THE ROLE OF SHOCK AND PLASMA CORTICOSTERONE IN THE RETENTION OF AN AVOIDANCE RESPONSE

by

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INTRODUCTION

The original impetus for the study of retention of learned behaviors was provided by Ebbinghaus. His classic studies on forgetting of nonsense syllables have been followed by a long series of experiments designed to describe retention of verbal material. Typical of these studies is one by Postman and Rau (1957). First, the subjects were required to learn a list of nonsense syllables or English words to a criterion of one perfect recitation. Then after intervals of 20-min, 24-, or 48-hrs, retention was measured by either a relearning, recognition, free recall, or anticipation method. The results were similar for both types of material: recognition produced the best retention and free recall and anticipation the poorest. Over the 48-hr interval, retention remained near the 100% level with the recognition method, but decreased linearly with the other three methods. These findings and similar studies have led to the general conclusion that the retention of verbal material in man is generally quite good and the little forgetting that occurs is typically a linearly decreasing function of the period of disuse.

The retention of classically and instrumentally conditioned responses has received only infrequent attention. Most of these studies revealed that conditioned responses show only a limited decrease in strength over time. Reports of the high resistance of conditioned responses to forgetting include the retention of a conditioned motor response in sheep for two years (Liddell, James, and Anderson, 1934); a conditioned eyelid reaction in dogs for 16

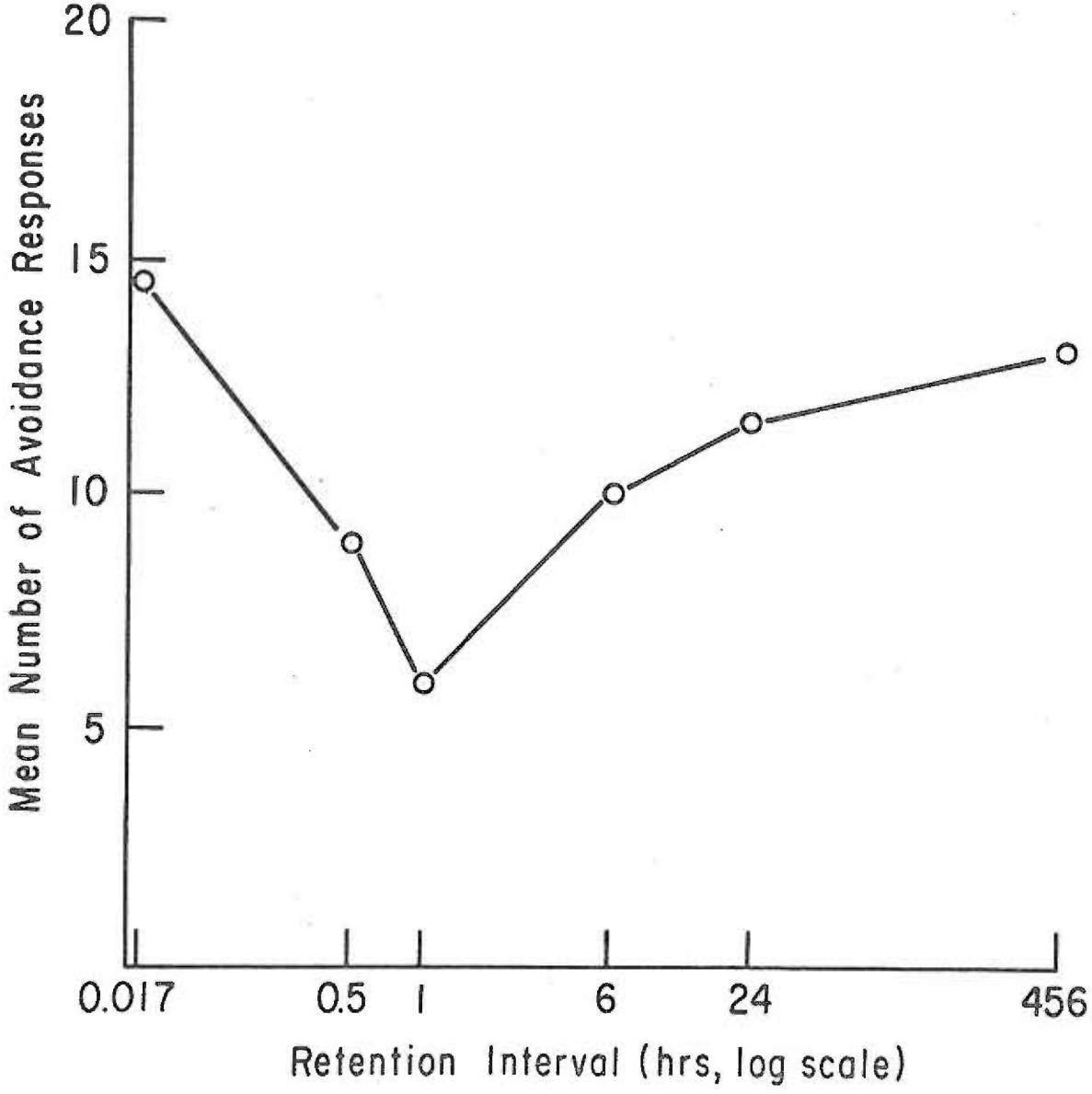
months (Marquis and Hilgard, 1936); a conditioned flexion reflex in dogs for 30 months (Wendt, 1937); a conditioned salivary response in man for 16 weeks (Razran, 1939); and a pecking response in pigeons for 4 years (Skinner, 1950).

