RETENTION OF PASSIVE AVOIDANCE LEARNING AND ITS HORMONAL CONTROL

By

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INTRODUCTION

Retention Function of an Aversively Motivated Response

Until recently, a detailed study of retention was confined mainly to the field of human verbal learning. There was no equivalent for the Ebbinghaus curve of retention within the conditioning literature. The exact form which a retention curve for conditioned responses might exhibit was not obvious, especially in the case of an aversively motivated response. The notion of incubation of fear (an increase of fear with passage of time) was first described by Diven (1937) and later by Haggard (1943). This notion was considered as a possibility for the retention function of an aversively motivated response, because there was some evidence that retention of avoidance learning might exhibit an incubation effect rather than a forgetting effect. The studies of electroconvulsive shock (ECS) by Brady (1952) and Brady, Stebbins, and Hunt (1953), as well as the studies of galvanic skin response (GSR) conditioning of Golin (1961) and Golin and Golin (1966) and the conditioned heart-rate acceleration studies by Breznitz (1967), all support the incubation hypothesis.

In 1957, Kamin made the rather surprising discovery that the retention of an incompletely learned avoidance response, as measured by relearning, is a U-shaped function of the time interval separating the original and retraining sessions (intersession interval - ISI), the minimum of the function being at 1 hr. He interpreted the data in terms of two independent processes: a forgetting process for the first segment extending from zero interval to 1 hr. and an incubation effect for the rising segment of the curve, extending from 1 hr. to 19 days (Kamin, 1957). This U-shaped function has been replicated by Denny, 1958; Denny and Thomas, 1960; Denny and Ditchman, 1962; and Brush, Myer, and Palmer, 1963. The

replications differ somewhat with regard to location of the minimum of the retention function; low points have been reported at 1, 2, and 4 hrs.

Kamin (1963) attempted to analyze further the retention curve of an incompletely learned avoidance response. He suggested that the U-shaped function appeared to be a composite of two factors: a warm-up decrement increasing monotonically with time (which he hypothesized might possibly be a dissipation of shock-induced emotionality) and an inverted U function which rises to a maximum 1 hr. after original learning and impedes performance. He thought that to attribute the latter function either to "recruited reticular activity" as Denny and Ditchman (1962) did or to a "parasympathetic overreaction" as Brush, Myer, and Palmer (1963) did was premature since there was no agreement yet as to whether the behavioral deficit after 1 hr. was due to too little or too much fear of the conditioned stimulus (CS) at that time or whether the behavioral deficit was dependent on emotional reactivity to the CS at all. He stated the need for an independent measurement of the emotional value of the CS and for careful parametric analysis of the warm-up phenomenon.

Brush (1964) showed that fear conditioning is the component of original training that is "necessary and sufficient" to produce the U-shaped function. He postulated that Kamin's (1963) failure to find this function after fear conditioning was probably due to an unfortunate selection of temporal or other parameters of fear conditioning. Recently, Brush (unpublished) has found that the retention of a learned avoidance response, as measured by extinction, is also a U-shaped function of the time separating training and extinction sessions.

Recent studies by McMichael (1966) and by Tarpy (1966) have attempted to determine the amount of fear present at various times following the

conditioning of fear in order to test the Denny-Ditchman hypothesis, which proposes that the curvilinear function depends on an incubation of fear followed, after a time interval, by its dissipation (Denny and Ditchman, 1962). They concluded that their results indicated an incubation of fear with time and thus supported the Denny-Ditchman explanation of the Kamin effect. However, their data demonstrated only an increase of fear and not a decrease, both of which are required by the hypothesis. In a second experiment McMichael used a passive avoidance measure to evaluate the Denny-Ditchman hypothesis. He reported that with increased intervals between training and testing, retention of the passive avoidance response decreased and then increased, with the point of poorest performance at the 4 hr. ISI. Thus, McMichael interpreted his results as providing support for the incubation-disruption explanation of the Kamin effect. However, McAllister and McAllister (1967) interpreted the results of McMichael's (1966) second experiment as not providing supportive evidence for the incubation notion and the Denny-Ditchman hypothesis; because with the exception of the 4-hr. delay group, all shocked groups showed retention of the passive avoidance response, although retention declined slightly as the duration of the retention interval increased.

The Hypothalamo-hypophyseal-adrenocortical System and Its Possible Relation to the U-shaped Retention Function

The time scale which describes these relations between performance and retention interval is particularly significant in examining the possible determinants of the U-shaped retention function. Since the time scale describing the temporal relation between the onset of noxious stimuli and the pattern of the physiological stress response is the right order of magnitude, it has been hypothesized that the Kamin effect might be a result

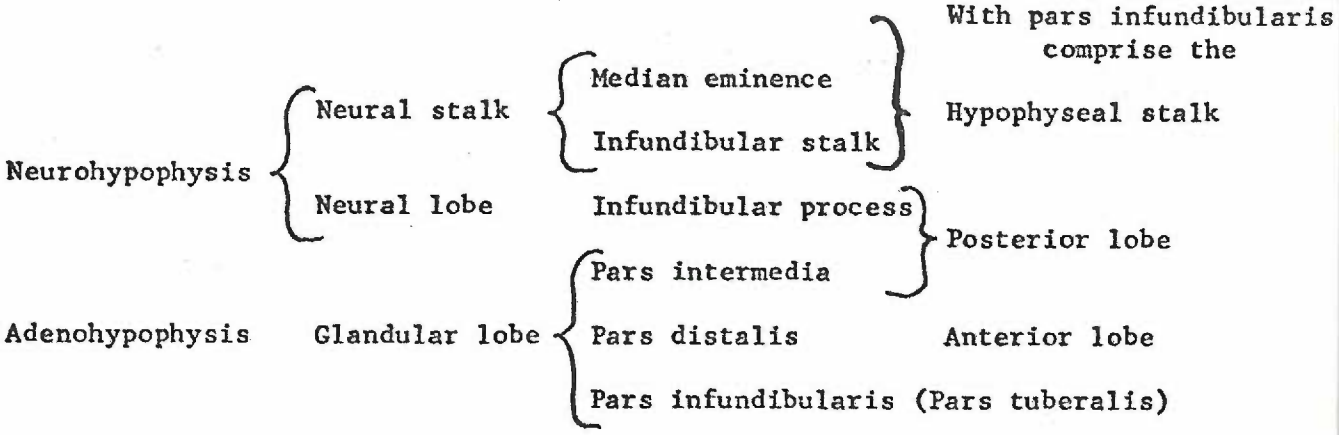
of changes in the activity of the hypothalamo-hypophyseal-adrenocortical (HHA) system. Before beginning a discussion of the studies designed to evaluate this hypothesis, let us briefly review the HHA system.

Description of HHA System

One of the well documented effects of environmental stress is increased pituitary-adrenocortical activity (Selye, 1946 and Selye, 1955). To understand this stress reaction requires some knowledge of the adrenal and pituitary glands and their functional interrelations.

The adrenal gland consists of an outer cortex and an inner medulla, both of which are enclosed by a thick capsule that extends into the cortex to varying depths. The blood supply of the gland comes from three principal groups of arteries. The superior suprarenal arteries arising from the inferior phrenic artery appear to be the major source. In addition, there are the middle suprarenals arising from the aorta and the inferior suprarenals, which are branches of the renal artery. Arteries from the above-mentioned sources form a plexus in the capsule from which the cortical arteries arise. Some of the major arterial branches from the capsule run through the cortex giving off few or no branches until they reach the medulla. The medulla thus has a dual supply - via the capillaries from the cortical arteries and via the medullary arteries that run from the capsule directly to the medulla. The capillaries of the cortex and of the medulla drain into a common venous system which emerges from the adrenal gland as the suprarenal vein (Bloom and Fawcett, 1968).

The pituitary gland or hypophysis is also divided into two main parts, the adenohypophysis and the neurohypophysis often incorrectly referred to as the anterior lobe and posterior lobe, respectively. The various classifications and subclassifications of the hypophysis are as follows (Bloom and Fawcett, 1968):



The blood supply of the hypophysis is involved in the control of the secretory activity of the gland. The inferior hypophyseal arteries, from the internal carotid, branch into the posterior lobe and to a lesser extent into the anterior lobe. The superior hypophyseal arteries, arising from the internal carotid and the posterior communicating artery of the circle of Willis, branch into the median eminence of the hypothalamus and base of the pituitary stalk. The capillaries in the median eminence are collected into veins that are located around the hypophyseal stalk and branch into the adenohypophysis. The venules connecting capillaries in the median eminence with the capillaries of the anterior lobe constitute the hypophyseoportal system. The drainage of the hypophysis is mainly through the cavernous sinus (Bloom and Fawcett, 1968). Different cell types can be distinguished in the normal pituitary on the basis of their affinity for various dyes - acidophilic, basophilic, and chromophobic. Kobayashi (1965) has demonstrated that adrenocorticotrophic hormone (ACTH) is produced by basophile cells in the pars distalis of the adenohypophysis. Knutson (1966) has evidence that chromophobe cells in the pars distalis are also engaged in the synthesis of ACTH. ACTH stimulates the secretion of the glucocorticoids (the hormones involved in carbohydrate metabolism)

by the adrenal cortex and appears to have an effect on the cholesterol-pregnenolone stage in the biosynthesis of the glucocorticoids.

The adrenocorticotrophic function of the pituitary gland appears to be regulated by 1) hormonal auto-regulation and 2) nervous control. Endroczi, Lissak, and Tekeres (1960) reported that an increase in the corticoid content of the peripheral blood is capable of inhibiting the ACTH secretion of the anterior pituitary. This finding supports the "negative feed-back" or "corticoid titre" hypothesis of Sayers and Sayers (1947). This hypothesis for the control of pituitary ACTH activity was based on findings that ACTH secretion could be prevented by physiological doses of corticoids. The inhibitory action of corticoids on pituitary ACTH synthesis has been demonstrated by Fortier and de Groot (1959) and Hodges and Vernikos (1960). However, there is other evidence which indicates that corticoids play a part in regulating ACTH release but that the mechanism is slow and not directly dependent upon changes in their concentration in the blood (Hodges and Jones, 1963 and Smelik, 1963). Bajusz (1964) concluded that regulation of ACTH secretion during various circumstances of stress does not always conform to the specifications of a negative feed-back system. Yates (1967) has postulated that there are two functionally distinct input pathways to the adrenal glucocorticoid control system, one being corticosteroid-sensitive and the other being corticosteroid-insensitive. He also hypothesized that there are two classes of stimuli. Class I stimuli are corticosteroid-sensitive, ACTH-releasing stimuli, and Class II stimuli are ACTH-releasing stimuli which are not completely inhibited by corticosteroids (Yates, 1967).

There is general agreement that the hypothalamic control of pituitary secretion of ACTH is by way of corticotrophin-releasing factor (CRF), a

neurohumoral agent. CRF appears to be released by neurons in the median eminence of the hypothalamus. Vernikos-Danellis (1965) has shown that extracts of the median eminence of the hypothalamus of rats will cause both release of stored ACTH and synthesis of new ACTH. There is evidence that CRF is carried in the blood via the hypophyseoportal system to the pars distalis of the adenohypophysis where it stimulates cells to release ACTH. Relatively little is known about the chemical nature of CRF. Guillemin (1964) has reviewed studies devoted to the identification of CRF. Hedge and Smelik (1968) suggest that CRF may have a cholinergic component.

Evidence of the Involvement of the HHA System in Acquisition and Retention

A number of investigators have hypothesized that the Kamin effect might be a result of changes in pituitary-adrenocortical activity. Brush and Levine (1966), for example, found that the descending limb of the U-shaped function was correlated with a corresponding decrease in plasma concentration of corticosterone. They have also found that high levels of performance are maintained by exogenous adrenocorticotrophic hormone (ACTH) administration and also, probably by cortisol replacement; whereas corticosterone replacement does not have this effect (Levine and Brush, 1967). However, the literature does not clearly indicate what role, if any, the pituitary-adrenal hormones play in the acquisition of a conditioned avoidance response. Nevertheless, there is evidence that these hormones are involved in the maintenance or retention of a conditioned avoidance response.

Extinction studies provide a clear differentiation of the behavioral effects of ACTH as opposed to the behavioral effects of glucocorticoids. Murphy and Miller (1955) were the first to demonstrate the effects of ACTH on extinction of a conditioned avoidance response. They found that ACTH

did not affect the acquisition of a shuttlebox avoidance response; however, if ACTH was administered during extinction, the extinction was significantly inhibited or retarded. Brush (unpublished) also found that administration of ACTH appeared to inhibit extinction. Miller and Ogawa (1962) demonstrated that ACTH inhibits extinction in the adrenalectomized rat. A series of studies by De Wied and his co-workers have indicated that ACTH retards extinction of a conditioned avoidance response (De Wied, 1966), whereas glucocorticoids facilitate extinction of the same response (De Wied, 1967). De Wied (1967) observed that adrenalectomy resulted in inhibition of extinction, whereas hypophysectomy restored extinction to normal. These findings indicate that an excess of circulating ACTH facilitates retention of active avoidance responding as measured by a delay or inhibition of extinction, whereas the absence of ACTH and glucocorticoids results in normal extinction of the conditioned avoidance response. He also found that both dexamethasone and corticosterone facilitates extinction in hypophysectomized animals. These studies indicate that the behavioral effects of ACTH and glucocorticoids are independent of, and opposite to, each other. However, it must be mentioned that Bohus and De Wied (1966) reported the surprising discovery that the behavioral effects of two different forms of the same fraction of the ACTH molecule were also independent of, and opposite to, each other! They found that the polypeptide chain which constitutes the first ten amino-acids of the ACTH molecule inhibits extinction of a shuttlebox avoidance response; but if the phenylalanine molecule in the seventh position of this peptide is replaced by its dextrorotary form, extinction is facilitated.

Thus far, all of the studies considered have dealt with active avoidance responses. There are several studies which indicate that the pituitary

and adrenocortical systems are also involved in passive avoidance. Endroczi, Telegdy, and Lissak (1957) found that rats which showed little passive avoidance learning had significantly less adrenal ascorbic acid depletion to a subsequent stress of unilateral adrenalectomy than did rats which showed greater passive avoidance conditioning. Since adrenal ascorbic acid depletion is a reflection of circulating levels of ACTH and steroid release, this study indicates that the greater the ACTH or steroid response to stress the better the acquisition of the passive avoidance response. However, in evaluating work in which adrenal ascorbic acid depletion is used as a measure of levels of ACTH and steroid release, it must be remembered that Hedner and Rerup (1962) found that simultaneous estimation of adrenal ascorbic acid and plasma corticoid levels showed that the latter parameter estimated endogenous corticotrophin release when the former did not. This indicates that invalid conclusions may be made about agents blocking corticotrophin release if the only measure used is adrenal ascorbic acid depletion.

