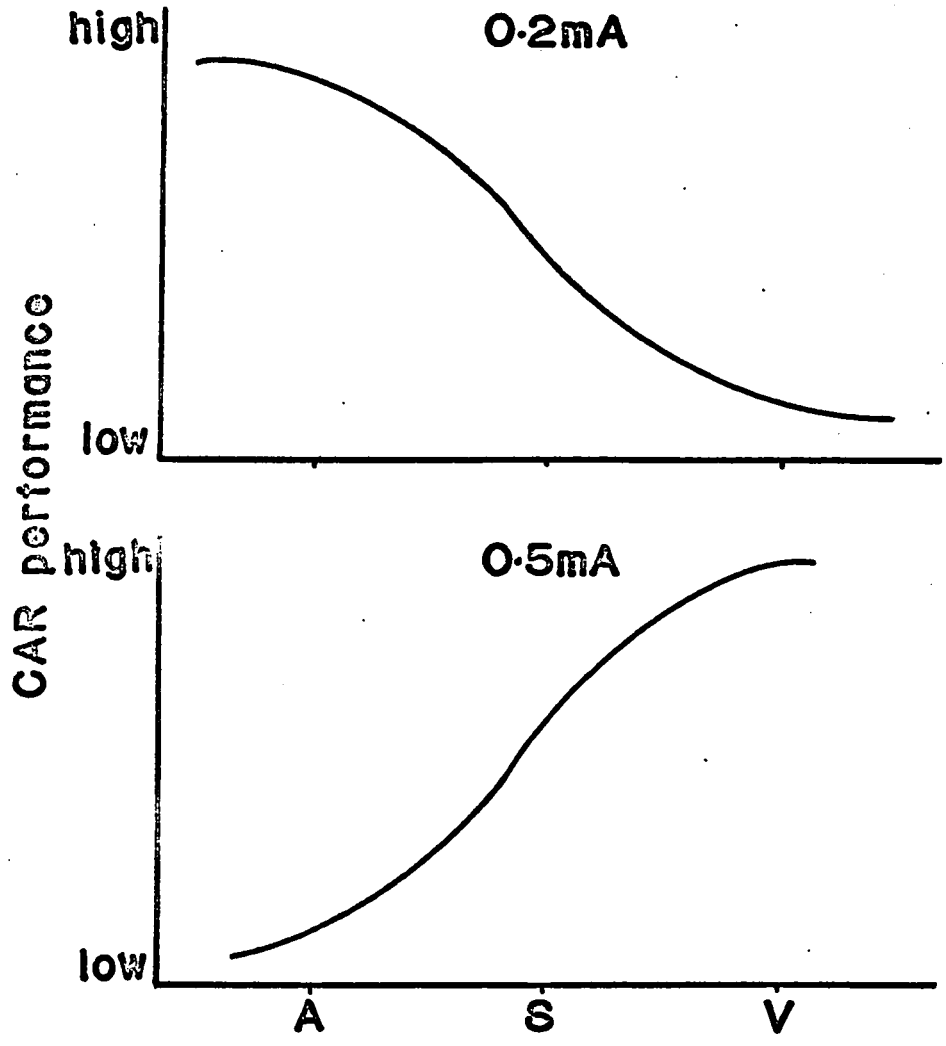


Figure 12. A possible theoretical interpretation of the extinction performance relationships among the 'Low' Long-Evans Hooded males treated with ACTH (A), saline (S), and vehicle (V).