After reviewing these studies, Kamin (1957) proposed that the main reason for the absence of any significant decrement in the strength of these conditioned responses was the extensive training given to the subjects over several weeks and in some cases over several years. He suggested that with such training the response decrements would be extremely small and the retention tests could have been insensitive to such slight decrements. He further proposed that a larger response decrement over a retention interval might be present if less extensive training of the conditioned response was given. To test this hypothesis, several groups of rats were given relatively few conditioning trials in a single training session. The training session consisted of 25 two-way active avoidance trials in a two-compartment shuttlebox. At the start of the session, the subject was placed in one side of the shuttlebox, and after a 2-min adaptation period was presented with the first trial. Each trial began with the onset of a buzzer in the compartment occupied by the subject. If the subject ran to the opposite compartment within a 5-sec interval, it avoided a 1.1 ma shock (unconditioned stimulus or US) and the buzzer was immediately turned off. Thus, the buzzer served as a warning stimulus or conditioned stimulus (CS) for each shock. If the subject failed to respond during the 5-sec interval between the onset of the CS and the onset of the US (CS-US) interval,

it could escape the shock by running to the opposite compartment, at which time both the US and CS terminated. Each trial was separated by a 1-min intertrial interval (ITI). The subject's performance was expressed as the number of times during the 25 trials that it successfully avoided the shock. The mean number of avoidance responses for all subjects during original training was 5.0. After completion of the 25 original training trials, the subjects were randomly divided into six groups to receive an additional 25 training trials. These trials were given in the same apparatus after intersession intervals (ISIs) of 0-, 0.5-, 1-, 6-, 24-, or 456-hrs. Figure 1 shows the results of Kamin's experiment: the group mean number of avoidance responses during the second training session reliably decreased from 0- to 1-hr and then increased from 1- to 456-hrs. This curvilinear retention function over the 24-hr period after original training was an unexpected finding in light of the general opinion that a linearly decreasing function would describe the retention of a learned avoidance response. This phenomenon has been subsequently labeled the "Kamin effect" or U-shaped retention function.

The training procedures employed by Kamin were first repeated by Denny (1958). All rats were given 25 trials of two-way active avoidance training in a shuttlebox, then divided into three groups to receive an additional 25 training trials after ISIs of either 0-, 1-, or 24-hrs. Although Denny's subjects showed a higher level of avoidance responding during original

Figure 1 Results reported by Kamin (1957), expressed as the mean number of avoidance responses during the second training session as a function of the intersession interval.


20
15
10
5
Mean Number of Avoidance Responses
0.017
0.5
1
6
24
456
Retention Interval (hrs, log scale)

training than Kamin's (10.5 vs 5.0 mean number of avoidance responses), the results of his study closely replicated Kamin's original findings; that is, retention at the 1-hr interval was inferior to that at the 0- and 24-hr intervals. Taken together, the two experiments indicated that the curvilinear or U-shaped retention function was invariant with changes in original avoidance performance over a 2:1 range.

To determine the low point in the function more precisely, Denny and Ditchman (1962) plotted the time course of the U-shaped retention function in 15-min intervals around the 1-hr minimum. A 70-db buzzer located outside the shuttlebox served as the CS, and an electric shock with a maximum flow of 1.7 ma was the US. The CS-US interval was 5-sec and both the CS and US were terminated by the running response. Rats were given 25 trials of two-way active avoidance training, followed by 25 retraining trials at retention intervals of 0.5-, 0.75-, 1-, 1.25-, and 1.5-hrs. Again a significant U-shaped retention function was found, with the mean number of avoidance responses during retraining decreasing between 0- and 1-hr and then increasing from 1- to 1.5 hrs. Minimum retention was between the .75- and 1.5-hr intervals, with the lowest point representing the maximum decrement in performance at the 1-hr interval.

Although these early studies on the U-shaped retention function dealt exclusively with a two-way active avoidance response, a study by Brush (1964) suggested that the retention of other learned responses may also be U-shaped if particular requisites are present. Brush trained rats in an automatic

shuttlebox, with an illumination and noise increase serving as the CS and a .3 ma electric shock as the US. Male rats were given 25 original training trials under one of three procedures: (1) an escape training procedure in which the CS-US interval was 0.5-sec and both the CS and US were terminated by the running response. The use of a very short CS-US interval reduced the probability of a subject's avoiding the shock, and thus subjects received shock on every trial; (2) a fear conditioning procedure in which subjects were confined to one compartment of the shuttlebox and given paired presentations of the CS and US. The CS-US interval was .5-sec and shock duration was .5-sec; (3) an unsignalled shock procedure in which the subjects were confined to one compartment and simply given shocks lasting .5-sec. Subjects trained under each of the three procedures were then randomly assigned to one of the following retraining intervals: 0.08-, 1-, 4-, 24-, or 168-hrs. The retraining session for all subjects consisted of 40 active avoidance training trials, with a 5-sec CS-US interval. A 1-min intertrial interval was used throughout the original and retraining sessions. The results revealed that a U-shaped function occurred after both the escape training and the fear conditioning procedures, but not after the unsignalled shock procedure. Since the only apparent difference between the unsignalled shock and the fear conditioning procedures was the omission of the CS, Brush concluded that Pavlovian fear conditioning to a CS is the necessary and sufficient requisite to produce the U-shaped function.