Levine and Jones (1965) also tested the effects of ACTH in a passive avoidance situation. They observed complete suppression of an operant response in all animals if ACTH was administered during the 7-day test period. However, if ACTH was administered only during that period of time prior to and terminating with the end of passive avoidance training, the animals showed a much more rapid return to operant responding. To explain these results they hypothesized that following treatment with ACTH, ACTH release is subsequently suppressed and that this suppression of ACTH facilitated extinction of the passive avoidance response; whereas the continued administration of ACTH inhibited the extinction of the passive avoidance response. Anderson, Winn, and Tam (1968) demonstrated a similar effect of

ACTH on passive avoidance behavior in hypophysectomized animals. They found that ACTH-injected groups maintained near complete response suppression over all recovery periods which ranged from 1 to 7 days. The other groups, including a cortisol-injected group, showed gradual return of bar-press rates to prepunishment levels. Recently, Weiss, McEwen, Silva, and Kalkut (1969) have found that hypophysectomized rats show less passive avoidance than normal rats, whereas adrenalectomized rats show greater passive avoidance than normal rats. They suggest that because hypophysectomized rats lack ACTH (which appears to increase arousal or emotionality) whereas adrenalectomized rats lack certain adrenal steroids (which appear to inhibit excitatory effects), ACTH and certain adrenal steroids have opposite effects in regulating fear-motivated behavior. When evaluating the studies mentioned above, the fact that a hypophysectomized animal lacks not only ACTH but also glucocorticoids (because of the lack of ACTH) and that injection of ACTH into an animal with intact adrenal glands not only increases ACTH but also increases glucocorticoids should be kept in mind.

Purpose and Rationale of Study

Experiment I

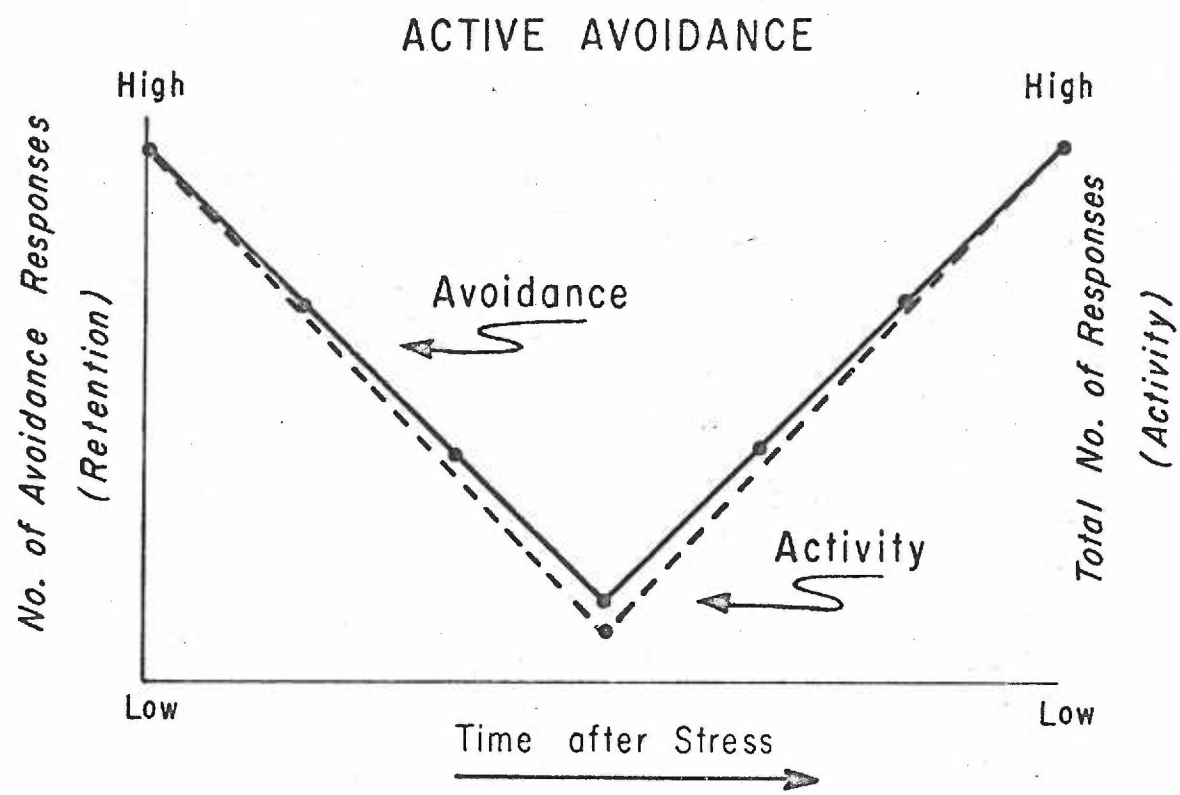
The basic objective of Experiment I is to determine whether the U-shaped retention curve which characterizes active avoidance learning can be found in a passive avoidance paradigm. If the Kamin effect can be demonstrated in a passive avoidance situation, it will indicate that the U-shaped function is not a mere peculiarity of active shuttlebox avoidance learning but rather that it is a more general phenomenon of aversively motivated learning. It has been shown in active avoidance that the low point of the U-shaped function corresponds both to the lowest number of avoidance responses

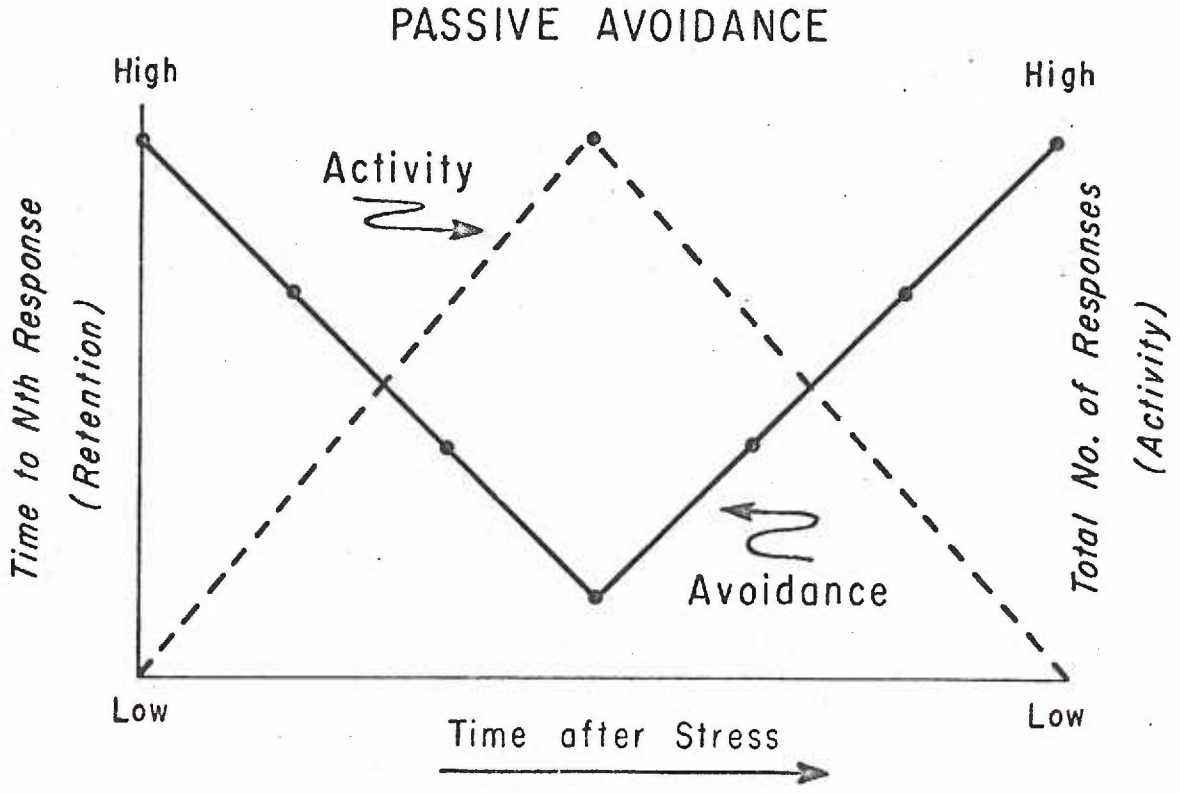
(measure of learning) and to the lowest amount of activity (total responses, avoidance plus intertrial responses). However, in the passive avoidance paradigm, learning and retention are indicated by a lack of responding, whereas an activity effect is indicated by increased responding. Thus, the low point of retention of the passive avoidance response (measure of learning) corresponds to the high point of activity using total number of responses which the subject learns not to make (for the passive avoidance response of "not responding") as an index of activity (see Figure 1).

Experiment II

If the Kamin effect can be found in a passive avoidance situation, an additional objective of this investigation is to differentiate the roles of ACTH and glucocorticoids in this U-shaped retention curve with respect to changes in activity and changes in retention. In active avoidance learning the literature clearly indicates that ACTH inhibits extinction (or facilitates retention) of the response, whereas glucocorticoids facilitate extinction (or inhibit retention) of the response. In passive avoidance learning the literature is less clear; however, the indications are that ACTH facilitates retention (or inhibits extinction) of the passive avoidance response (PAR) just as it does of the active avoidance response. A glucocorticoid effect has not been demonstrated. It must be remembered that these results are from experiments using intersession intervals (usually 24 hrs.) that are longer than those proposed for use in this study. Only the studies by Levine and Brush (1967), in which administration of ACTH or hydrocortisone maintained performance of an active avoidance response, and by Brush (unpublished), in which administration of ACTH inhibited extinction of an active avoidance response, used intervals similar to Kamin's and to those proposed in this study. Nevertheless, the general conclusions drawn from the bulk of the literature cited above

Figure 1. Comparison of retention-activity relation in active and passive avoidance.


ACTIVE AVOIDANCE
High
High
No. of Avoidance Responses
(Retention)
Avoidance
Activity
Total No. of Responses
(Activity)
Low
Low
Time after Stress


PASSIVE AVOIDANCE
High
High
Time to Nth Response
(Retention)
Activity
Avoidance
Total No. of Responses
(Activity)
Low
Low
Time after Stress

must be taken into account since they have at least an indirect relationship to the problem.

If ACTH actually inhibits extinction (facilitates retention), a group with high levels of ACTH tested at the low point of the U-shaped function should show facilitated retention (or inhibited extinction) of the PAR independent of glucocorticoid effects. In other words, it should raise the low point and thus change the U-shaped curve to a more linear function. This result would also give support to the conclusion that ACTH is not merely increasing activity, as could be argued in the case of active avoidance responding, since in passive avoidance learning, raising the low point of the U-shaped function would indicate a decrease of activity and an increase of retention of the PAR.

On the other hand, if the literature which indicates that glucocorticoids facilitate extinction (or inhibit retention) of active avoidance responses is correct and if this conclusion also applies to passive avoidance behavior, a glucocorticoid administered to the group at the low point of the U-shaped function should result in the lowering of this point (or if the point cannot be lowered, it should at least remain the same). However, if the glucocorticoids (especially cortisol) maintain the performance of an active avoidance response as reported by Levine and Brush (1967) and have the same effect on a passive avoidance response, the low point of the U-shaped function should be raised. This result would indicate that glucocorticoids actually facilitated retention or maintenance of the response.

MATERIAL AND METHODS

Experiment I

Experimental Design

The first experiment was designed to determine whether or not a U-shaped intersession interval function could be found in a passive avoidance paradigm. Subjects were trained to press a bar for food pellets on a continuous reinforcement (CRF) schedule in daily sessions until they had received a preset number of reinforcements. After reaching the reinforcement criterion, they were given passive avoidance training. Following passive avoidance training, subjects were assigned to various intersession intervals (ISIs). After their respective ISIs, subjects were tested for their retention of passive avoidance learning.

Subjects

The subjects were 60 experimentally naive, male, hooded rats (Long-Evans) obtained from the University of Oregon Medical School Animal Farm. They were 71-114 days of age and their weight ranged from 241-392 grams. The animals were housed in individual cages under constant illumination and were maintained under continuous lighting for three weeks prior to experimentation in an attempt to randomize their diurnal cycles of glucocorticoid activity. Critchlow (1963) found that male rats maintained in constant illumination showed no prominent peaks in plasma levels of corticosterone. In a 24-hr. period, the levels ranged from approximately 10 to 20 μg corticosterone per 100 ml plasma.

Apparatus

Four identical Skinner boxes were used. The inside dimensions of the boxes were 11 1/2 in. long, 9 1/2 in. wide, and 7 1/2 in. high. Three of the walls were aluminum and the door and roof were plexiglass. On a

side wall of each box was a lever located 4 in. above the floor and 1 1/2 in. above the food hopper. A bar press delivered a 45-mg Noyes pellet to the hopper by activating a Gerbrand feeder. A 6-W, 110-V, white light, which was constantly on, provided background illumination. The floor of each enclosure was constructed of 18 copper rods, 1/8 in. in diameter, running parallel to the side containing the lever and food hopper. Shock was delivered to the grid floor through a shock scrambler for multiple boxes (Brush, 1967). The circuit consisted of a 339 VAC source, delivered through 760 K resistance in series with the rat. The boxes were contained in light-proof, sound-attenuating ice chests and were ventilated by fans which provided a continuous masking noise.

Procedure

Because food pellets were used for reinforcement in bar press training, subjects were maintained at 75% normal (ad libitum) body weight by a schedule of limited food intake.To reduce subjects to 75% of ad lib body weight, they were maintained on ad lib food for two days and weighed on each day. The average of the two weights was then used as the normal ad lib body weight for each subject from which to compute the 75% body weight. Subjects were put on total food deprivation for the next three days and on partial food deprivation thereafter until 75% of normal body weight was attained. Subjects were maintained at that weight throughout the experiment. Water was available ad lib for the duration of the experiment.