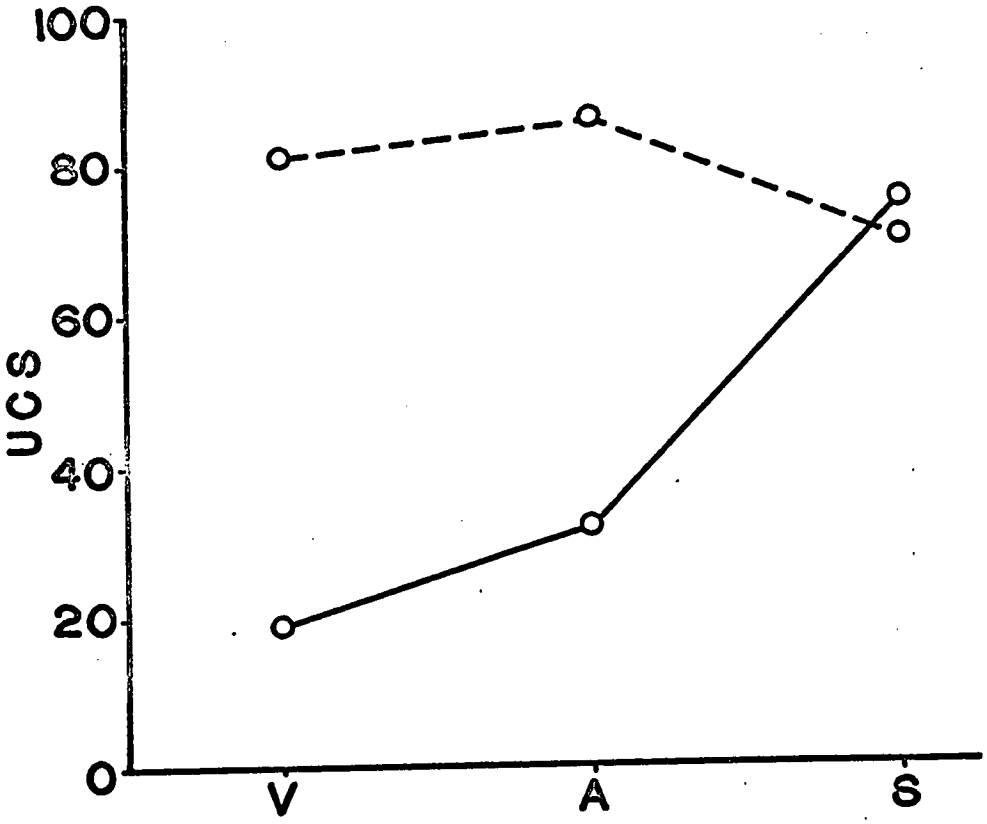


Figure 13. UCS to acquisition or in 100 trials for Long-Evans Hooded male rats trained at 1.0 mA UCS and treated with either vehicle (V), ACTH (A), or physiological saline (S). ——— handled subjects; – – – – – non-handled subjects. See Experiment V for definition of handling.

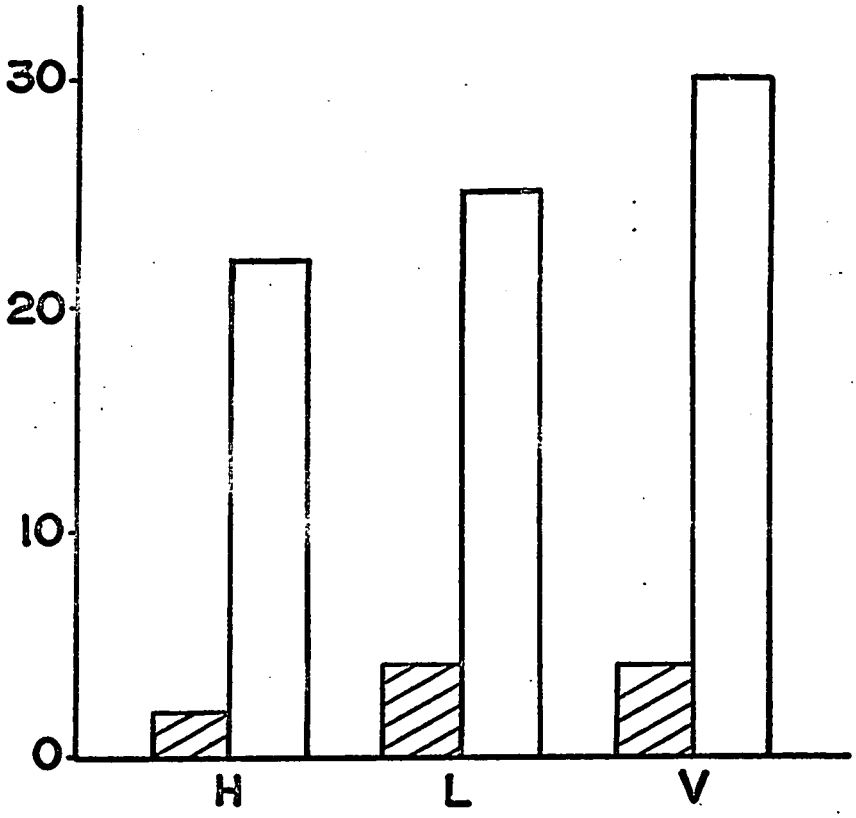


Figure 14. Performance of 'handled' Long-Evans Hooded male rats during ten non-reinforced pre-training trials in the shuttlebox: ▭ inter-trial crossings; ▨ crossings within five seconds of CS onset (avoidance). Subjects received a high (H) or a low (L) dose of ACTH or control vehicle (V).