On the basis of the preceding experiment, conditioning procedures that include Pavlovian fear conditioning to a CS, other than the two-way active avoidance procedure, should also be followed by a U-shaped retention function. Subsequent studies have supported this reasoning. Klein and Spear (1969) reported a U-shaped retention function after a one-way active avoidance training procedure. The one-way avoidance task is similar to the two-way task, except the subject is only required to run from one compartment (painted white) to another compartment (painted black), after which it is picked up by the experimenter and placed back in the white compartment to await another trial. An avoidance response occurs when the subject crosses from the white to the black compartment before the shock is delivered in the white compartment. Using a pulsing light CS and a 1-min ITI, Klein and Spear trained rats to avoid a 1.6 ma shock. After intersession intervals of either 10-min, 1-, 4-, or 24-hrs, a significant U-shaped retention function was found during the retraining sessions. A number of other studies have also reported significant U-shaped performance after one-way active avoidance training (e.g., Baum, 1968; Klein and Spear, 1970a, b).

Pinel and Cooper (1966a) reported a U-shaped retention function after a passive avoidance training procedure. Although the procedure involved the conditioning of fear to a CS, the passive avoidance task is quite different from the previously mentioned active task. First, subjects were trained for several days to press a lever for continuous water reinforcement. When a stable rate of lever pressing had been established, the subjects were placed in the box

and after a 20-sec delay, the first lever press initiated a 0.01-sec, 0.5 ma electric foot shock. Three groups were then tested for retention of the lever press response at intervals of either 1-min, 2-, or 8-hrs after the shock. Measured as the difference between the number of lever presses during the last 10-min of the final continuous reinforcement session and the number of presses during the 10-min retention session after the shock, performance was a U-shaped function with the minimum at the 2-hr interval.

In addition to the preceding studies, U-shaped retention has also been reported after wheel-running avoidance training in rabbits (Gabriel, 1968); signalled escape training (Brush, Myer, and Palmer, 1963; Brush, 1964); and Pavlovian conditioning of fear (Bintz, Brand, and Brown, 1970; Brush, 1964; Brush and Levine, 1966; Walrath, 1968).

The experiments reported thus far indicate that U-shaped retention is apparently a reliable phenomenon not unique to a single training paradigm. Despite differences in apparatus specifications, location and type of CS, intensity of US, CS - US interval, species of subject, sex of subject, and retention intervals, U-shaped retention was a common finding. However, several studies have failed to obtain it (Irwin and Banuazizi, 1966; Adams and Calhoun, 1970; Clark, 1967). Although there is not a readily available explanation for these failures, it should be noted that in each study a passive avoidance procedure was used that involved the suppression of a relatively unlearned response. A reasonable conclusion is that conditioning procedures

can be arranged in such a way that the probability of obtaining a U-shaped retention function is minimized. However, the remainder of this paper will be mainly concerned with those procedures and experiments that successfully produced the effect.

The number of original training trials necessary to produce the U-shaped retention function has not been systematically investigated. Differences in the number of trials between experiments would indicate that the amount of original training above a minimum of at least 1 trial does not determine the occurrence or nonoccurrence of the function. For example, Kamin (1957), Denny (1958), and Brush (1964) gave 25 original training trials, Brush (1968, unpublished) used 10 trials, Walrath (1968) gave 5 trials, Bintz, Brand, and Brown (1970) presented 2 trials, and Pinel and Cooper (1966a) gave only 1 trial, yet each experiment reported a significant U-shaped retention function. Other investigators have set as their criterion a specific number of avoidance responses during original training and allowed the actual number of training trials to vary from subject to subject. Again, variations both between and within experiments with respect to the avoidance criteria used during original training failed to affect the occurrence or nonoccurrence of the U-shaped retention function. A study by Klein and Spear (1969) illustrates this point. In Experiment I, all subjects received one-way active avoidance training to a criterion of one avoidance response (after at least one failure to avoid) and were then retrained at retention intervals of either 10-min, 1-, 4-, or 24-hrs. In Experiment II, a performance criterion of 5 avoidance responses during

original training was used, and retraining occurred following approximately the same intersession intervals. Despite the different original training criteria, a reliable U-shaped retention function was reported in both experiments.

Although neither the number of original training trials nor the performance criterion appear to affect the occurrence or nonoccurrence of the U-shaped function, they have been shown to affect the locus of the interval of minimum retention. After an exhaustive review of the literature, Brush (1971) reported that subjects that learn the avoidance response rapidly show an earlier minimum (closer in time to the original training session) than subjects that learn the avoidance response more slowly. The interval of minimum retention, then, is an inverse function of the rate of conditioning, where rate is defined as the percentage number of avoidance responses.

Although there is general agreement about the nonlinearity of the retention function after aversively motivated learning and many of the procedural variables that affect the phenomenon, the same cannot be said of the theoretical interpretations of the function. Kamin (1957) proposed two processes to account for the U-shaped retention function, one for each segment of the curve. To account for the descending segment extending from 0- to 1-hr, Kamin proposed that the subjects forget the avoidance response. When a second training session occurs at 1-hr, the subjects must relearn the avoidance response or at least require more trials to "warm-up" and begin avoiding again. The ascending segment of the function between 1- and 456-hrs after original training was

thought to result from an incubation effect, i.e., a linear increase in the animal's conditioned emotional response (fear) or possibly a consolidation of the avoidance habit as a function of time after original training. Under either of these influences there should be an increase in the probability of occurrence of the avoidance response. Subsequently, Kamin (1963) revised his proposal of a linear increase in fear in favor of an inverted U function, where avoidance performance is impaired as the function rises to a maximum 1-hr after original training. He did not, however, attribute this latter function to any known process or condition such as fear or physiological reactions. Rather, Kamin concluded that this was the most parsimonious interpretation and that more experimentation was necessary to reveal the exact nature of the mechanisms involved in producing the U-shaped retention function.