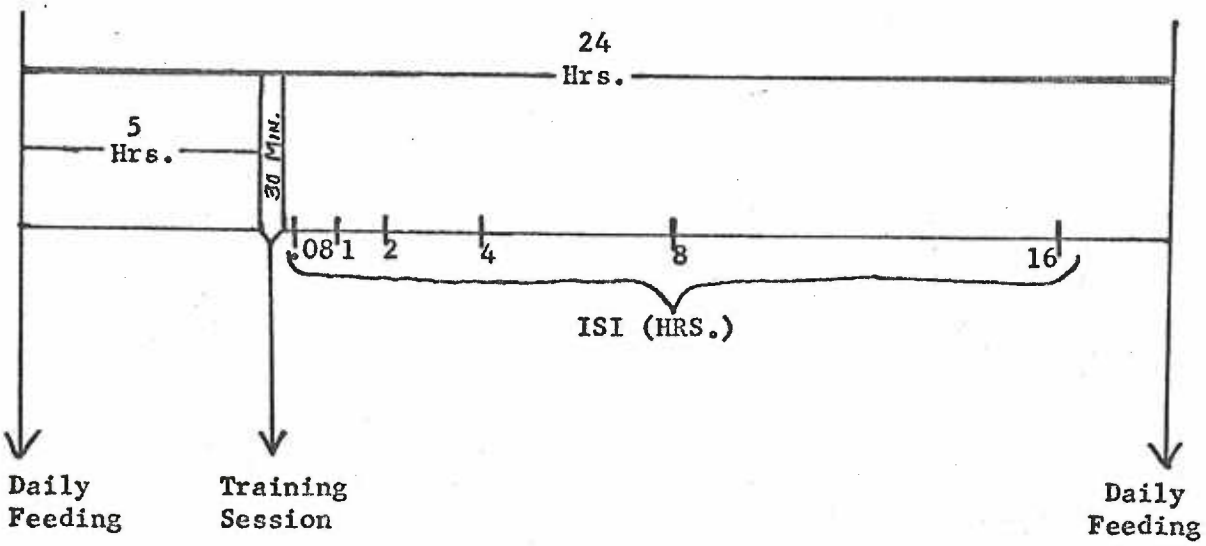
On the first day of training subjects received magazine training and CRF. On the following days they remained on a CRF schedule, each daily session being continued until approximately 90-100 reinforcements were earned. Daily training sessions were conducted until each subject earned about the same number of reinforcements (from 690-890). Attention was

paid to controlling the number of reinforcements for all subjects because Lawson and Born (1964) found that number of pre-punishment reinforcements has an effect on recovery from punishment. On the day following the attainment of the reinforcement criterion (the tenth day, for most animals), passive avoidance training was initiated. A scrambled ac shock (.3 ma intensity and .55 sec. duration) was administered through the grid, contingent upon a bar press and upon a fixed interval (FI) 2-min. intermittent schedule. In other words, the subject was shocked for the first bar press 2 min. after the preceding punished response. Passive avoidance training continued until a criterion of 10 min. of no responding was met.

Ten subjects were assigned to each ISI group, the ISIs being .08, 1, 2, 4, 8, and 16 hr. Subjects were assigned to ISIs in order to match the groups for medians of number of shocks received, amount of time spent in the passive avoidance training session, and time of day of session. After reaching the passive avoidance criterion, subjects were returned to their home cages for their respective ISIs. Following the ISI, subjects were given a 60-min. test session, during which no shock was administered; however, bar presses continued to be reinforced on a CRF schedule. Minutes until each of the first five responses were used to measure extinction of the PAR. Total responses were used to measure activity.

Subjects were fed daily between approximately 0700 and 1200. However, each individual subject was fed daily at about the same time, which was approximately 5 hr. before his training session. Thus, the subjects were trained daily between approximately 1200 and 1800. Again, each subject was trained daily at about the same time of day. Because subjects were fed 5 hr. before their training sessions began, none of the groups were fed during the ISI between the passive avoidance training session and the

test session. The 16-hr. group (the group with the longest ISI) was run in the test session approximately 3 hr. before regular daily feeding time. The following diagram illustrates the feeding schedule in relation to the training and test sessions.



Experiment II

Experimental Design

The second experiment was designed to test the effects of high and low levels of ACTH and glucocorticoids on the passive avoidance behavior of subjects at the low point of the U-shaped function as determined in Experiment I. This low point was found to be the 2-hr. ISI. The design was a 2 x 2 factorial with four combinations of levels being tested. The combinations were as follows: high glucocorticoid-high ACTH, for which normal rats were injected with ACTH; high glucocorticoid-low ACTH, for which normal rats were injected with glucocorticoids; low glucocorticoid-high ACTH, for which adrenalectomized rats were used; and low glucocorticoid-low ACTH, for which hypophysectomized rats were used. (The rationale for using the preparations stated above for each cell of the table will be given later.) The design is depicted in the following diagram.

GLUCOCORTICOIDS	ACTH: HIGH	ACTH: LOW
HIGH	ACTH INJECTED	GLUCOCORTICOID INJECTED
LOW	ADRENALECTOMIZED	HYPOPHYSECTOMIZED

Subjects and Apparatus

The subjects were 111 experimentally naive rats of the same sex and strain as in Experiment I. They were 73-101 days of age. The weight of the normal subjects ranged from 217-339 grams; of the adrenalectomized subjects, from 200-228 grams; and of the hypophysectomized subjects, from 200-277 grams. As in Experiment I, the animals were housed in individual cages with constant illumination. The apparatus was identical to that used in Experiment I.

Procedure

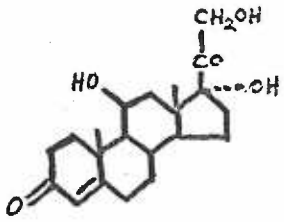
Normal rats who were later injected with ACTH or glucocorticoids were reduced to 75% of ad lib body weight. Their food deprivation, maintenance, and training was the same as that of the subjects in Experiment I. The adrenalectomized and the hypophysectomized animals were reduced to 85% of ad lib body weight instead of 75%, since it was found that there was a significant increase in mortality at body weights below 85%, especially for the adrenalectomized rats. Food was available ad lib for 1 or 2 days. Subjects were weighed on days of ad lib food and the average of the two weights (or the single weight alone) was used as the normal body weight from which to compute the 85% body weight. The adrenalectomized and hypophysectomized subjects were never on total food deprivation. Their partial

deprivation consisted of daily feedings under the same time conditions as those of the subjects in Experiment I in addition to several hours (3-6) of access to 5% glucose-1% saline solution beginning at 2100 or later. (This solution was never given before 2100 so that its availability would not interfere with the 2-hr. ISI on the day of passive avoidance.) Water (1% saline solution) was available ad lib throughout the experiment. Aureomycin (3 cc/400 ml solution) was added to the 1% saline solution and to the 5% glucose-1% saline solution to prevent development of infection in the adrenalectomized and hypophysectomized rats. With the exception of the food deprivation schedule, the maintenance and training of the adrenalectomized and hypophysectomized animals was the same as that of the subjects in Experiment I.

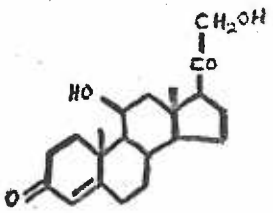
A group of ten normal subjects received ACTH injections in order to produce high levels of ACTH and glucocorticoids. According to Holzbauer and Vogt (1957), Lipscomb and Nelson (1960), and Turner (1966), administration of ACTH to intact animals is followed by adrenocortical hypertrophy and a consequent increase in the production of glucocorticoids. The ACTH (Organon Cortrophin-Zinc) was an aqueous suspension (made isotonic with NaCl) of purified corticotrophin with alpha zinc hydroxide and was of porcine origin. One cc, containing 40 U.S.P. units of corticotrophin with 1 mg alpha zinc hydroxide, was injected subcutaneously in the flank of each rat. The injection was administered immediately after the passive avoidance training session and thus 2 hr. before the test session. (All injections reported hereafter in Experiment II were administered under these conditions.) This group is referred to as the "ACTH-injected group".

Two groups of ten normal subjects each were used to test for glucocorticoid effects. One group was injected with an 85% corticosterone-15%

cortisol mixture. According to Williams (1962), this ratio approximates that of the naturally occurring glucocorticoids secreted by the rat adrenal cortex. The rats were injected with a 1 cc physiological saline suspension containing 17 mg corticosterone and 3 mg cortisol (both from Merck, Sharp, and Dohme). The following are the structures of the glucocorticoids.



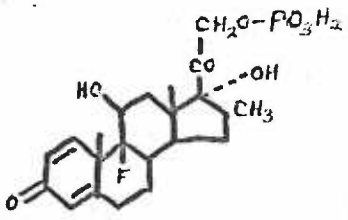
CORTISOL



CORTICOSTERONE

This group is designated as the "B and F group" because Kendall referred to corticosterone as compound B and called cortisol compound F. This group supposedly had high levels of glucocorticoids and low levels of ACTH since, due to the feedback system of the pituitary-adrenocortico-axis, steroids inhibit ACTH secretion when their blood concentration increases (Yates, Leeman, Glenister, and Dallman, 1961; and Dallman and Yates, 1968).

The other group to test for glucocorticoid effects was injected with 1 cc of dexamethasone sodium phosphate (Decadron) in physiological saline. Each cc contained 1.2 mg of dexamethasone 21-phosphate, the structure of which is as follows:



DEXAMETHASONE
21-PHOSPHATE

Dexamethasone, a highly potent synthetic glucocorticoid, causes an almost complete block of ACTH release (Itoh, 1966). A group of 10 subjects injected with 1 cc of physiological saline was run as an injection control group for all of the injected groups. All conditions for this control group, except for the saline injection, were the same as those of the other injected groups.

Six adrenalectomized rats were used to test the effects of high levels of ACTH and low levels of glucocorticoids. Gemzell, Van Dyke, Tobias, and Evans (1951), Brodish and Long (1956), Cox, Hodges, and Vernikos (1958), and Hodges and Vernikos (1959) found that adrenalectomy results in a chronic increase in circulating levels of ACTH reaching a maximal level 3 weeks after adrenalectomy. The animals used in this group were adrenalectomized at least 4 weeks before being run in the experiment. Adrenalectomy was performed bilaterally by the dorsal route under ether anesthesia. Adrenalectomy was confirmed at the end of the experiment by giving animals food and water ad lib but depriving them of the 1% NaCl solution. Animals that died within 7 days were considered adrenalectomized. A group of eight sham adrenalectomized animals were run to control for operative effects. The sham adrenalectomies also were done at least 4 weeks before the experiment and were performed in exactly the same way as the adrenalectomies except that while both adrenals were visualized they were not disturbed. With the exception of the adrenalectomy, this control group was treated exactly like the adrenalectomized group.

Seven hypophysectomized rats were used for the group with low levels of both glucocorticoids and ACTH. Because these animals had no pituitary gland, and thus no circulating ACTH, this preparation served as the equivalent of hypophysectomy and adrenalectomy. Shima, Matsuba, and Pincus (1968)

reported that hypophysectomy greatly reduces the concentration of corticosterone in whole adrenals as soon as 2 hr. after the operation and the de novo formation of corticosterone by 96 hr. post operation. Rats from the same colony as others used in this investigation were sent to Jackie Ehlert, University of California at Berkeley, for hypophysectomies. The operation was performed using the parapharyngeal approach under ether anesthesia. At the end of the experimental period, hypophysectomies were confirmed by visual inspection for absence of the pituitary. If no pituitary tissue could be found with the dissection microscope at 10 power, the animals were considered hypophysectomized. To control for the operative effects of hypophysectomy, a group of eight sham controls was used. All conditions for this control group were the same as those of the hypophysectomized group except that the pituitary gland was exposed but not removed.

In order to determine endogenous levels of corticosterone of the .08-hr. and 2-hr. ISI groups at the beginning of the passive avoidance test session, two control groups (8 animals in the .08-hr. ISI group and 10 animals in the 2-hr. ISI group) were maintained and trained identically to animals in these ISI groups. However, the control groups were decapitated after their respective ISIs instead of being run in their test sessions.

Two control groups, each containing four animals, were treated identically to the ACTH group and the dexamethasone group, respectively. However, 2 hr. after they reached criterion in the passive avoidance training session and received their respective injections, the animals were decapitated at the time they would have been run in the passive avoidance test session. The purpose of these two control groups was to determine the

circulating levels of corticosterone for the ACTH and dexamethasone groups at the beginning of the passive avoidance test session.

Another control group, containing 8 subjects, was decapitated before passive avoidance training to determine corticosterone levels in animals whose weight was 75% of their normal body weight and who had received bar press training. A ninth control group (8 animals) was decapitated before passive avoidance training to determine corticosterone levels in animals whose weight was 85% of normal body weight, who had been maintained on 1% salt solution instead of pure water, who had access to the 5% glucose-1% saline solution daily, for the same amount of time as the adrenalectomized and hypophysectomized groups, and who had received bar press training.

Immediately after decapitation of animals, blood samples were collected in heparinized beakers and centrifuged. The plasma was then collected and frozen. Later it was analyzed for corticosterone levels using the microfluorometric method of Glick, Von Redlich, and Levine (1964) as modified by Brush (1969) from Kendall's (1969) simplified macrofluorometric method. Blood samples were obtained from all animals in Experiment II, except those in the adrenalectomized and hypophysectomized groups, after the 60-min. passive avoidance test session to determine what their corticosterone levels were by the end of the session.

RESULTS

Experiment I

Pre-ISI Measures

Group medians for pre-ISI measures are presented in Table 1. Three Kruskal-Wallis one-way analyses of variance revealed no significant differences among the six ISI groups in 1) total number of reinforcements earned, 2) time of day of the passive avoidance training session, and 3) number of shocks received in the passive avoidance training session.

A significant difference in number of responses made in the passive avoidance training session by the six ISI groups was indicated by a Kruskal-Wallis one-way analysis of variance ($H = 46.75$, $df = 5$, $p < .001$). A Mann-Whitney test (two-tailed) revealed a significant difference between the 8-hr. and 1-hr. ISI groups at the .02 level ($U = 17$, $n_1 = n_2 = 10$), the 8-hr. group showing a greater total number of responses in the passive avoidance training session. Mann-Whitney tests revealed no significant differences between the 2-hr. and 1-hr., the .08-hr. and 1-hr., and the 16-hr. and 1-hr. ISI groups.

A Kruskal-Wallis one-way analysis of variance on the amount of time spent in the passive avoidance training session was significant at the .05 level ($H = 13.06$, $df = 5$). A Mann-Whitney test (two-tailed) showed that a significant difference occurred, again, between the 8-hr. and 1-hr. ISI groups ($U = 10$, $n_1 = n_2 = 10$, $p < .002$). The differences between these measures for the two groups are consistent, the 8-hr. group making a greater total number of responses and spending a longer period of time in the passive avoidance training session than the 1-hr. group. However, these differences apparently do not affect performance in the post-ISI passive avoidance test session, because the 1-hr. and 8-hr. ISI groups were not significantly different from each other on any post-ISI measures. A significant difference was

Table 1. Intersession interval group medians for pre-ISI measures.

Table 1

ISI Group	Total Reinforcements	Time of Day of Training Session	Number of Shocks Earned	Total Responses in Training Session	Total Time in Training Session (min)
.08	726	1452.5	5	64	26.5
1	722.5	1610	4	52	25
2	728.5	1615	5	65.5	33.5
4	744.5	1550	4.5	58.5	28.5
8	733	1340	5.5	62	37
16	724	1452.5	4	60.5	29.5

also found between the .08-hr. and 8-hr. ISI groups ($U = 20.5$, $n_1 = n_2 = 10$, $p < .05$), the 8-hr. group spending more time in the training session. However, the existence of this difference in passive avoidance training, like the other differences mentioned above, does not appear to be related in any systematic way to the subsequent observed effects of ISI which are described below. Therefore, it can be concluded that groups differed on these measures only because of sampling error. Mann-Whitney tests revealed no significant differences between the 4-hr. and 8-hr. and between the 16-hr. and 8-hr. ISI groups on amount of time spent in the training session.