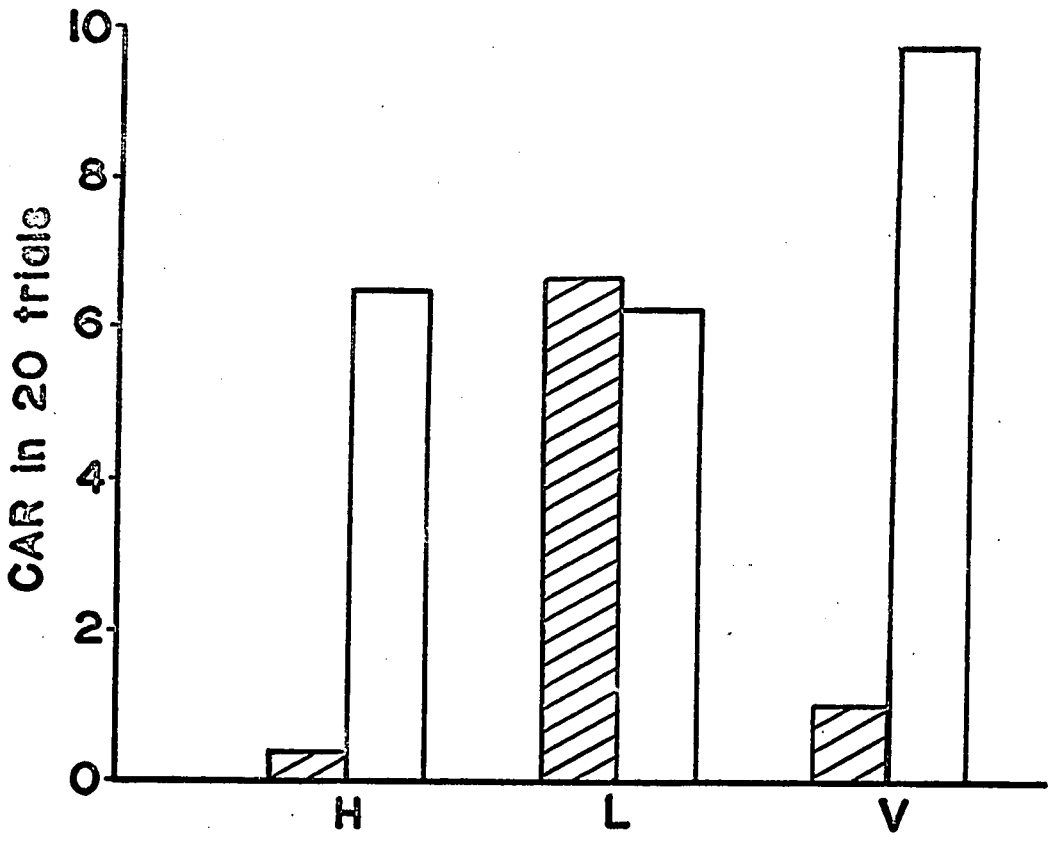


Figure 15. Shuttlebox acquisition performance of 'handled' Long-Evans Hooded male rats receiving a high (H) or a low (L) dose of ACTH or control vehicle (V). ▨ Subjects trained at 0.5 mA UCS; ▭ subjects trained at 1.0 mA UCS.