Denny (1958) suggested that a single inverted U-shaped process could account for retention after aversive conditioning. The inverted function represented an initial incubation (increase) of anxiety which reached its peak at about 1-hr after original training, followed by a gradual waning of this anxiety to a "basal" level within 24-hrs after original training. The decrement in performance at the 1-hr retention interval was thought to be a consequence of competing responses such as freezing interfering with the instrumental running response.

Two more recent positions attributed the U-shaped retention function to a process similar to that suggested by both Kamin (1957) and Denny (1958).

Denny and Ditchman (1962) proposed an explanation in terms of an "incubation of anxiety" concept (Bindra and Cameron, 1953; Diven, 1937; Golin, 1960). According to their interpretation, fear begins to increase immediately after the original training session, reaching a peak after approximately 1-hr. This heightened state of fear produces responses incompatible with the avoidance response (e.g., freezing), and the effect is poor avoidance behavior around the 1-hr interval. As fear dissipates after the 1-hr interval, good performance returns. Pinel and Cooper (1966a), on the other hand, suggested that the 1-hr ISI decrement in performance is a result of a decrease in fear from 0- to 1-hr after original avoidance training. When fear increases between the 1-and 24-hr test sessions, performance improves.

There are several reasons to suggest that theories based on a change in fear over time are untenable. First, the phenomenon of "incubation of fear" has recently been questioned by McAllister and McAllister (1967). Their conclusion from an extensive review of the literature was that attributing changes in performance to incubating fear is experimentally unjustified, and that in every case other explanations, equally as plausible could be offered. Second, whereas Brush (1964) reported that fear conditioning alone is the necessary and sufficient condition for producing the U-shaped function, subsequent studies have failed to substantiate this finding under different training procedures (Desiderato, Butler, and Meyer, 1966; McAllister and McAllister, 1965). Third, it seems improbable that the incubation of fear should have two different time courses, one resulting in a decline in retention between an immediate and a 1-hr retention interval (Denny and

Ditchman, 1962), and a second causing an improvement in performance between the 1- and 24-hr tests (Pinel and Cooper, 1966a, 1966b). Therefore, the interpretations mentioned thus far, at least in their present form, are probably not sufficient to explain the U-shaped retention function.

A physiological analysis of the U-shaped function was presented by Brush, Myer, and Palmer (1963). They proposed that the procedures associated with original avoidance training such as shock and handling establish within each subject an autonomic state of sympathetic dominance. When the subject is returned to its home cage after training, the autonomic nervous system overshoots to a state of "parasympathetic dominance" (Mason, Brady, Polish, Bauer, Robinson, Rose, and Taylor, 1961; Mason, Brady, and Sidman, 1957). If the subject is given avoidance training at the peak of this parasympathetic overreaction (presumably 1-hr after original training), it is unable to cope with the stress of such training and avoidance behavior is impaired. The assumption that this parasympathetic overreaction is peculiarly dependent on fear conditioning was cited as being compatible with existing data on ulcer formation (Brady, Porter, Conrad, and Mason, 1958). Additional evidence for this hypothesis was provided by Denny (1958), who reported that after 25 trials of two-way active avoidance training, a decrement in retention was not observed at the 1-hr interval when the subjects remained in the shuttlebox instead of being returned to their home cages during the intersession interval. Brush et al. (1963) suggested that these data are consistent with their hypothesis, since a parasympathetic overreaction would probably not occur in the presence of

fear-eliciting apparatus cues.

This position, however, was later revised in favor of one suggesting a more direct involvement of the hypothalamic-pituitary-adrenal system. Based on a number of studies which indicated a causal relationship between ACTH and avoidance behavior, Brush and Levine (1966) hypothesized that the descending segment (0- to 1-hr after original training) of the U-shaped retention function is associated with a refractory hypothalamic-pituitary state and a homeostatic decrease in the level of circulating plasma steroids. To support their hypothesis, they cited studies that have shown that (1) the exogenous administration of ACTH facilitates avoidance learning (Beatty, 1969); (2) an ACTH deficiency (adenohypophysectomy) slows the learning of an avoidance response (deWied, 1964); (3) the exogenous administration of ACTH inhibits the extinction of an avoidance response (Miller and Ogawa, 1962; deWied, 1966); and (4) the inhibition of ACTH facilitates the extinction of an avoidance response (deWied, 1967).