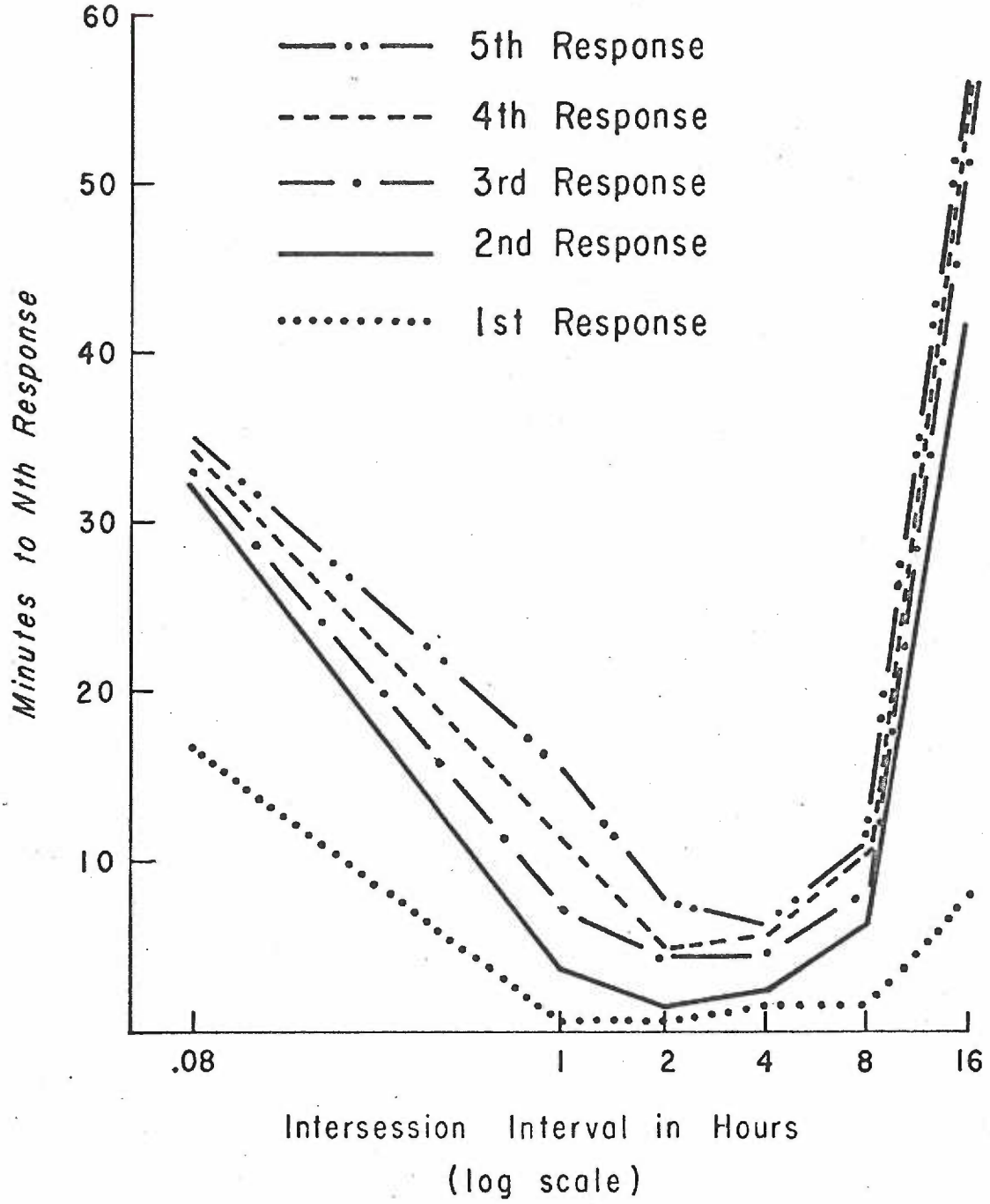
Post-ISI Measures

1) Latency of the first five responses

A definite U-shaped ISI function was found in passive avoidance learning as measured by minutes to each of the first five responses. Figure 2 illustrates this U-shaped function. A Kruskal-Wallis one-way analysis of variance showed that for latency to the first response, the effect of ISI was significant at the .01 level ($H = 16.96$, $df = 5$). Mann-Whitney tests (one-tailed) revealed a difference between the .08-hr. and 2-hr. ISI groups ($U = 15$, $n_1 = n_2 = 10$, $p < .01$) and between the 16-hr. and 2-hr. groups ($U = 16$, $n_1 = n_2 = 10$, $p < .01$).

For latencies to the second and third responses, Kruskal-Wallis one-way analyses of variance showed that the ISI effect was significant at the .001 level in each case. Mann-Whitney tests (one-tailed) for latencies to the second response showed that the .08-hr. and 2-hr. ISI groups and the 16-hr. and 2-hr. ISI groups were different at the .001 level ($U = 3$, $n_1 = n_2 = 10$ for .08 hr. versus 2 hr., and $U = 8$, $n_1 = n_2 = 10$ for 16 hr. versus 2 hr.). Mann-Whitney tests (one-tailed) for latencies to the third response also demonstrated a significant difference between the .08-hr. and 2-hr. ISI groups and between the 16-hr. and 2-hr. ISI groups ($U = 9$,

Figure 2. Median latencies (mins.) to each of the first five responses for intersession interval groups.


5th Response
4th Response
3rd Response
2nd Response
1st Response
Minutes to Nth Response
60
50
40
30
20
10
.08
1
2
4
8
16
Intersession Interval in Hours
(log scale)

$n_1 = n_2 = 10$, $p < .001$ for .08 hr. versus 2 hr., and $U = 10$, $n_1 = n_2 = 10$, $p < .001$ for 16 hr. versus 2 hr.).

Kruskal-Wallis one-way analyses of variance demonstrated a significant difference among ISI groups for latencies to the fourth response ($H = 20{,}334$, $df = 5$, $p < .01$) and to the fifth response ($H = 19.88$, $df = 5$, $p < .01$). Mann-Whitney tests (one-tailed) between .08-hr. and 2-hr. ISI groups and between the 16-hr. and 2-hr. ISI groups for latencies to the fourth response were significant at the .01 level ($U = 11$, $n_1 = n_2 = 10$ for both .08 hr. versus 2 hr. and 16 hr. versus 2 hr.). Mann-Whitney tests (one-tailed) between the .08-hr. and 2-hr. ISI groups and between the 16-hr. and 2-hr. ISI groups were also significant at the .01 level for latencies to the fifth response ($U = 12$, $n_1 = n_2 = 10$ for .08 hr. versus 2 hr. and $U = 11$, $n_1 = n_2 = 10$, for 16 hr. versus 2 hr.). For each of the above latency measures, the 2-hr. ISI group always exhibited a shorter latency than either the .08-hr. or the 16-hr. ISI groups. Mann-Whitney tests showed that the 1-hr. and 2-hr. ISI groups and the 8-hr. and 2-hr. ISI groups were not significantly different on latencies to responses one through five.

2) Latency to a criterion response rate

Latencies to responses beyond the fifth were not measured since inspection of the data revealed that by the fifth response animals appeared to be responding regularly as indicated by very small differences in inter-response time (IRT) between the third and fourth responses and between the fourth and fifth responses (See Figures 3 and 4). However, in order to have a quantitative measure of resumption of responding, a time to criterion measure was used on all of the ISI groups. The measure was computed from the median IRTs of the first five responses of the animals

Figure 3. Median interresponse time intervals (mins.) of responses one through five for each of the intersession interval groups.

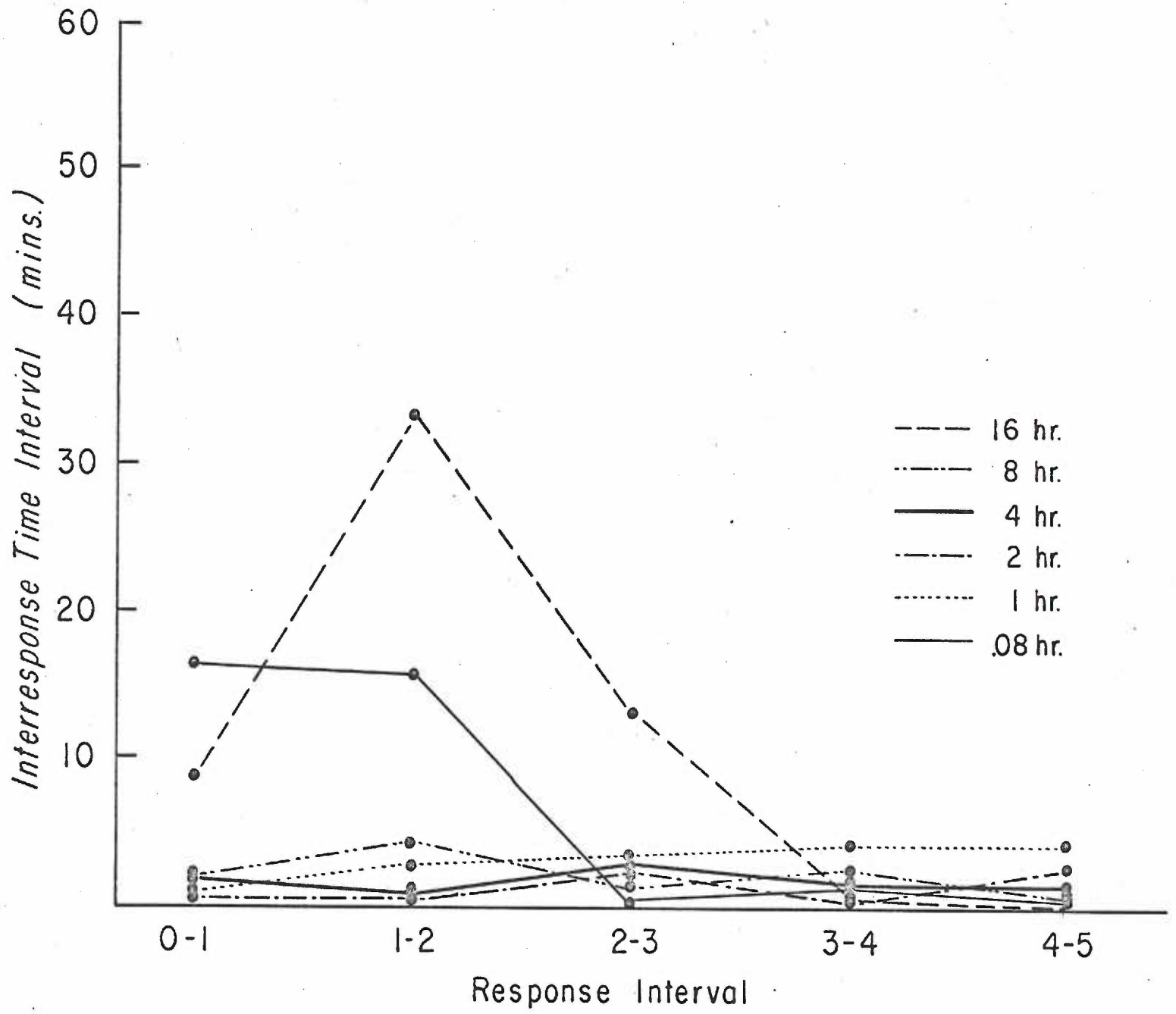
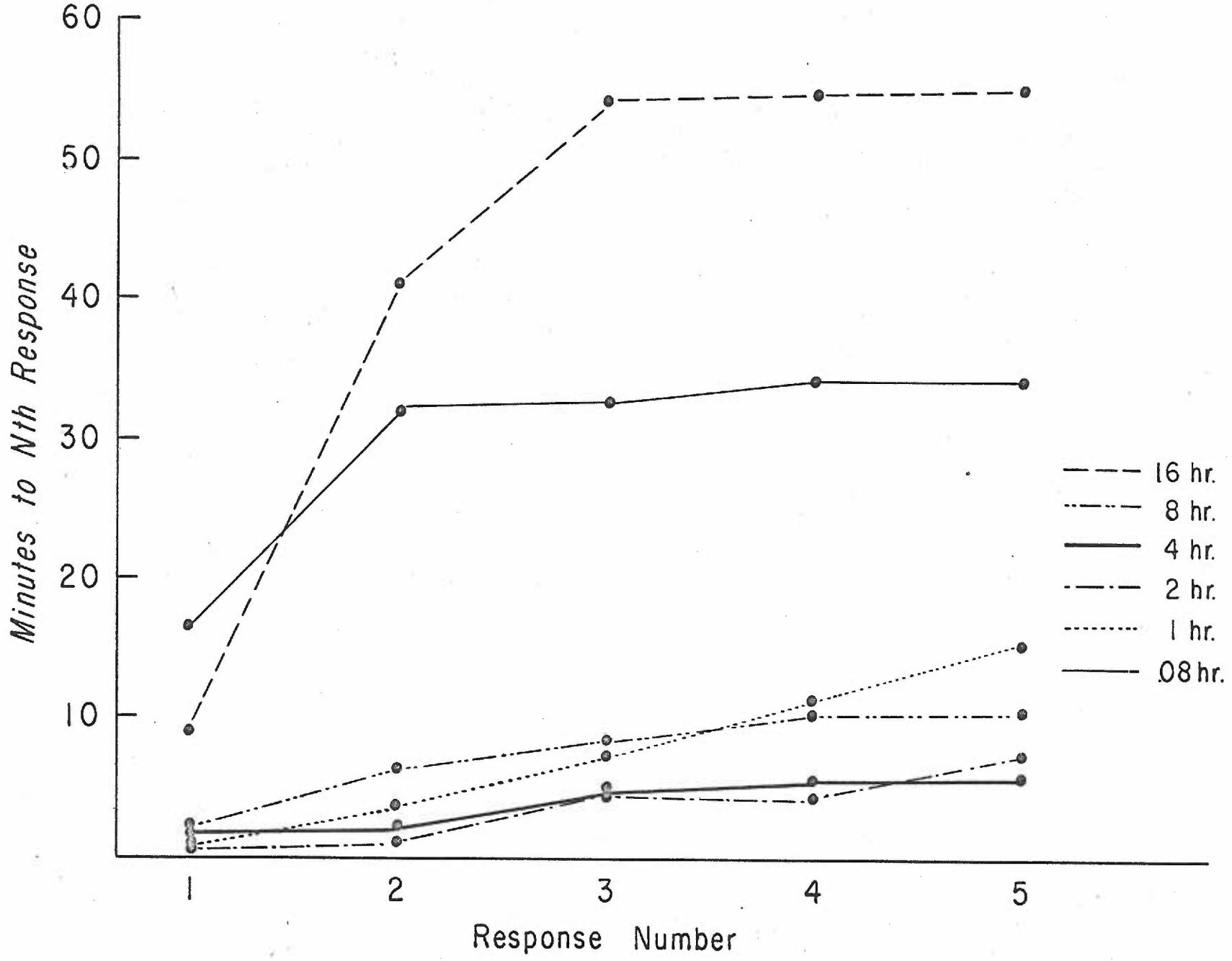

60
50
40
30
20
10
Interresponse Time Interval (mins.)
16 hr.
8 hr.
4 hr.
2 hr.
1 hr.
.08 hr.
0-1
1-2
2-3
3-4
4-5
Response Interval

Figure 4. Median latencies (mins.) of responses one through five for each of the intersession interval groups.