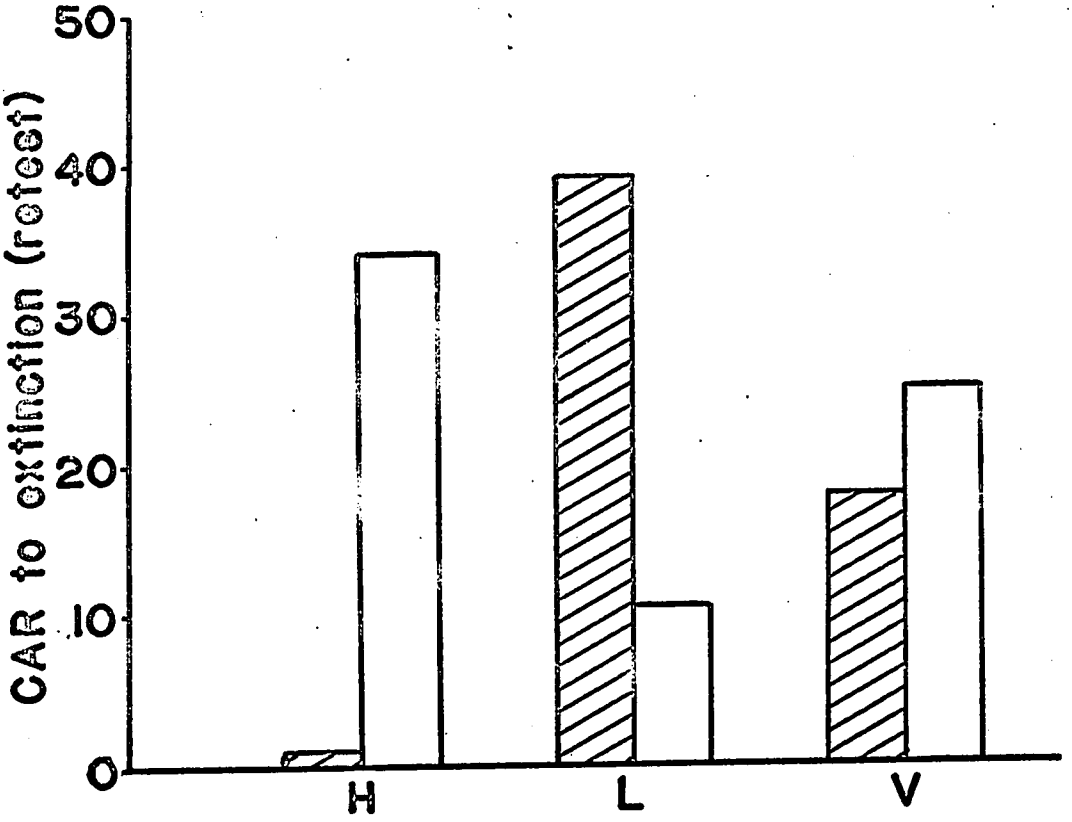


Figure 16. Shuttlebox extinction performance of 'handled' Long-Evans male rats tested one week after training. During training Ss received a high (H) or a low (L) dose of ACTH or control vehicle (V). ▨ Subjects trained at 0.5 mA UCS; ▭ subjects trained at 1.0 mA UCS.

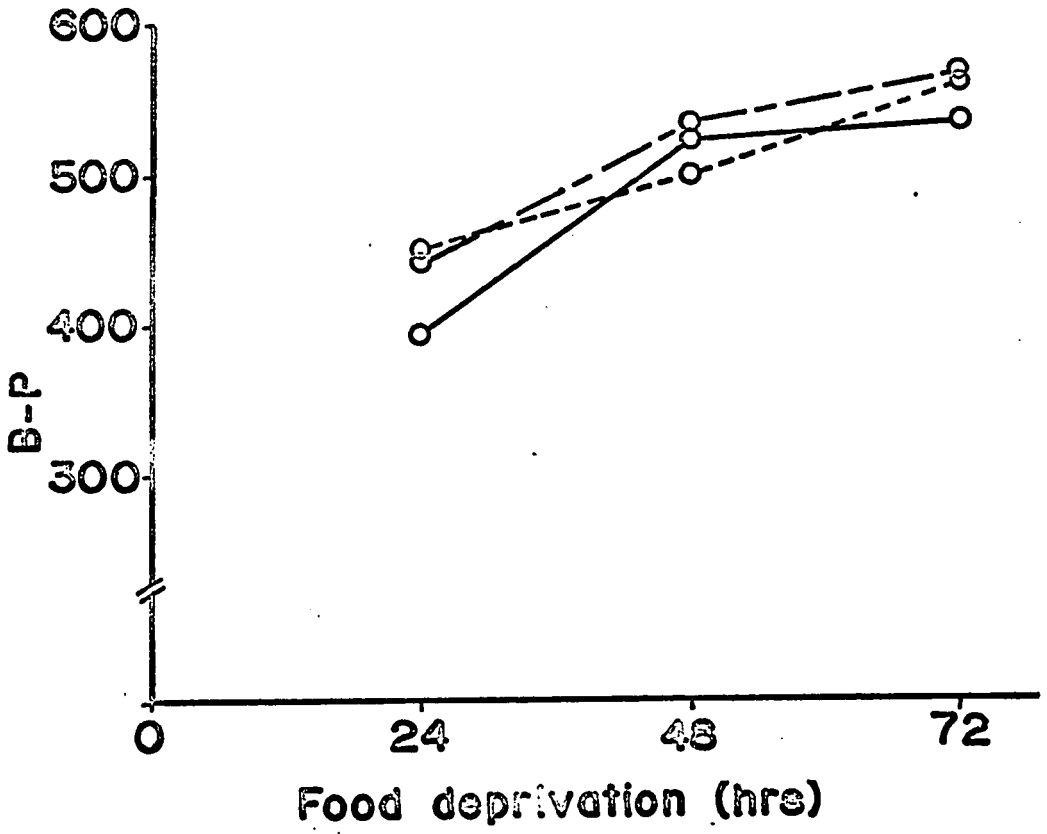


Figure 17. Operant level of bar pressing of Long-Evans Hooded male rats on a VI:35 reinforcement schedule at 24, 48, and 72 hours food deprivation. Subjects received injections of ACTH (————), vehicle (——–——) or physiological saline (----------).