On the basis of these findings, Brush and Levine (1966) conducted an experiment to demonstrate a relationship between the U-shaped function and the hypothalamic-pituitary-adrenal system. Hooded rats were trained in shuttleboxes with a light and noise CS and a 0.3 ma shock US. Original training consisted of 25 fear conditioning trials with a 0.5-sec CS-US interval and a 0.5-sec US, after which each subject was assigned to one of the following ISIs: 0.08-, 1-, 4-, 24-, or 168 hrs. After the appropriate ISI, half the

subjects were decapitated for a determination of plasma corticosterone levels and half received 40 avoidance training trials. The results clearly showed that groups that had been given the sequence fear conditioning, ISI, and avoidance training performed during retraining in a U-shaped manner, with the minimum interval between 1- and 4-hrs. More important to their hypothesis, however, were data from groups that had been given the sequence fear conditioning, ISI, then decapitated. In these groups the mean level of plasma corticosterone decreased from 0- to 1-hr after fear conditioning, and thereby paralleled the descending segment of the U-shaped avoidance performance function.

Although these data provided evidence in support of Brush and Levine's (1966) theory, data from control groups that had received unsignalled shock did not support it. Brush (1964) had previously shown that unsignalled shock does not significantly affect subsequent avoidance performance. Therefore, to determine the plasma corticosterone response in groups that do not show U-shaped performance, they confined 40 rats to one side of the shuttlebox and gave them 25 unsignalled shocks (no CS) of a 0.3 ma intensity and 0.5-sec duration. These subjects were then assigned to ISIs of either 0.08- or 1-hr. After the appropriate interval, half were given 40 avoidance training trials and half were decapitated for a determination of plasma corticosterone. As predicted, avoidance performance after unsignalled shock did not decrease from 0- to 1-hr. However, the decapitated groups did show a decrease in

plasma corticosterone from 0- to 1-hr after unsignalled shocks. To reconcile these discrepant data, Brush and Levine suggested that whereas unsignalled shock is sufficient to produce the "motivational" changes involved in U-shaped performance during retraining, signalled shock was necessary for these motivational changes to be detected behaviorally.

A subsequent study by Levine and Brush (1967), in which they manipulated plasma corticosterone levels, provided additional support for the hypothesis that a direct relationship exists between plasma corticosterone levels and the retention of an avoidance response. After original avoidance training to a criterion of 3 avoidance responses, subjects were assigned to retention intervals of either 0.08-, 1-, 4-, 24-, or 168-hrs. After the appropriate interval, ten subjects at each interval were given another 40 training trials. Immediately after original training, three additional groups of 10 subjects each were given a 0.2 cc injection of either ACTH, hydrocortisone acetate, or corticosterone to maintain a high level of plasma corticosterone through the 1-hr interval. According to their hypothesis, this level should abolish the performance decrement normally present at that interval. A single control group was injected with physiological saline immediately after original training, then all four treatment groups were retrained 1-hr after original training. The results showed that those groups given the sequence original training, ISI, then retraining performed following a U-shaped function with the minimum at the 1-hr interval. Whereas the groups injected with corticosterone and saline also showed the usual performance decrement at the

1-hr interval, the groups injected with ACTH and hydrocortisone acetate did not. Avoidance responding in these groups was maintained at a level similar to the 0.08- and 24-hr groups. Levine and Brush concluded that these data support their hypothesis since maintaining high levels of plasma corticosterone through exogenous administration of ACTH or hydrocortisone precluded the decrease in avoidance performance usually observed between the 0- and 1-hr intervals. The somewhat paradoxical finding that corticosterone itself failed to abolish the 1-hr ISI performance decrement may have been the result of an inadequate dose or perhaps an indication that corticosterone is not necessarily the corticosteroid that exerts CNS control. In either case, it presents interpretational difficulties for the hypothalamic-pituitary-adrenal hypothesis.

In both the Brush and Levine (1966) and the Levine and Brush (1967) study, plasma corticosterone measurements taken 24-hrs after original training posed further problems for the hypothalamic-pituitary-adrenal hypothesis. The level of plasma corticosterone in subjects decapitated 24-hrs after training was similar to the level observed 1-hr after training. Thus, the improvement in avoidance performance between the 1- and 24-hr retention intervals was not paralleled by a rise in plasma corticosterone. King (1969) concluded from these results that a second variable must be acting to effect the improvement in performance between the 1- and 24-hr intervals. He proposed that U-shaped retention was a function of two factors: (a) the level of adrenal activity indexed by plasma corticosterone and (b) the cue value of olfactory material generated during original training. Each

factor was assumed to change over the 24-hr period after original conditioning: adrenal activity decreases from a high level at the end of original conditioning (0-hr interval) to a low basal level by the 2-hr interval, and remains at the basal value thereafter; the cue value of odor is low at the end of original conditioning, then increases in value between 6- and 24-hrs after conditioning. King further proposed that both the adrenal activity and the cue value of odor directly affect the retention of acquired fear, and when either factor is present at a high value, the retention of acquired fear is good. By transposing the changes in adrenal activity and olfactory cue value after avoidance training on top of one another, King found that the two functions formed a U-shaped curve, with the point at which both factors are at a low value falling between the 2- and 6-hr intervals. He proposed that after original avoidance training, performance decreases between the 0- and 2-hr interval as a result of decreasing adrenal activity and a constant low value of odor cues. Performance then improves between 6- and 24-hrs as a result of an increase in the cue value of odor, even though adrenal activity remains low throughout this period. To support his hypothesis, King (1969, Experiments 2 and 3) demonstrated that when the olfactory material is removed immediately after original avoidance training or when fresh olfactory material is placed in the apparatus just before the retraining session, the subjects fail to show the usual improvement in performance between 6- and 24-hrs after original conditioning. Thus, King retrained for his theory Brush and Levine's hypothesis of direct hypothalamic-pituitary-adrenal involvement and added an olfactory component to account for the improvement in performance by 24-hrs.