60
50
40
30
20
10
Minutes to Nth Response
1
2
3
4
5
Response Number
16 hr.
8 hr.
4 hr.
2 hr.
1 hr.
.08 hr.

in the 2-hr. ISI group. The median between successive pairs of responses for the first five responses were .50, .38, .42, and .31 min., respectively. The median of these four IRTs is .40 min. or 24 sec., and the criterion was set at three consecutive responses with IRTs of 24 sec. or less. Figure 5 presents the median time to reach criterion for each ISI group. A Kruskal-Wallis one-way analysis of variance of time to criterion revealed a significant difference at the .02 level ($H = 14.64$, $df = 5$). Mann-Whitney tests (one-tailed) showed that there was a difference in time to criterion between the .08-hr. and 2-hr. ISI groups and between the 16-hr. and 2-hr. ISI groups, the .08-hr. and 16-hr. groups taking more time to reach criterion. ($U = 26.5$, $n_1 = n_2 = 10$, $p < .05$ for .08 hr. versus 2 hr., and $U = 20$, $n_1 = n_2 = 10$, $p < .025$ for 16 hr. versus 2 hr.). However, Mann-Whitney tests (one-tailed) indicated that the differences between the 1-hr. and 2-hr., the 8-hr. and 2-hr., and the 2-hr. and 4-hr. ISI groups were not significant. Thus, it can be assumed that this measure is very similar to that of latency to the fifth response and, therefore, that measuring latencies beyond the fifth response to describe recovery from passive avoidance was not necessary.

3) Total responses

Figure 6 presents the median total number of responses made by each ISI group during the 60-min. passive avoidance test session. A Kruskal-Wallis one-way analysis of variance of these data indicates that there is a significant overall effect of ISI ($H = 12.44$, $df = 5$, $p < .05$). Mann-Whitney tests (two-tailed) between the 1-hr. and 16-hr. ISI groups ($U = 23$, $n_1 = n_2 = 10$) and between the 2-hr. and 16-hr. ISI groups ($U = 10.5$, $n_1 = n_2 = 10$) were significant at the .05 level and at the .02 level, respectively. It was thus assumed that there was a significant

Figure 5. Median time (mins.) to reach criterion for each intersession interval group.

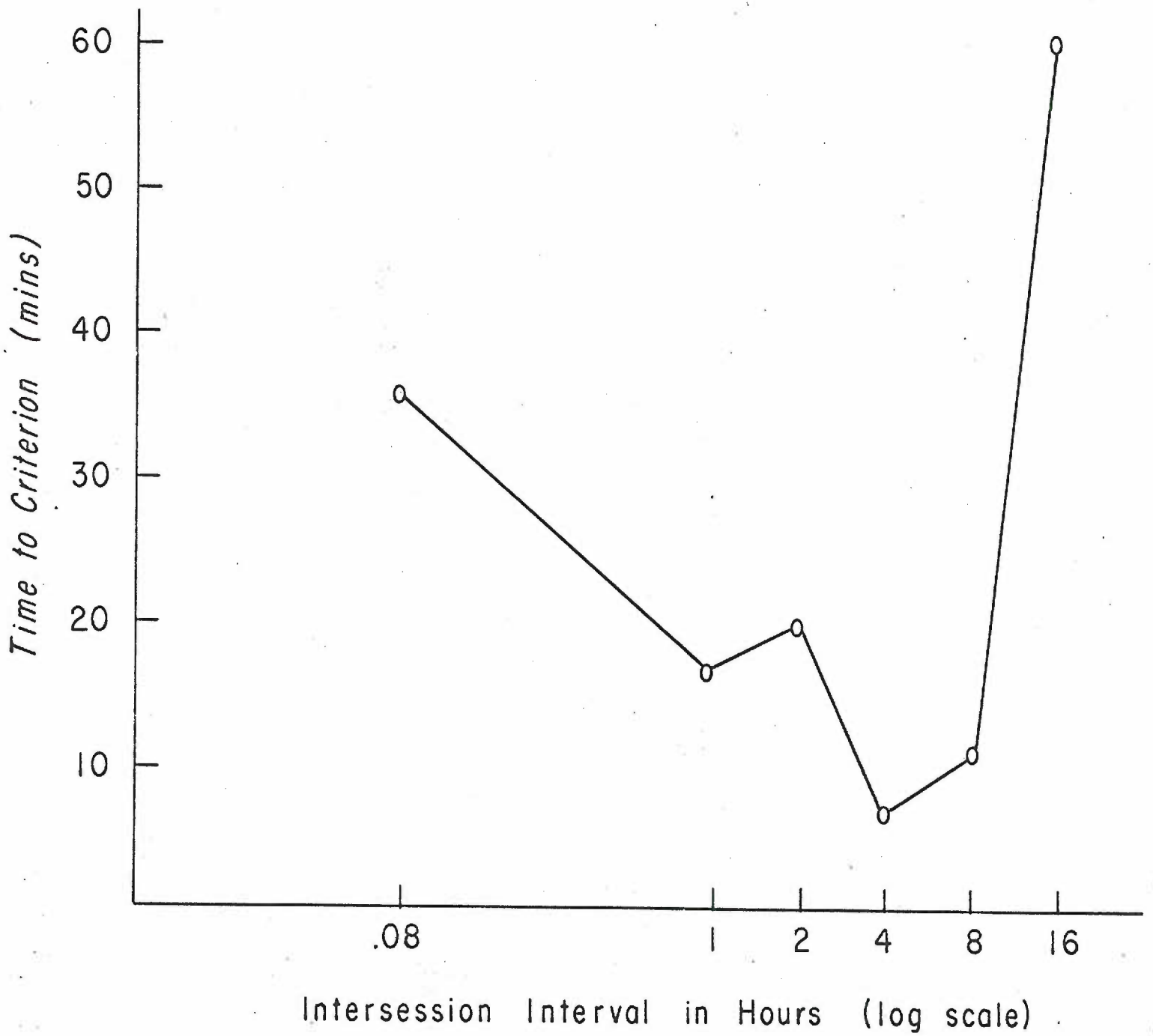
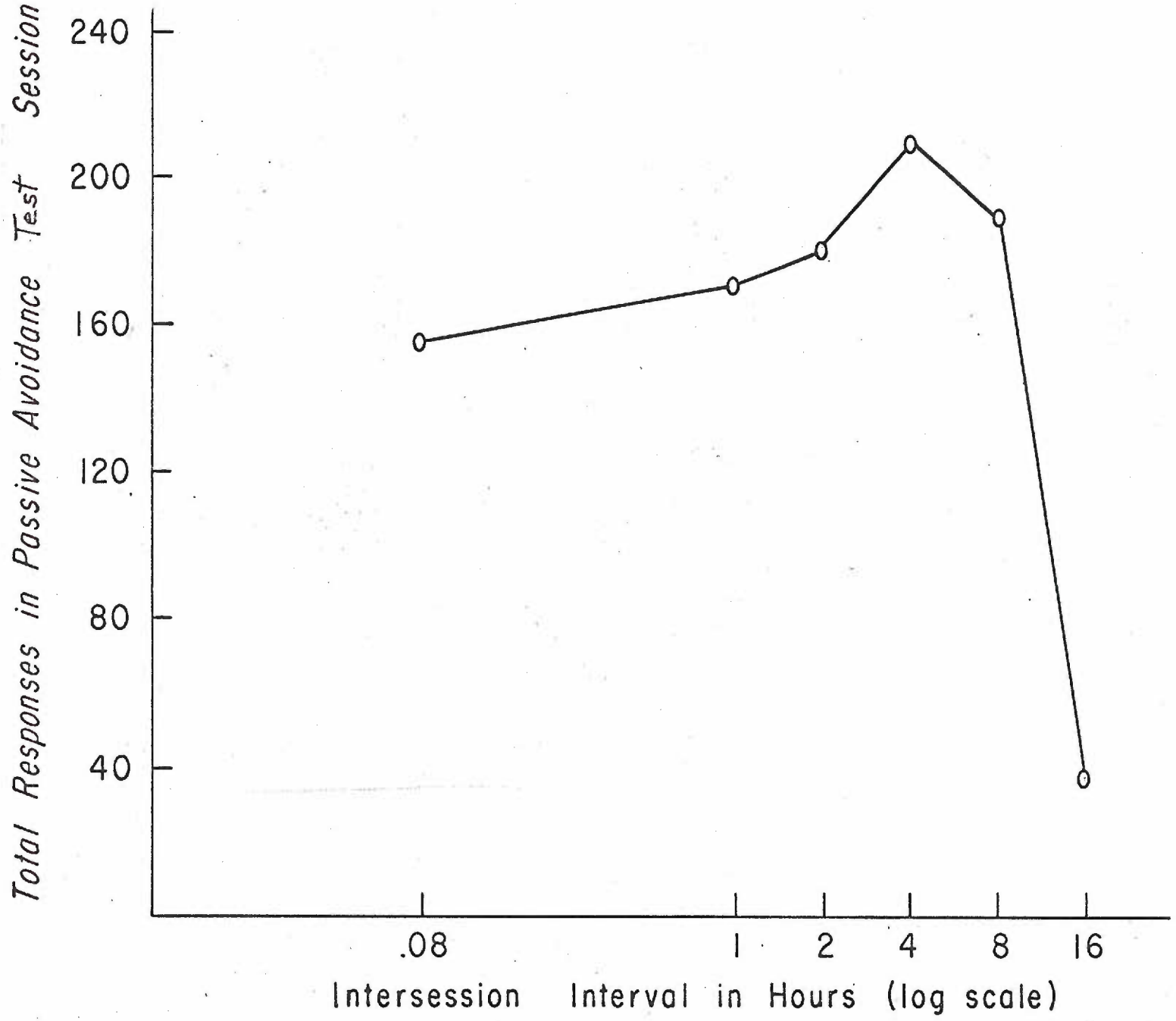

60
50
40
30
20
10
Time to Criterion (mins)
.08
1
2
4
8
16
Intersession Interval in Hours (log scale)

Figure 6. Median total number of responses made during passive avoidance test session for each intersession interval group.


Total Responses in Passive Avoidance Test Session
240
200
160
120
80
40
.08
1
2
4
8
16
Intersession Interval in Hours (log scale)

difference at the .05 level between the 4-hr. and 16-hr. ISI groups and between the 8-hr. and 16-hr. ISI groups, because the differences between their respective rank totals (Rx - Ry) exceeded that of the 1-hr. and 16-hr. ISI groups. Mann-Whitney tests (two-tailed) on the .08-hr. and 16-hr. ISI groups, the .08-hr. and 4-hr. ISI groups, and the .08-hr. and 2-hr. ISI groups revealed no significant differences. Thus, the 16-hr. ISI group was significantly different from all other ISI groups with the exception of the .08-hr. group; and the .08-hr. group was not significantly different from any of the other groups. It thus might be argued that the function in Figure 6 is not an inverted U-shaped function. However, it should be noted that although the .08-hr. ISI group is not significantly different from the 4-hr. ISI group, it is also not significantly different from the 16-hr. ISI group. Therefore, one can still conclude that the function in Figure 6 resembles an inverted U-shaped function but cannot be unequivocally identified as such.

Experiment II

Pre-ISI Measures

Table 2 presents the group medians for pre-ISI measures. Kruskal-Wallis one-way analyses of variance revealed no significant differences among all groups used in Experiment II plus the 2-hr. behavioral control group in 1) total number of reinforcements earned and 2) time of day of the passive avoidance training session or decapitation. The same kind of analysis indicated no difference in number of shocks received among the following groups: 2-hr. behavioral control, 85% body weight-procedural control, sham adrenalectomized, sham hypophysectomized, saline, ACTH, B and F, and dexamethasone. However, Mann-Whitney tests between the hypophysectomized group and its sham control and the adrenalectomized group and its sham

Table 2. Group medians for pre- ISI measures.

TABLE 2

GROUP	TOTAL REINFORCEMENTS	TIME OF DAY OF TRAINING SESSION	NUMBER OF SHOCKS EARNED	TOTAL RESPONSES IN TRAINING SESSION	TOTAL TIME IN TRAINING SESSION (MIN.)
2-HR. BEHAVIORAL CONTROL	728.5	1615	5	65.5	33.5
85% BODY WEIGHT PROCEDURAL CONTROL	729	1605	3	54	20.5
SHAM ADRENALECTOMIZED	730	1410	4	44	26
SHAM HYPOPHYSECTOMIZED	739	1440	4.5	60	28.5
SALINE	759.5	1405	4	54	24.5
ACTH	733.5	1457.5	4	48.5	23.5
B AND F	739.5	1418.5	4	54	25.5
DEXAMETHASONE	725	1444.5	4	48.5	21
ADRENALECTOMIZED	725	1416.5	4	22.5	24.5
HYPOPHYSECTOMIZED	727	1608	3	18	21

DECAPITATED GROUP	TOTAL REINFORCEMENTS	TIME OF DAY OF DECAPITATION
75% BODY WEIGHT PROCEDURAL CONTROL	720.5	1537.5
.08-HR. DECAPITATED	751.5	1530
2-HR. DECAPITATED	735.5	1555

control showed that the hypophysectomized animals received fewer shocks than their controls ($U = 9$, $n_1 = 7$, $n_2 = 8$, $p < .014$) but that there was no difference between the adrenalectomized animals and their controls.

A Kruskal-Wallis one-way analysis of variance on the 2-hr. behavioral control, 85% body weight-procedural control, sham adrenalectomized, sham hypophysectomized, saline, ACTH, B and F, and dexamethasone groups revealed no significant differences in total responses in the passive avoidance training session. However, Mann-Whitney tests showed that the sham adrenalectomized group made more responses than the adrenalectomized group ($U = 9$, $N_1 = 6$, $n_2 = 8$, $p < .03$), and the sham hypophysectomized group made more responses than the hypophysectomized group ($U = 7$, $n_1 = 7$, $n_2 = 8$, $p < .007$).