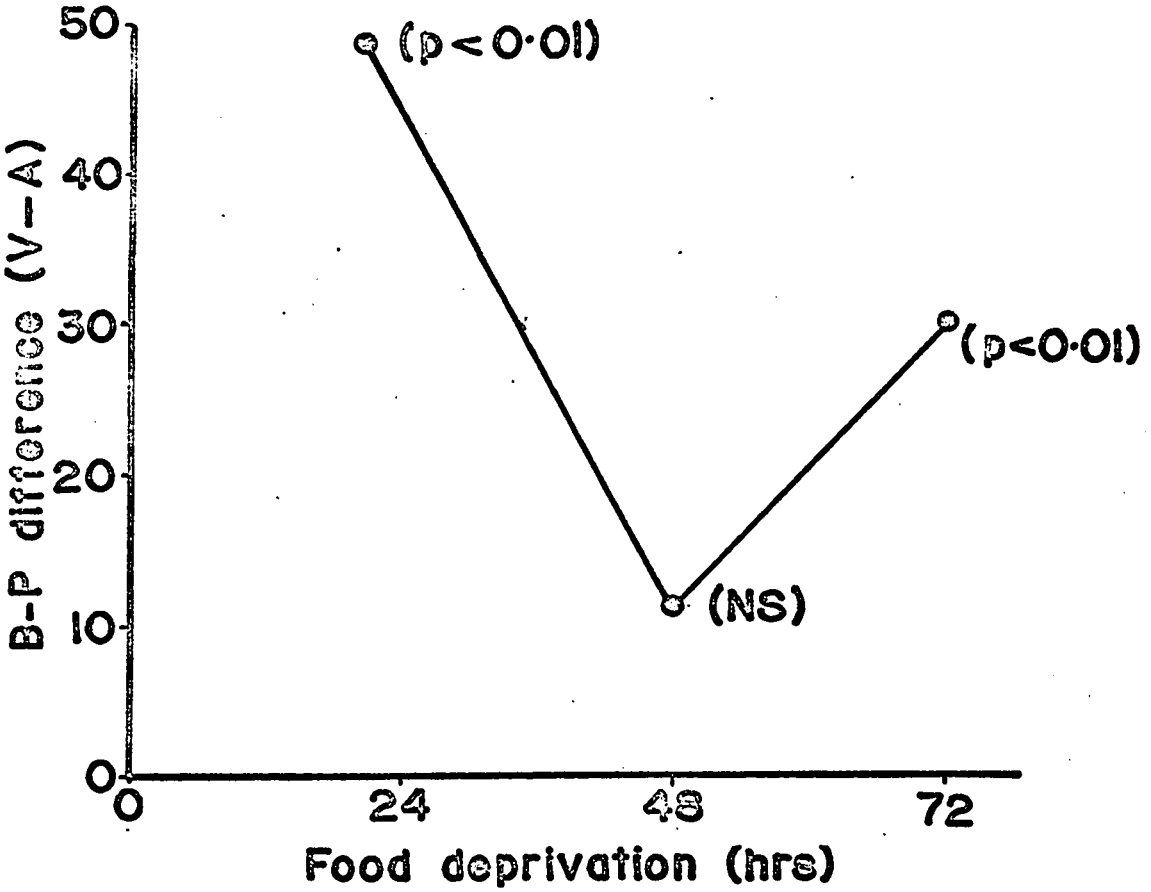


Figure 18. Difference score (vehicle minus ACTH) of Long-Evans Hooded male rats on a VI:35 reinforcement schedule at 24, 48, and 72 hours food deprivation. The associated probability levels are in parenthesis.

REFERENCES

Ader, R.
Gastric erosions in the rat: effects of immobilization at different points in the activity cycle.
Science, 1964, 145, 406-497.

Anderson, D., Winn, W., and Tam, T.
Adrenocorticotrophic hormone and acquisition of a passive avoidance response: a replication and extension.
Journal of Comparative and Physiological Psychology, 1968, 66, 497-499.

Appley, M.H.
Endocrine factors in avoidance learning.
Invited address: Canadian Psychological Association, 1964.

Beatty, Patricia Ann, Stolle, T.H., and Beatty, W.W.
Failure of exogenous ACTH to influence the acquisition or reversal of a spatial discrimination in rats.
Psychonomic Science, 1969, 17, 156-157.

Belanger, D.
Effects of hypophysectomy on the learning of an escape-avoidance reaction.
Canadian Journal of Psychology, 1958, 12, 171-178.

Bohus, B. and de Wied, D.
Inhibitory and facilitatory effect of two related peptides on extinction of avoidance behavior.
Science, 1966, 153 (3733), 318-320.