Although there is some evidence to support the theories that advocate a direct involvement of the hypothalamic-pituitary-adrenal system in the retention of aversively motivated behaviors, contrary results have also been reported. Kasper-Pandi, Hansing, and Usher (1970) obtained a U-shaped retention function when the corticosterone response was blocked by dexamethasone injections, and Marquis and Suboski (1969) reported the function in hypophysectomized rats. In the latter study, hypophysectomy eliminated changes in ACTH and corticosteroid levels which could not, therefore, have differentially affected retention performance. More recent evidence was provided by Snider, Marquis, Black, and Suboski (1971) in Sprague-Dawley rats, which have a minimum amount of extra-adrenal corticosterone. Thirty subjects underwent bilateral adrenalectomy and thirty received a sham operation 24 days before experimentation. Another thirty subjects were unoperated. After 30 active avoidance training trials, ten subjects from each operative condition were given 20 retraining trials after ISIs of either 1-min, 1-, or 24-hrs. The results for all three operative conditions showed that avoidance performance during retraining was a U-shaped function of the ISI.

Although the data of Kasper-Pandi et al. (1970), Marquis and Suboski (1969), and Snider et al. (1971) indicate that the hypothalamic-pituitary-adrenal system is not necessary for the U-shaped retention function, this system may, in fact, play a significant role in intact animals. When animals are subjected to surgical intervention or drug administration, normal functioning may be so altered that their subsequent behavior cannot be appropriately

compared with their pretreatment behavior. Moreover, behavior observed after such procedures often defies physiological specification. For example, deWied (1964) showed that the debilitating effect of hypophysectomy on learning can be completely reversed by either the administration of ACTH or a "cocktail" mixture (thyroxine, cortisone, testosterone). In addition, it is impossible to account for all the behavioral effects of a drug while it remains physiologically active in an organism. Perhaps a more critical test of the hypothalamic-pituitary-adrenal hypothesis would be to investigate the response of this system to stress as a function of time after aversive conditioning. At present, such experimentation has not been conducted and a final answer concerning the involvement of the hypothalamus and its activity, the pituitary and its hormones, or the adrenal gland and its steroids is yet to come.

A final hypothesis concerning the mechanisms involved in producing the U-shaped retention function was recently presented by Klein and Spear (1969; 1970a; 1970b) and Spear, Klein, and Riley (1971). In an initial experiment, Klein and Spear (1969) trained rats in a one-way active avoidance apparatus to a criterion of 5 successive avoidances. A flashing light CS and a 1.6 ma shock US were employed. The avoidance response occurred when the rat hurdled from a white to a black compartment before a 5-sec CS-US interval elapsed. After the fifth consecutive avoidance, all subjects were returned to their home cage to await retraining at ISIs of either: 0.08-, 1-, 4-, or 24-hrs. The retraining test, however, was not an active but a passive avoidance task,

which required the rat to remain for 60-sec in the white compartment where it had previously received shock. A hurdle response within the 60-sec period resulted in a 1-sec shock. It should be noted that the two tasks, active avoidance during original training and passive avoidance during retraining, require opposite or conflicting responses for the successful avoidance of shock. Therefore, the stronger the tendency to perform the active avoidance response during retraining, the slower the acquisition of the passive avoidance response.

The results of their experiment indicated that rats tested 0.08- and 24-hr after active avoidance training acquired the passive avoidance response significantly more slowly than naive rats tested at the same intervals but without previous active avoidance training. In other words, rats tested 1- or 4-hrs after active avoidance training acquired the passive avoidance response in about the same number of trials as naive rats and reliably faster than the 0.08- and 24-hr groups. To test whether the superior passive avoidance performance at the 1- and 4-hr retention intervals was due to a freezing response (Denny, 1971) or to a general physical debilitation (e.g., fatigue), the training sequence was reversed. In a second experiment, rats were first given passive avoidance training to a criterion of three consecutive 60-sec suppressions followed by active avoidance training under the same stimulus conditions employed in their first experiment. Again, the results indicated that the retraining task was acquired significantly faster at the 1- and 4-hr retention intervals than at the 0.08- and

24-hr intervals. Since the retraining response in the second experiment was an active response, the possibility that freezing or fatigue was involved in producing the effect was rejected.

Klein and Spear (1970a) hypothesized that these results can be accounted for in terms of "state-dependent learning." Essentially, the concept of state-dependent learning states that the memory of a learned task is dependent on the presence of the same internal stimulus cues as those present during the learning of the task. When an animal learns a task under a given set of internal stimuli, a change in or absence of these stimuli at the time of retraining results in failure to recall the original task. Klein and Spear proposed that after the original training session, the internal stimuli associated with that task were present at the 0.08- and 24-hr retention intervals, but were no longer present and could not be elicited at the intermediate retention intervals (1- and 4-hrs.). Accordingly, acquisition of the second task was most rapid at the 1- and 4-hr intervals because the memory of the original conflicting task was absent. However, because of the presence of the same internal stimuli at the 0.08- and 24-hr intervals as those during original training, the memory of the original task interfered with the performance of the conflicting retraining task.