A significant difference in amount of time spent in passive avoidance training was indicated by a Kruskal-Wallis one-way analysis of variance on the 2-hr. behavioral control, 85% body weight-procedural control, sham hypophysectomized, sham adrenalectomized, saline, ACTH, B and F, and dexamethasone groups ($H = 18.62$, $df = 7$, $p < .01$). Mann-Whitney tests demonstrated that the sham hypophysectomized group spent more time in passive avoidance training than the 85% body weight-procedural control ($U = 6$, $n_1 = 6$, $n_2 = 8$, $p < .01$). However, Mann-Whitney tests showed no significant differences on this measure between the 2-hr. behavioral control and the sham hypophysectomized groups, between the 2-hr. behavioral control and the sham adrenalectomized groups, or between the 85% body weight-procedural control and the sham adrenalectomized groups.

A Mann-Whitney test showed there was no significant difference in time spent in passive avoidance training between the adrenalectomized group and its sham control; however, a difference was found between the

hypophysectomized group and its sham control ($U = 5$, $n_1 = 7$, $n_2 = 8$, $p < .003$), the hypophysectomized animals taking less time to reach criterion in passive avoidance training.

Thus, the hypophysectomized animals made fewer responses in passive avoidance training, spent less time in passive avoidance training, and received fewer shocks there than did their sham controls. The adrenalectomized animals made fewer responses in passive avoidance training than their sham controls but were not different from their controls in time spent in the training session and number of shocks received.

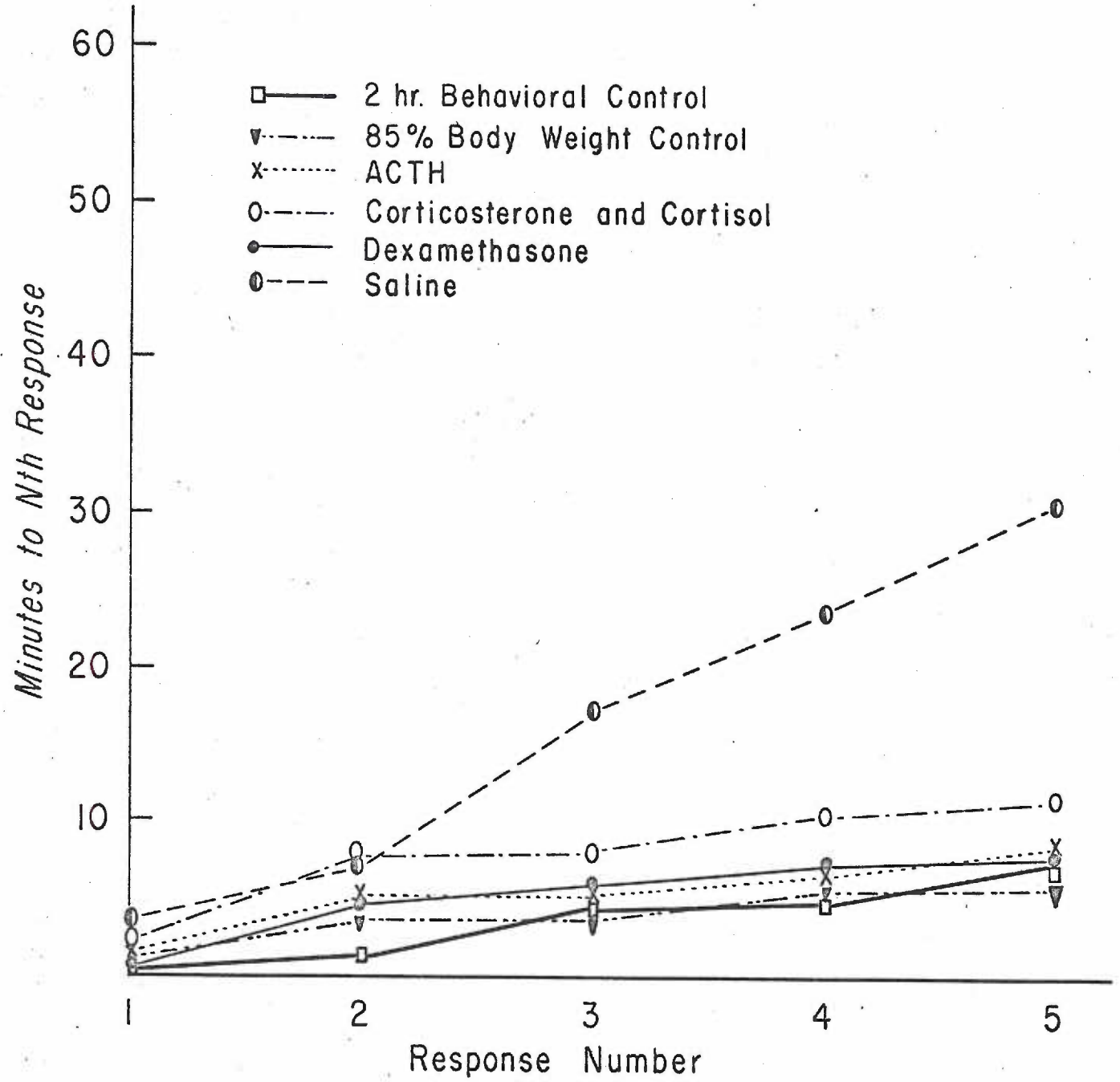
Ratios of the number of responses divided by the number of shocks were calculated to determine whether the adrenalectomized and hypophysectomized animals had a higher rate of acquisition of the passive avoidance response or whether the fewer number of responses resulted in a higher probability of punishment of a response in those groups. The median responses per shock for the adrenalectomized animals was 7.63 and for the sham controls, 13.03. The median responses per shock for the hypophysectomized group was 6 and for its sham control, 14.55. Mann-Whitney tests showed that the adrenalectomized animals had lower ratios compared to their sham controls ($U = 6$, $n_1 = 6$, $n_2 = 8$, $p < .01$) and that the hypophysectomized animals had lower ratios compared to their sham controls. Thus, the adrenalectomized and hypophysectomized animals, having a lower response rate, were more likely to be punished for responding and, hence, required fewer shocks and/or less time to meet criterion than their controls.

Post-ISI Measures

1) Latency measures

Figure 7 presents the median latencies to the nth responses for the 2-hr. behavioral control, saline, B and F, dexamethasone, and ACTH groups.

Figure 7. Median latencies to nth response for 2-hr. behavioral control, saline, B and F, dexamethasone, and ACTH.


60
50
40
30
20
10
Minutes to Nth Response
2 hr. Behavioral Control
85% Body Weight Control
ACTH
Corticosterone and Cortisol
Dexamethasone
Saline
1
2
3
4
5
Response Number

Latency to the fifth response was significantly greater in the saline injection control group than in the 2-hr. behavioral control group as indicated by a Mann-Whitney test ($U = 1$, $n_1 = n_2 = 10$, $p < .001$). The effect of the injection of the vehicle made it difficult to interpret results of other injected groups.

A Kruskal-Wallis one-way analysis of variance of latency to the fifth response of the saline, B and F, dexamethasone, and ACTH groups just missed significance at the .05 level ($H = 7.3748$, critical value = 7.815, $df = 3$). However, A Mann-Whitney test, a more powerful test than the Kruskal-Wallis one-way analysis of variance, indicated a significant difference at the .05 level between the saline and dexamethasone groups ($U = 16.5$, $n_1 = 8$, $n_2 = 10$), and a Mann-Whitney test on the saline and ACTH groups also indicated a significant difference at the .05 level ($U = 14$, $n_1 = 8$, $n_2 = 10$). The saline group had the longer latency in each case. These results indicate that dexamethasone and ACTH have an effect on latency to the fifth response, because these groups received exactly the same treatment as the saline group, except that they were injected with a drug in addition to the saline vehicle, and yet their behavior was significantly different. There was no significant difference in latency to the fifth response between the saline and B and F groups.

A Kruskal-Wallis one-way analysis of variance on the 2 x 2 factorial groups (adrenalectomized, hypophysectomized, ACTH, B and F, and dexamethasone) revealed no significant differences in latency to the fifth response. However, it is difficult to evaluate results of this analysis because of the saline injection effect. The ACTH, B and F, and dexamethasone groups were injected with drugs in a saline medium, whereas the adrenalectomized and hypophysectomized groups were not. Furthermore, the hypophysectomized

and adrenalectomized groups were maintained at 85% body weight and with special food and water conditions, whereas the other groups were not; but a Mann-Whitney test between the 85% body weight-procedural control and the 2-hr. behavioral control revealed no significant difference.

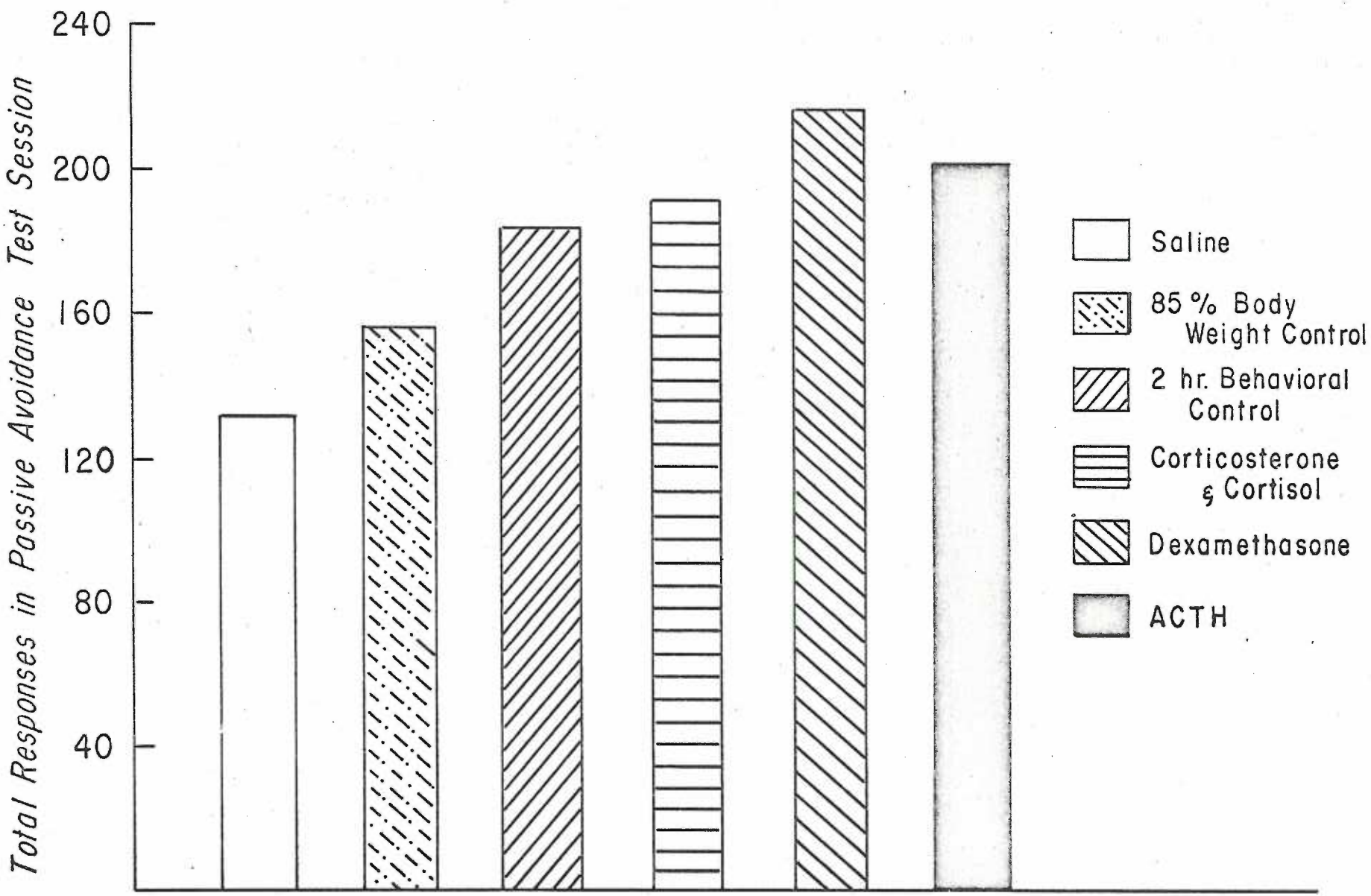
Mann-Whitney tests demonstrated no significant differences in latency to the fifth response between the hypophysectomized group and its sham control or between the adrenalectomized group and its sham control. Mann-Whitney tests also revealed no significant differences in latency to the fifth response between the 2-hr. behavioral control group and the sham adrenalectomized or sham hypophysectomized groups, or between the 85% body weight-procedural control group and the sham adrenalectomized or sham hypophysectomized groups. The groups which were tested for latency to the fifth response were also tested for latency to the first response. No significant differences in this measure were found between or among groups.

2) Total responses

Figure 8 depicts the total responses for the saline, 85% body weight-procedural control, 2-hr. behavioral control, B and F, dexamethasone, and ACTH groups. A Mann-Whitney test (two-tailed) on the saline group and 2-hr. behavioral control group was significant ($U = 15.5$, $n_1 = 8$, $n_2 = 10$, $p < .05$), the saline group making fewer total responses in the passive avoidance test session than the 2-hr. behavioral controls. A Mann-Whitney test on the 85% body weight-procedural controls and the 2-hr. behavioral controls showed no significant difference on this measure.