Broadhurst, P.L.
Emotionality and the Yerkes-Dodson law.
Journal of Experimental Psychology, 1957, 54, 345-352.

Bronson, F.H. and Eleftheriou, B.E.
Adrenal response to fighting in mice: separation of physical and psychological cause.
Science, 1965, 147, 627-628.

Broverman, D.M., Klaiber, E.L., Kobayashi, Y., and Vogel, W.
Roles of activation and inhibition in sex differences in cognitive abilities.
Psychological Review, 1968, 75, 23-50.

Calhoun, J.B.
Population density and social pathology.
Scientific American, 1962, 206, 139-147.

Christian, J.J. and Davis, D.E.
Journal of Mammalogy, 1956, 37, 475.

Christian, J.J., Lloyd, J.A. and Davis, D.E.
The role of endocrines in the self-regulation of mammalian populations.
In: Recent Progress in Hormone Research, 1965, 21, 501-578.

Cleghorn, R.A.
Psychological and behavioral reactions as side effects of steroid administration.
In: Ciba Foundation Colloquia on Endocrinology, v. 3, New York: Blakiston, 1952.

Corson, J.A.
Selected aspects of neuroendocrine function: role in learning and memory.
Paper presented at the Canadian Psychological Association Symposium, "Biological Basis of Learning: Frontiers of Research", 1966.

Corson, J.A., Kirkpatrick, Dhalia, and Ley, K.F.
Adrenocorticotrophic hormone (ACTH). Injections influence food motivated discrimination in the cat.
In preparation, 1970.

Cox, G.S., Hodges, J.R., and Vernikos, J.
The effect of adrenalectomy on the circulating level of adrenocorticotrophic hormones in the rat.
Journal of Endocrinology, 1958, 17, 177-180.

de Groot, J.
Limbic and other neural pathways that regulate endocrine function
In: Martini, L. and Ganong, W. (Eds.), Neuroendocrinology.
New York: Academic Press, 1966.

de Wied, D.
Influence of anterior pituitary on avoidance learning and escape behavior.
American Journal of Physiology, 1964, 207, 255-259.

de Wied, D.
Inhibitory effect of ACTH and related peptides on extinction of conditioned avoidance behavior in rats.
Proceedings of the Society for Experimental Biology and Medicine, 1966, 122, 28-32.

de Wied, D., Bohus, B., and Greven, H.M.
Influence of pituitary and adrenocortical hormones on conditioned avoidance behavior in rats.
In: Endocrinology and Human Behavior. R.P. Michael (Ed.). London: Oxford, 1968.

Doty, P.
Proteins.
Scientific American, 1957, 197(3), 173-184.

Eggleston, L.V. and Krebs, H.A.
Strain differences in the activities of rat liver enzymes.
Biochemical Journal, 1969, 114, 877-879.

Endröczi, E. and Lissák, K.
Spontaneous goal-directed motor activity related to the alimentary conditioned reflex behavior and its regulation by neural and humoral factors.
Acta Physiologica Hungaricae, 1962, 21, 265-583.

Endröczi, E., Telegdy, G.Y., and Lissák, K.
Analysis of the individual variations of adaptations in the rat, on the basis of conditioned reflex and endocrine studies.
Acta Physiologica Hungaricae, 1957, XI, 393-398.

Festinger, L.
An exact test of significance for means of samples drawn from populations with an exponential frequency distribution.
Psychometrika, 1943, 8(3), 153-160.

Fisher, A.E.
Chemical stimulation of the brain.
Scientific American, 1964, 210, 60-68.

Friedman, H., and Derks, P.
Stress induced by active and passive avoidance training: an analogue to stress induced by crowding.
A research proposal submitted to the National Aeronautics and Space Administration, 1966.

Friedman, S.B., Ader, R., Grota, L., and Larson, T.
Plasma corticosterone response to parameters of electric shock stimulation in the rat.
Psychosomatic Medicine, 1967, XXIX, 323-328.

Gaito, J., Mottin, J., Davison, J., and Rigler, Joanne.
The effect of hypophysectomy on avoidance behavior and on molecular neurochemistry.
Molecular Psychobiology Laboratory research report no. 6, Toronto: York University, 1966.

Hebb, D.O.
A Textbook of Psychology.
New York: Saunders, 1966 (second edition).

Hodges, J.R., and Jones, M.T.
Changes in pituitary corticotrophic function in the adrenalectomized rat.
Journal of Physiology, 1964, 173, 190-200.

Ivanov, V.M.
Changes in higher nervous activity in rats after hypophysectomy.
Pavlov, 1961, 11, 126-130.