The same interpretation was given to account for the U-shaped retention function: the slower relearning of a previously learned avoidance response at intermediate retention intervals (1- to 4-hrs) was the result of the absence

of the internal stimulus cues to which the response was conditioned, and the consequent failure to recall the original training experience. To test this hypothesis, Klein and Spear (1970b) gave rats active avoidance training following the same procedures previously employed (Klein and Spear, 1969), except that the second training session occurred at ISIs of either 10-min, 2.5-, or 24-hrs. Five minutes before retraining, half the subjects assigned to each interval were given 5 unsignalled shocks to initiate the internal physiological reactions and the associated internal stimuli that had accompanied original training. Presumably, the most salient internal stimuli were thought to be shock produced, and it was felt that unsignalled shock would result in stimuli similar to those produced by the signalled shock during original training.

As predicted, the groups that received unsignalled shocks showed no difference in the retention of the active avoidance response at the 10-min, 2.5-, and 24-hr intervals. Thus, the shocks eliminated the decrement in performance usually observed at the 2.5-hr retention interval. Furthermore, this study revealed that the unsignalled shocks impaired the acquisition of a passive avoidance response at the 2.5-hr interval. These results were interpreted to mean that the unsignalled shocks improved the retention of an active avoidance response and impaired the learning of a passive avoidance response at the 2.5-hr interval because they reactivated the internal stimulus cues conditioned to the original active avoidance response. Klein and Spear hypothesized that at least part of the complex of internal stimuli reactivated by the shocks was produced by the hypothalamic-pituitary-adrenal system.

EXPERIMENT I

Klein and Spear (1970a) have proposed that during the learning of an avoidance response, the shocks associated with training result in physiological reactions which provide internal cues that become conditioned to the avoidance response. Moreover, the tendency to perform the avoidance response at a later time depends in a major way on the presence of these shock-produced cues. To account for the U-shaped retention function, they suggested that at intermediate retention intervals after avoidance training, the retraining shocks do not produce the same internal cues as those produced during original training. Thus, retraining at these intervals results in poor avoidance performance because many of the internal cues to which the avoidance response was conditioned during original training are absent. According to this hypothesis, a U-shaped retention function should also occur after unsignalled shocks interpolated between original training and retraining. This follows since the unsignalled shocks should act in the same way as the shocks associated with training in their effect on the internal state of the subject. Thus, retraining at intermediate intervals after unsignalled shock would result in different internal cues from those produced during original training.

Only one experiment (Denny, 1958) has provided data relevant to this question. In that study, rats were given 25 active avoidance training trials, then returned to their home cages for 23-hrs. At the end of this interval, the

subjects were placed in a different apparatus from that of original training (a clear plexiglas, single compartment box), and given a short series of unsignalled shocks. One hour after these shocks (24-hrs after original training), all subjects were tested for retention of the active avoidance response. The results indicated that retention was as poor as that of animals tested 1-hr after original training. Although these results provide some support for the hypothesis that a U-shaped retention function occurs after unsignalled shocks, Denny's study was never published and so it is difficult to assess the reliability of his results. Moreover, Denny used only one retention interval which does not warrant the conclusion that avoidance performance after the unsignalled shock is a U-shaped function of time. For example, exposure to unsignalled shock could permanently suppress avoidance responding, as was demonstrated under somewhat similar circumstances by Seligman and Maier (1967).

The purpose of Experiment I was to measure the retention of an avoidance response at several intervals after a series of unsignalled shocks given 23-hrs after original avoidance training. Retention was measured by 25 retraining trials presented at intervals of 0.08-, 1-, 2-, 4-, or 24-hrs after the unsignalled shocks. The findings have a bearing on the state-dependent hypothesis advanced by Klein and Spear (1970a), since their hypothesis predicts that such a retention function would be U -shaped. In addition, the experiment was designed to provide detailed information about the nature of avoidance

responding after both original training and unsignalled shock. These data have not been previously presented in such a way as to allow an examination of specific response differences between subjects that maintain high levels of avoidance responding during retraining and subjects that do not.

METHOD

Subjects

The subjects were 165 experimentally naive, male, Sprague-Dawley albino rats obtained from Simonsen Laboratories (Gilroy, California). All subjects were 50 to 60 days old, weighing 200 to 220 grams at the time of original training, and were individually housed under conditions of ad libitum food and water and continuous illumination.

Apparatus

The subjects were trained in a shuttlebox 16 in long, 5.5 in wide, and 9 in high. The shuttlebox was made of aluminum and divided into two identical compartments by a center partition. A 3 x 3 in top-hinged swinging door permitted passage between compartments at any time. The CS was the onset of a 6-watt incandescent light and white noise at 54-db in the compartment occupied by the subject. The US was a .32 ma electric shock delivered to a grid floor made of 3/16 in stainless steel rods spaced 7/8 in apart. The shock was generated by a high-voltage, high-resistance circuit and scrambled across the grid floor 10/sec. The stimulus events were programmed automatically by equipment located in a room adjacent to the experimental chambers. Basically two photocell units, one located in each compartment of the shuttlebox, were used to detect a subject's presence in one side or the other. Two horizontally mounted solenoids wired to the output of each photocell unit

pulled an armature back and forth as a subject moved from one compartment to the other. During each change of state, a microswitch was momentarily closed, providing an impulse for counting the number of crossings, and for terminating the CS and US at the time of the response. The movement of the armature also opened or closed a second microswitch. This switch was used for selectively presenting the CS to the compartment occupied by the subject (see Brush and Knaff, 1959, for circuitry details). Two identical experimental units were used, each housed in a sound shielded chamber equipped with a fan to provide adequate ventilation and to mask extraneous sounds which might penetrate the subject's compartment. Printing counters recorded the latency ($\pm$ 0.1-sec) of the first response after the CS onset.