A Kruskal-Wallis one-way analysis of variance on the saline, B and F, dexamethasone, and ACTH groups indicated a difference in total responses in the test session ($H = 10.03$, $df = 3$, $p < .02$). Mann-Whitney tests

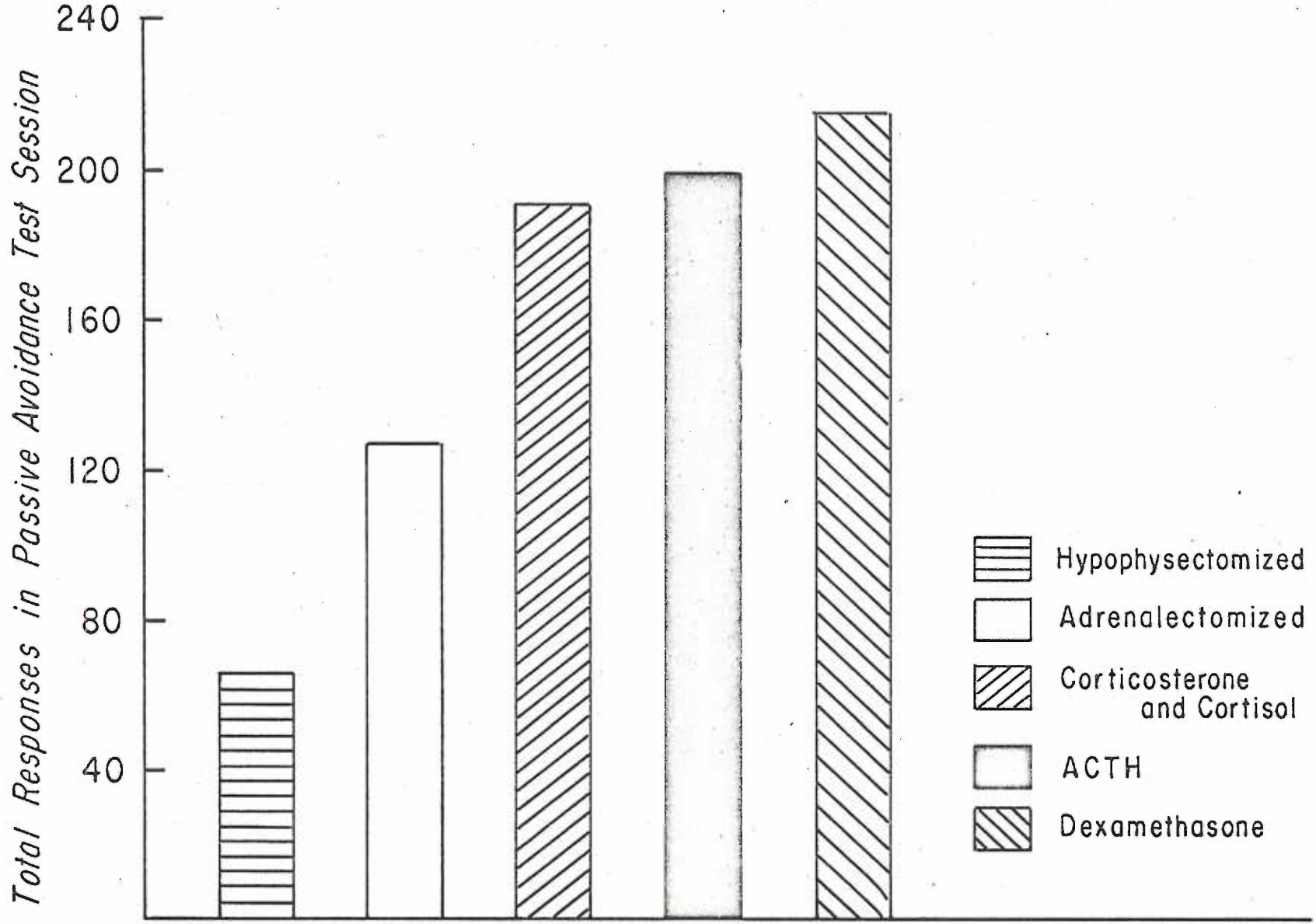
Figure 8. Median total responses in passive avoidance test session for saline, 85% body weight-procedural control, 2-hr. behavioral control, B and F, dexamethasone, and ACTH groups.


Total Responses in Passive Avoidance Test Session
240
200
160
120
80
40
Saline
85 % Body Weight Control
2 hr. Behavioral Control
Corticosterone & Cortisol
Dexamethasone
ACTH

revealed that the saline group made significantly fewer responses than the other three injection groups. The saline group differed from the dexamethasone group at the .02 level ($U = 11$, $n_1 = 8$, $n_2 = 10$); from the ACTH group, also at the .02 level ($U = 11.5$, $n_1 = 8$, $n_2 = 10$); and from the B and F group at the .05 level ($U = 17$, $n_1 = 8$, $n_2 = 10$). The B and F group was not significantly different from the dexamethasone group. The above results, again indicate a significant effect since all injected groups were treated like the saline group with the exception of having received their respective hormones along with the saline solution. The effect of these hormones, in all cases, is to increase the total number of responses relative to the saline injection control.

Total responses in the passive avoidance test session for the 2 x 2 factorial groups (adrenalectomized, hypophysectomized, ACTH, B and F, and dexamethasone) are represented in Figure 9. A Kruskal-Wallis one-way analysis of variance for total responses in the test session on the 2 x 2 factorial groups indicated significant differences at the .01 level ($H = 16.53$, $df = 4$). Mann-Whitney tests revealed that the injected groups made more total responses in the passive avoidance test session than the hypophysectomized or adrenalectomized groups. The B and F group was significantly different from the hypophysectomized ($U = 12$, $n_1 = 7$, $n_2 = 10$, $p < .05$) as was the dexamethasone group ($U = 10$, $n_1 = 7$, $n_2 = 10$, $p < .02$). Since the differences of the rank totals ($Rx - Ry$) of the following pairs were greater than the difference of the rank totals of the dexamethasone and hypophysectomized groups, it was assumed that the following pairs differed significantly in total responses in the test session at the .02 level: B and F group versus adrenalectomized group and ACTH group versus hypophysectomized group. A Mann-Whitney test showed that the ACTH group

Figure 9. Median total responses in passive avoidance test session for the 2 x 2 factorial groups.


Total Responses in Passive Avoidance Test Session
240
200
160
120
80
40
Hypophysectomized
Adrenalectomized
Corticosterone and Cortisol
ACTH
Dexamethasone

differed significantly from the adrenalectomized group at the .002 level.

The hypophysectomized group and its sham control just missed being significantly different from each other at the .05 level in total number of responses in the test session ($U = 14$, $n_1 = 7$, $n_2 = 8$, $p < .06$). However, a Mann-Whitney test indicated a significant difference between the adrenalectomized group and its sham control ($U = 5$, $n_1 = 6$, $n_2 = 8$, $p < .006$), the adrenalectomized animals making fewer total responses in the passive avoidance test session. Mann-Whitney tests showed no significant differences between the 2-hr. behavioral control group and the sham adrenalectomized or sham hypophysectomized groups and between the 85% body weight-procedural control group and the sham adrenalectomized or sham hypophysectomized groups and in total responses made in the test session.

Corticosterone Levels

Table 3 lists the median plasma corticosterone levels of the groups decapitated before training or test sessions. A Kruskal-Wallis one-way analysis of variance of these data was done on the .08-hr. ISI group, the 2-hr. ISI group, and the 75% body weight-procedural control group, the .08-hr. and 2-hr. groups having been decapitated immediately before the passive avoidance test session and the 75% group, before the passive avoidance training session. A significant difference was found at the .01 level ($H = 12.86$, $df = 2$). Mann-Whitney tests revealed a difference between the 75% and 2-hr. groups ($U = 8.5$, $n_1 = 8$, $n_2 = 10$, $p < .01$) but no differences between the .08-hr. and 2-hr. ISI groups or between the .08-hr. and 75% groups.

Table 4 lists the median plasma corticosterone levels of the groups decapitated after the test session. The pre- and post-test session corticosterone levels of the ACTH groups indicated that the ACTH injection

Table 3. Median plasma corticosterone levels (μg/100 ml plasma) of groups decapitated before training or test sessions.

Table 4. Median plasma corticosterone levels (μg/100 ml plasma) of groups decapitated after test session.

Table 3

Group	ug% corticosterone
Before Training Session:	
75% body weight-procedural control	19.25
Before Test Session:	
.08 hr. decapitation control	17.25
2 hr. decapitation control	11.25
ACTH	66.00
Dexamethasone	6.00

Table 4

Group	ug% corticosterone
ACTH	50.5
Dexamethasone	4.0
B and F	52.5
85% body weight-procedural control	23.5
Saline	15.25
Sham hypophysectomized	11.5
Sham adrenalectomized	8.5

produced high levels of both glucocorticoids and ACTH. Both the pre- and post-test session levels of corticosterone indicate high levels of ACTH.

The pre- and post-test session corticosterone levels of the dexamethasone-injected group indicate that the drug was suppressing ACTH secretion throughout the test session and thus did produce a group with low levels of ACTH and high levels of (synthetic) glucocorticoid.

It can be assumed from the high post-test session corticosterone level of the B and F group that corticosterone levels were high throughout the test session and thus that ACTH levels were low during the same time period. Therefore, the B and F injection produced a group with low levels of ACTH and high levels of glucocorticoids. (All Mann-Whitney tests on data from Experiment II were two-tailed.)

DISCUSSION

Experiment I

The results of Experiment I clearly demonstrated a U-shaped curve of retention of the passive avoidance response. This indicates that the U-shaped function is not a peculiarity of active shuttlebox avoidance, but is a more general phenomenon of aversively motivated learning. Even though McMichael (1966) used a passive avoidance paradigm in testing the retention of a response after various ISIs, his results did not indicate a clear U-shaped function as noted by McAllister and McAllister (1967). However, the passive avoidance response in his investigation consisted of crossing from one side of a box to another, and passive avoidance learning was measured by the amount of time spent on the "safe" side. The shock intensity, duration, and frequency also differed from those used in this investigation. These are possible reasons for his ambiguous results.

The main significance of this experiment is that it demonstrates that the U-shaped function is not merely an activity effect. The absence of activity controls in the experiments showing a U-shaped function in active avoidance made it questionable as to whether this function was really a retention curve. It could also be claimed that it was an activity curve, the subjects at the low point showing a suppression of activity. However, the passive avoidance paradigm has a built-in activity control, since high activity levels (large total number of bar presses in this case, since bar presses were used as an index of activity) necessarily accompany low retention levels and vice versa (see Figure 1). The results of Experiment I indicate that the U-shaped function is probably a retention function.

It is difficult to interpret the measure of total responses in the passive avoidance test session. It can be viewed as an activity measure but, as such, is at least partially dependent upon the latency to criterion

(that point where the animal begins to respond regularly), because earlier resumption of regular responding would provide more time to make more responses. In this way, the total response measure is related to the latency measure. The total response curve (Figure 6) is similar to, but not the same as, the inverted form of the latency curve (Figure 2); the .08-hr. group being significantly different from the 2-hr. and 4-hr. groups in latencies to responses one through five but not in total number of responses in the passive avoidance test session.

It could be argued that what occurred in Experiment I was not merely acquisition of a passive avoidance response but also acquisition of a conditioned emotional response (CER). Spevack and Suboski (1967) found that shock administered either contingent or not contingent upon the response reduced bar press rates. A noncontingent shock control group was not feasible in this study because of the high rate of bar pressing. Any shock administered would have occurred shortly after a bar press and thus would have suppressed responding by adventitious punishment. However, a conditioned suppression measure could be used to indicate the amount of conditioned emotionality as a function of time after conditioning. Tarpy (1966) measured incubation of anxiety by suppression of bar pressing for food during presentation of a CS that was paired with shock during shuttle-box avoidance training. He demonstrated that suppression increased over time up to 4 hr. but after that remained at approximately the same level for the longer retention intervals (up to 1 week). McMichael (1966) studied conditioned suppression as a function of ISI and found that groups having instrumental avoidance training, as well as groups having classical aversive conditioning, showed an increase in magnitude of suppression to the CS from 1-6 hrs. following conditioning. With longer intervals, suppression

leveled off and finally declined slightly after 504 hrs. In neither of the above cases was the magnitude of the suppression (magnitude of the CER) a U-shaped function after conditioning. Thus, it seems likely that the U-shaped function obtained in Experiment I was due mainly to disruption of the passive avoidance response, not to changes in conditioned emotionality.

Experiment II

The U-shaped retention function of passive avoidance having been established, the next step was to attempt to determine the basis for it. Literature indicated that this function might be under hormonal control - particularly the hormones of the HHA system. Levine and Brush (1967) found that ACTH injected into normal rats maintained performance of an active avoidance response or, in other words, raised the low point of the U-shaped function. A similar but not statistically significant effect of hydrocortisone was also found in that experiment. Brush (unpublished) also found that ACTH inhibited the rapid extinction of an active avoidance response which otherwise occurred in normal, uninjected animals at the 2-hr. ISI. Murphy and Miller (1955), Miller and Ogawa (1962), and De Wied (1966, 1967) clearly indicate that ACTH inhibits extinction (or facilitates retention) of an active avoidance response. These effects are accompanied by a high level of activity. De Wied (1967) has also demonstrated that glucocorticoids facilitate extinction (or inhibit retention) of an active avoidance response. This effect is accompanied by a decrease in activity.

Given these results, it seemed reasonable to test groups with various combinations of levels of ACTH and glucocorticoids at the low point of the U-shaped retention curve. Two groups similar to those used in Levine and Brush's (1967) investigation were produced by injection of ACTH (high

ACTH-high glucocorticoids) and by injection of a B and F mixture (low ACTH-high glucocorticoids). Neither injection raised the latency measure above that of the 2-hr. behavioral control group. Thus, an increase in the retention of the passive avoidance response and a raising of the low point of the U-shaped function was not demonstrated. The total responses of each group were higher, but not significantly, than those of the 2-hr. behavioral control group. If anything, these results indicate that high ACTH with high glucocorticoids or high glucocorticoids alone are associated with high levels of activity.

It might be argued that the hormone dosage and postinjection time intervals of this investigation were not the same as those of Levine and Brush. However, their data indicated that the mean µg% corticosterone level of the ACTH-injected group was 59.41 immediately before entering the test session. In this investigation the median µg% corticosterone level of the ACTH-injected group was 66 immediately before entry. Thus, the two groups exhibited similar increases in hormone levels stimulated by the ACTH injections. The dosage of cortisol used by Levine and Brush was appreciably lower (2 mg per rat) than that of the B and F group of this investigation (3 mg cortisol plus 17 mg corticosterone per rat). Thus, the glucocorticoid groups are not strictly comparable.

De Wied (1967) found that ACTH injected into normal rats inhibited extinction (and, therefore, facilitated retention) of an active avoidance response. This effect corresponded with an increase in activity. He also found that dexamethasone, as well as corticosterone, injected into normal rats facilitated extinction (and, therefore, inhibited retention) of an active avoidance response. This corresponded with a decrease in activity. The ACTH group of this investigation did not show facilitated

retention but did have a high level of activity. The B and F and dexamethasone groups did not show decreases in activity but did show inhibited retention of the passive avoidance response. Comparing De Wied's results with those of this investigation, it could be concluded that ACTH injected into a normal rat (thus producing high levels of ACTH and glucocorticoids) increases activity and that a B and F mixture, as well as dexamethasone, injected into a normal rat inhibits retention of the avoidance response.

In this investigation, hypophysectomized animals (low ACTH-low glucocorticoids) and adrenalectomized animals (high ACTH-low glucocorticoids) both exhibited a short latency to the fifth response similar to that of the 2-hr. behavioral control; however, their total responses were lower than the above control, the hypophysectomized animals tending to make a small total number of responses in the test session. De Wied's (1967) adrenalectomized rats exhibited elevated resistance to extinction (and, therefore, facilitation of retention and high levels of activity). His hypophysectomized rats showed normal extinction (and, therefore, poor retention with low levels of activity). In this investigation, both hypophysectomized and adrenalectomized groups exhibited poor retention. However, the hypophysectomized animals did show a lower level of activity than did the adrenalectomized animals.