Joffe, J.M.
Avoidance learning and failure to learn in two strains of rats selectively bred for emotionality.
Psychonomic Science, 1964, 1, 185-186.

Kasper-Pandi, P., Hansing, R., and Usher, D.R.
Effect of dexamethasone blockade of ACTH release on avoidance learning.
Physiology and Behavior, 1970, 5, 361-363.

Korányi, L., Endröczi, E., Lissák, K., and Szepes, Eva.
The effect of ACTH on behavioral processes motivated by fear in mice.
Physiology and Behavior, 1967, 2, 439-445.

Levine, S.
UCS intensity and avoidance learning.
Journal of Experimental Psychology, 1966, 71, 163-164.

Levine, S.
Hormones and conditioning.
Nebraska symposium on motivation, XIV, 1968, 85-101.

Levine, S., and Jones, Lucy.
Adrenocorticotrophic hormone (ACTH) and passive avoidance learning.
Journal of Comparative and Physiological Psychology, 1965, 59, 357-360.

Li, C.H.
The ACTH molecule.
Scientific American, 1963, 209, 46-53.

Lissák, K., and Endrőczi, E.
Neurohumoral factors in the control of animal behavior.
In: Brain mechanisms and learning. Delafresnaye, J.F. (Ed.).
Springfield: Chas. Thomas, 1961, 293-308.

Malmo, R.B.
Activation: a neuropsychological dimension.
Psychological Review, 1959, 66, 367-386.

Malmo, R.B., and Belanger, D.
Related physiological and behavioral changes: what are their determinants?
In: Sleep and Altered States of Consciousness. Kety, S.S., Evarts, E.V., and Williams, H.L. (Eds.)
Baltimore: Williams and Wilkins, 1968.

Mangili, G., Motta, Marcella, and Martini, L.
Control of adrenocorticotrophic hormone secretion.
In: Neuroendocrinology. Martini, L., and Ganong, W. (Eds.).
New York: Academic Press, 1966.

Marquis, H., and Suboski, M.
Hypophysectomy and ACTH replacement in the incubation of passive and shuttlebox avoidance responses.
Proceedings of the 77th Annual Convention of the American Psychological Association, 1969, 4, 207-208.

Mason, J.W.
Psychological influences on the pituitary-adrenal cortical system.
In: Recent Progress in Hormone Research, 1959, 15, 345-389.

Miller, N.E.
Studies of fear as an acquirable drive: 1. Fear as motivation and fear-reduction as reinforcement in the learning of new responses.
Journal of Experimental Psychology, 1948, 38, 89-101.

Miller, R.E., and Ogawa, N.
The effect of adrenocorticotrophic hormone (ACTH) on avoidance conditioning in the adrenalectomized rat.
Journal of Comparative and Physiological Psychology, 1962, 55, 211-213.

Mirsky, I.A., Miller, R., and Stein, M.
Relation of adrenocortical activity and adaptive behavior.
Psychosomatic Medicine, 1953, 15, 574-584.

Moyer, K.E., and Korn, J.H.
Effect of UCS intensity on the acquisition and extinction of an avoidance response.
Journal of Experimental Psychology, 1964, 67, 352-359.

Murphy, J.V., and Miller, R.E.
The effect of adrenocorticotrophic hormone (ACTH) on avoidance conditioning in the rat.
Journal of Comparative and Physiological Psychology, 1955, 48, 47-49.

Ronziger, L., and Kindwall, J.A.
Thyroid therapy in some mental disorders.
Diseases of the Nervous System, 1948, 9, 2-12.

Rubin, L.S.
Patterns of adrenergic-cholinergic imbalance in the functional psychoses.
Psychological Review, 1962, 69, 501-519.

Saroff, J., and Wexler, B.
Metabolic clearance and production rates of corticosterone in male and female virgin and breeder rats.
Acta Endocrinologica, 1969, 62, 411-424.

Sawrey, W.L., Conger, J.J., and Turrell, E.S.
An experimental investigation of the role of psychological factors in the production of gastric ulcers in rats.
Journal of Comparative and Physiological Psychology, 1956, 49, 457-461.

Schildkraut, J.J., and Kety, S.S.
Biogenic amines and emotion.
Science, 1967, 156, 21-30.

Schwyzer, R.
Chemistry and metabolic action of nonsteroid hormones.
Annual Review of Biochemistry, 1964, 33, 259-286.

Seward, J.P., and Seward, G.H.
Psychological effects of estrogenic hormone therapy in the menopause.
Journal of Comparative and Physiological Psychology, 1937, 24, 377-392.

Sourkes, T.L.
Biochemistry of Mental Disease.
New York: Hoeber Division of Harper and Row, 1964.