Weiss et al. (1969), comparing hypophysectomized and adrenalectomized rats in a one-trial passive avoidance situation, found that adrenalectomized rats had significantly longer latencies (and, therefore, better retention) before re-entry into a "shock compartment" than did the hypophysectomized rats (who, therefore, exhibited poorer retention). These results agree with those of De Wied - that adrenalectomized animals show better retention and hypophysectomized animals poorer retention of an avoidance

response - but not with those of this experiment. A factor that may account for the results obtained from the adrenalectomized and hypophysectomized groups of this experiment, at least in part, is that they were maintained at 85% body weight, whereas those in the two above mentioned studies were at normal body weight.

If one looks at the 2 x 2 factorial groups in relation to levels of ACTH, there seems to be no consistent pattern either in the latency measure or the total response measure (see Figure 10). Groups with both high and low levels of ACTH exhibit short latencies and large total numbers of response. If one looks at these same groups in relation to levels of glucocorticoids, there appears to be no relation to the latency measure, since groups with both high and low levels exhibit short latencies. However, there seems to be at least some relation between corticosterone levels and total responses in the test session, the groups with trace levels making fewer responses than the rest of the groups (see Figure 11).

A very serious problem in the interpretation of the results of the injection groups in Experiment II is the significant saline effect. It was only in the saline group that the low point of the function was raised, the median latency to the fifth response for this group being 30.64 min. and that of the .08-hr. behavioral group being 34.21 min. It is difficult to interpret this saline injection effect. It could be postulated that the injection was stressful (which it indeed appeared to be judging by the extremely "emotional" response of the rats to the injection, i.e., squealing, urinating, and struggling) and that this stress was sufficient to stimulate the HHA system to a point which resulted, 2 hr. later, in long latency measures and small median number of total responses (130), comparable to that of the adrenalectomized animals (127).

Figure 10. Median latencies to the fifth response and median total responses in the test session in relation to levels of ACTH.

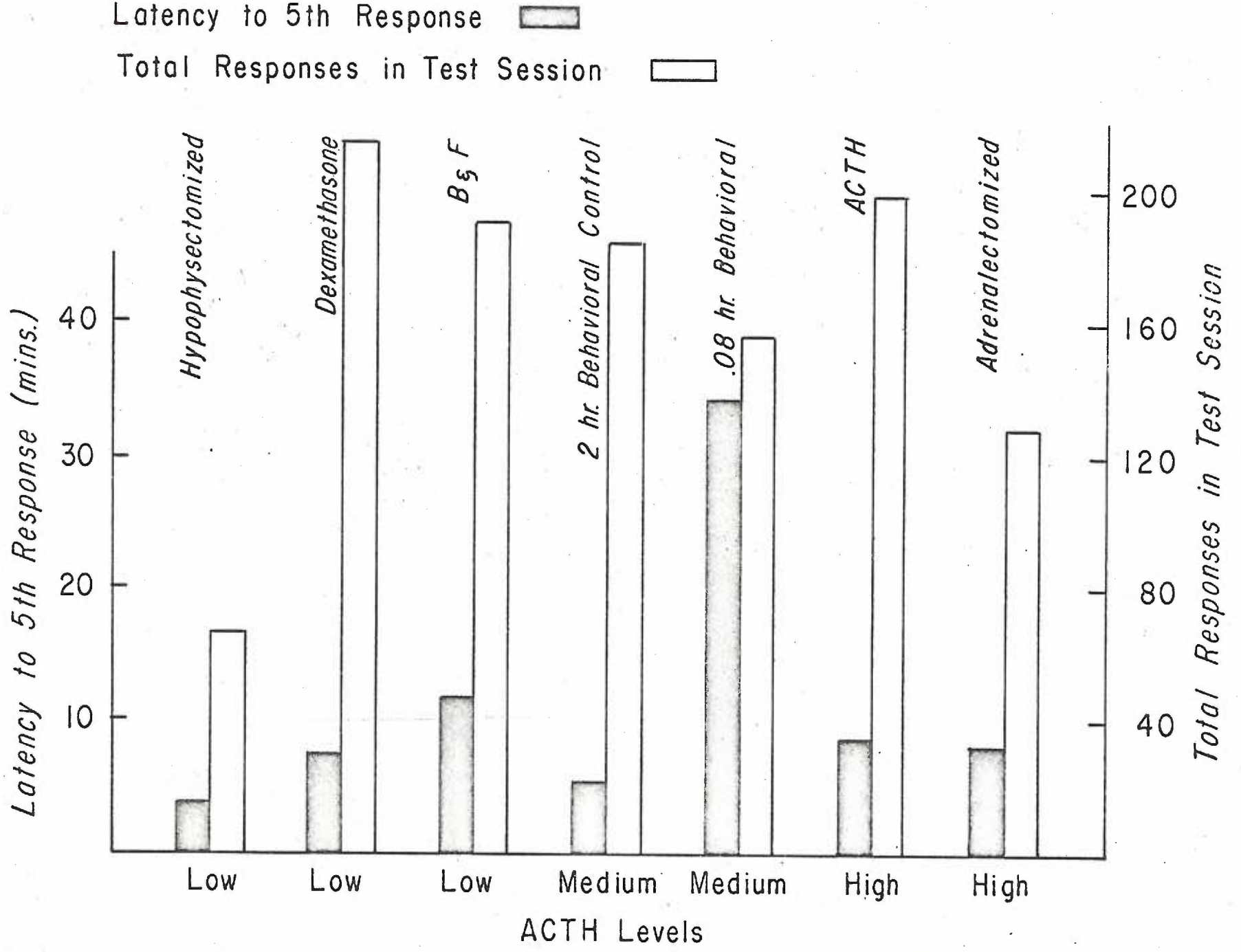
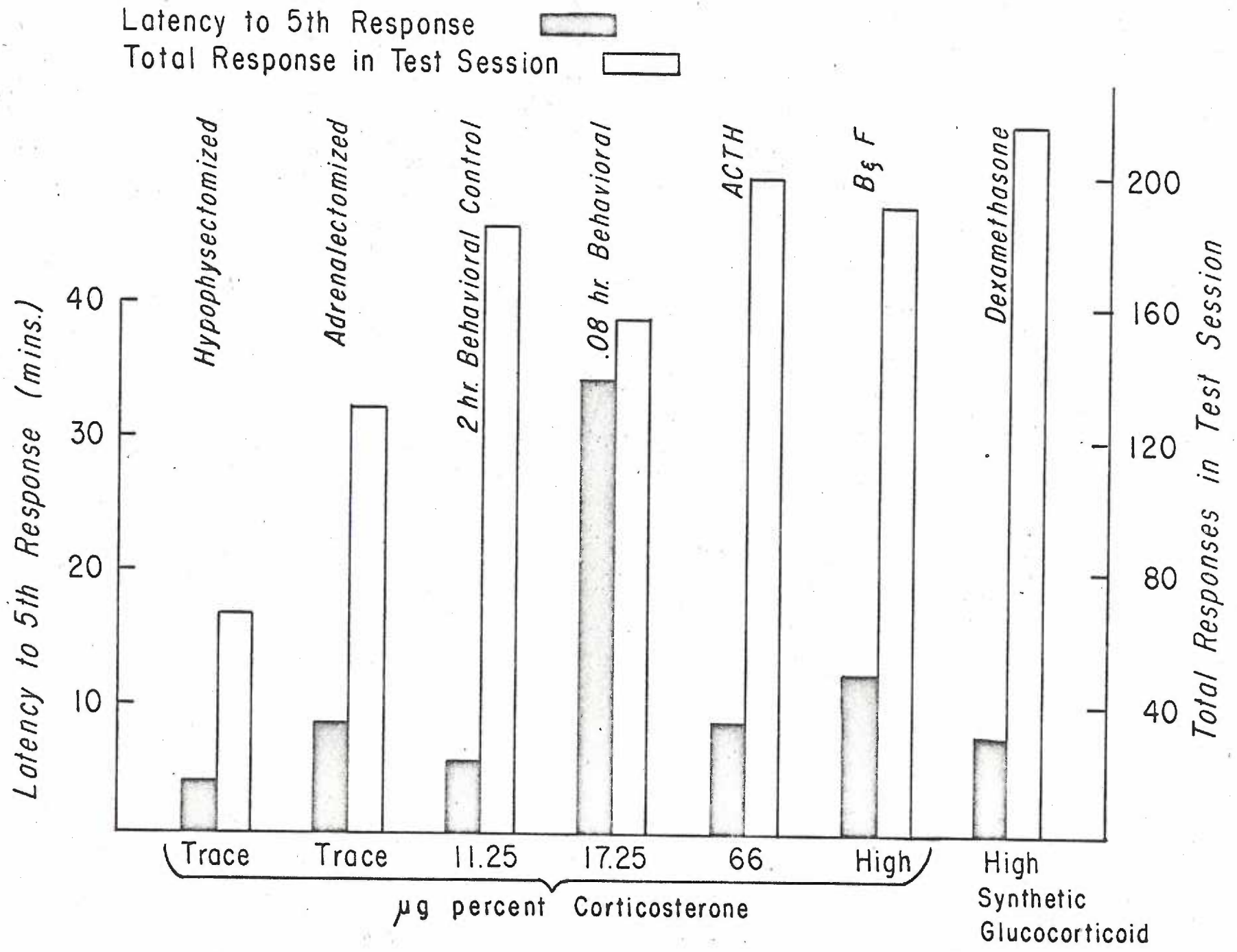

Latency to 5th Response
Total Responses in Test Session
Hypophysectomized
Dexamethasone
B & F
2 hr. Behavioral Control
.08 hr. Behavioral
ACTH
Adrenalectomized
Latency to 5th Response (mins.)
Total Responses in Test Session
10
20
30
40
40
80
120
160
200
Low
Low
Low
Medium
Medium
High
High
ACTH Levels

Figure 11. Median latencies to the fifth response and median total responses in the test session in relation to levels of corticosterone.


Latency to 5th Response
Total Response in Test Session
Hypophysectomized
Adrenalectomized
2 hr. Behavioral Control
.08 hr. Behavioral
ACTH
B ξ F
Dexamethasone
Latency to 5th Response (mins.)
40
30
20
10
200
160
120
80
40
Total Responses in Test Session
Trace
Trace
11.25
17.25
66
High
High
Synthetic
Glucocorticoid
μg percent Corticosterone

It must be remembered that the injection groups (ACTH, B and F, and dexamethasone) also received a saline injection as the vehicle for their respective hormones and that two of the groups (ACTH and dexamethasone) exhibited a significantly shorter latency to the fifth response and all three groups showed a significantly greater number of total responses compared to the saline group. Thus, it appears that all three drugs reduced the latency to the fifth response and increased total responses in the test session. Common to all three hormone-injected groups are high levels of glucocorticoids. It would seem feasible to conclude that high levels of glucocorticoids, therefore, reduce latency (retention) and increase activity. This would agree with De Wied's conclusion that glucocorticoids facilitate extinction. However, the 2-hr. behavioral control group exhibits a short latency and high activity and yet has low levels of glucocorticoids immediately preceding the test session.

The need for further investigation of the saline injection effect is apparent. Three groups could be trained according to the procedure described in this experiment. After passive avoidance training, one group could be injected with physiological saline; one with distilled water, for volume control; and one with nothing but merely punctured with a hypodermic needle. If all groups exhibited the same behavior in the test session, one could conclude that it was the result of the injection itself. However, if the saline group showed a different effect, it would appear that NaCl somehow affected latency and total response measures.

It is possible that fluctuations in levels of hormones of the HHA system are not the basis of the U-shaped function. Suboski, Marquis, Black, and Platenius (unpublished) studied neuroendocrine function in incubation of aversively conditioned responses by testing subjects after

short or long postconditioning intervals in three training procedures (passive avoidance, CER, and two-way shuttlebox responses). Adrenalectomized subjects showed acquisition and incubation of all three types of responses that were comparable to the sham-operated subjects. Hydrocortisone injections had no apparent effect. They concluded that the hypothesis of direct involvement of adrenal corticosteroids in the incubation of aversively conditioned responses was not supported.

Ideally, Experiment II should have been run with each of the 2 x 2 factorial groups (hypophysectomized, adrenalectomized, ACTH-injected, glucocorticoid-injected) tested at all of the ISIs used. For example, six groups of hypophysectomized animals should have been trained and then tested, one group at each ISI, and the same procedure repeated with adrenalectomized, ACTH-injected, and glucocorticoid-injected animals. The results would have indicated whether or not each of the 2 x 2 factorial groups exhibited a U-shaped function and, if so, whether it was identical to that of the normal behavioral group. The results from such an experiment would provide direction for future research in this area. For example, if it was found that the six ISI groups of each combination of levels of ACTH and glucocorticoids formed a U-shaped function, the results would indicate that this retention curve is due to factors other than ACTH and glucocorticoids. If some combinations resulted in a U-shaped function and others did not, the likely combinations involved in the function would at least be indicated.

SUMMARY AND CONCLUSIONS

A study of passive avoidance learning and its possible hormonal control was conducted in two parts. The purpose of the first experiment was to determine whether the U-shaped retention curve (Kamin effect) which characterizes active avoidance learning could also be found in a passive avoidance paradigm. Rats were trained to press a bar in a Skinner box for CRF. After the response was well established, they were given passive avoidance training which consisted of administration of electric shock contingent upon the first bar press after a fixed interval from the last punished response. After the passive avoidance training session, subjects were returned to their home cages for one of the following intersession intervals (ISIs): .08, 1, 2, 4, 8, and 16 hrs. At the end of the ISI, subjects were returned to the Skinner Boxes for a test session. Results showed a definite U-shaped function in passive avoidance. This indicated that the U-shaped function is not a peculiarity of active shuttlebox avoidance but rather, a more general phenomenon of aversively motivated learning. It also suggested that the U-shaped function is probably a retention effect.

The relation of the pituitary-adrenocortical hormones (ACTH and glucocorticoids) to the U-shaped function was investigated in the second experiment. Rats were trained and tested using the same procedures as that of the first experiment. By surgical intervention or administration of hormones, various combinations of high and low levels of ACTH and glucocorticoids were induced in various groups. All groups were tested at the low-point ISI which was 2 hrs. No clear-cut relation between levels of ACTH or glucocorticoids and latency to the fifth response or total number of responses was found. The only discernable pattern was that of total number of responses being lower with trace levels of corticosterone than normal levels or high levels. A saline injection effect was revealed, the

saline injection control group having a longer latency to the fifth response and making a smaller total number of responses than uninjected controls and hormone-injected groups. The relation of the pituitary-adrenocortical hormones to the U-shaped function was discussed.

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