Srole, L., Langner, T., Michael, S., Opler, M., and Rennie, T.
Mental Health in the Metropolis.
New York: Blakiston Division of McGraw-Hill, 1962.

Thoman, Evelyn, and Levine, S.
Role of maternal disturbance and temperature change in early experience studies.
Physiology and Behavior, 1969, 4, 143-145.

Turner, C.D.
General Endocrinology.
Philadelphia: W.B. Saunders Co., 1966.

Weiss, J.M., McEwen, B.S., Silva, Teresa, and Kalkut, Marcia.
Pituitary-adrenal influences on fear responding.
Science, 1969, 163, 197-199.

Welch, B.L., and Welch, A.S.
Graded effect of social stimulation upon d-amphetamine toxicity, aggressiveness and heart and adrenal weight.
Journal of Pharmacology and Experimental Therapeutics, 1966, 151, 331-338.

Werner, A.A.
Syndrome accompanying deficiency or absence of the ovarian follicular hormone.
Endocrinology, 1935, 19, 695-700.

Wertheim, G.A., Conner, R.L., and Levine, S.
Adrenocortical influences on free-operant avoidance behavior.
Journal of the Experimental Analysis of Behavior, 1967, 10, 555-563.

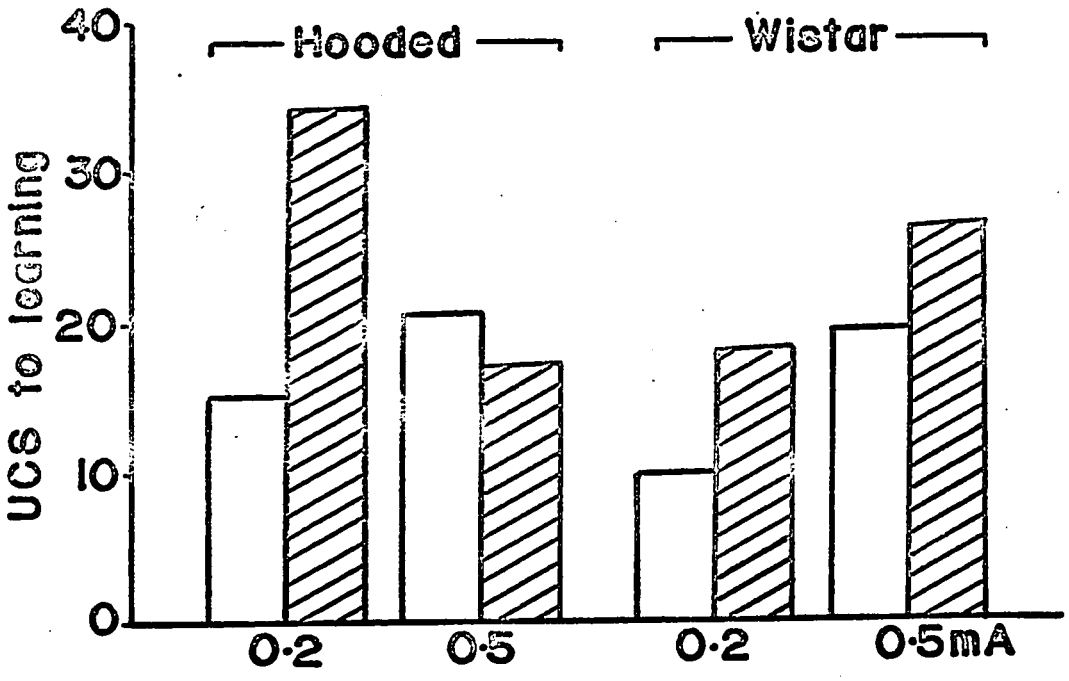
Wertheim, G.A., Conner, R.L., and Levine S.
Avoidance conditioning and adrenocortical function in the rat.
Physiology and Behavior, 1969, 4, 41-44.

White, A., Handler, P., and Smith, E.
Principles of Biochemistry.
New York: McGraw-Hill, 1964.

Winer, B.J.
Statistical principles in experimental design.
New York: McGraw-Hill, 1962.

Woodbury, D.M.
Effect of hormones on brain excitability and electrolytes.
In: Recent progress in hormone research. G. Pincus (Ed.).
1954, X, 65-107.

Young, W.C., and Rundlett, B.
The hormonal induction of homosexual behavior in the spayed female guinea pig.
Psychosomatic Medicine, 1939, 1, 449-460.



APPENDIX A

Comparison of shuttlebox acquisition of Long-Evans Hooded males and Wistar albino males, 160±20 grams, given massed training (▭) as in Experiment IV, or spaced training (▨) as in Experiment I.