The subjects were given unsignalled shock in a 10 in square plexiglas box with a grid floor made of 1/8 in stainless steel rods spaced 5/8 in apart. The construction of this apparatus was as different as possible from the avoidance training apparatus to minimize stimulus generalization from one situation to the other. The characteristics and source of shock in the unsignalled shock apparatus were the same as in the shuttleboxes.

Procedure

Original Avoidance Training. All subjects were trained in the following manner. Each subject was placed in one side of the shuttlebox and allowed a 2-min interval to acclimate to the apparatus. After this period, 10 pretest trials were given during which only the CS was presented. Each trial was

separated by a 2-min interval and the CS terminated either when the subject ran to the opposite compartment or after remaining on for 45-sec. If during the pretest trials a subject responded during the first 5-sec of the CS on more than 5 of the 10 trials or on 3 of the last 5, it was discarded. Immediately after the 10 pretest trials and without removing the subject from the apparatus, 40 active avoidance training trials were presented in which a 5-sec CS-US interval and a 1-min intertrial interval were used. During each trial, the subject could avoid the shock and simultaneously terminate the CS by running to the opposite compartment during the 5-sec CS-US interval; the same response occurring after the CS-US interval escaped the shock and simultaneously terminated the CS.

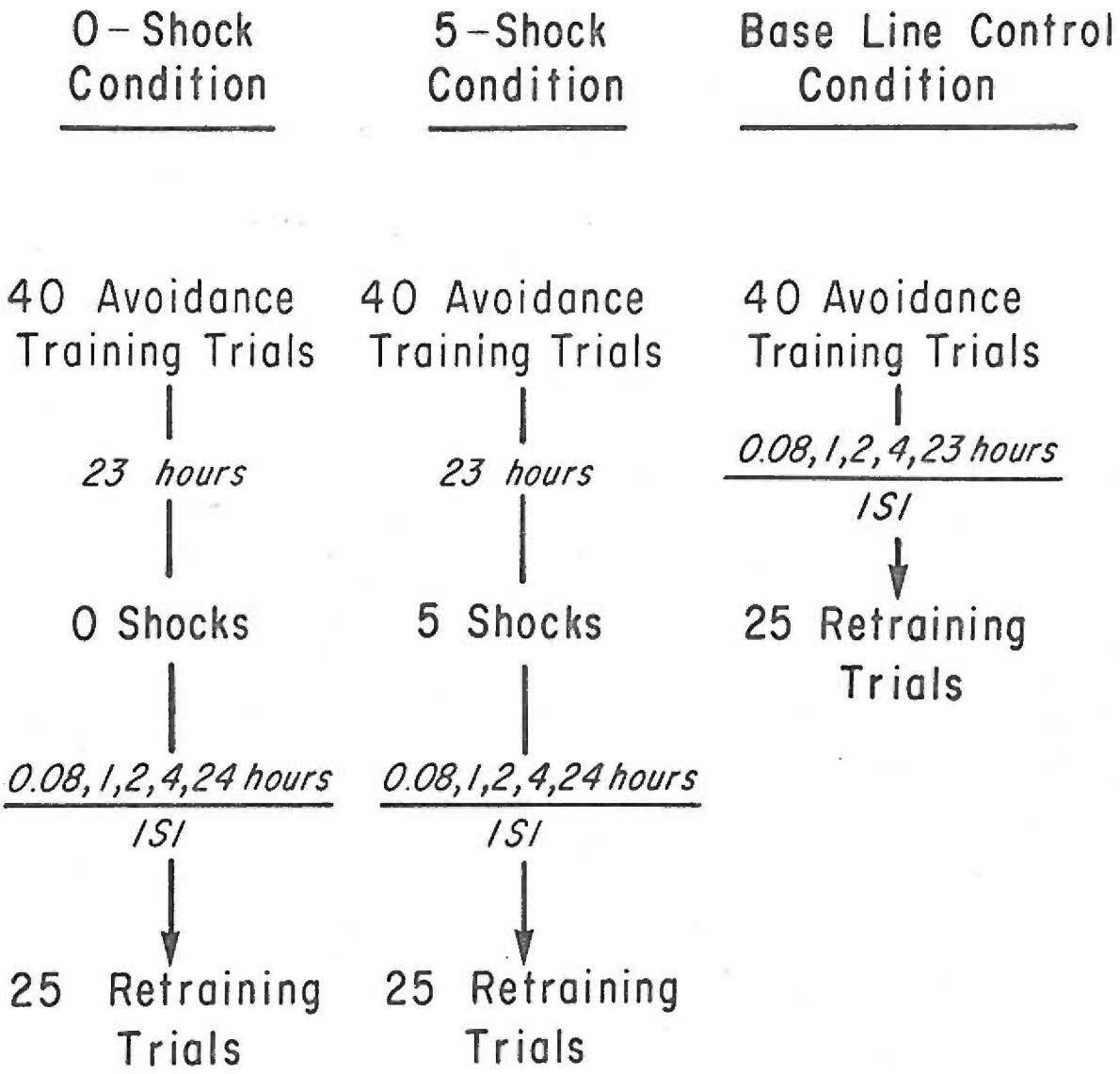
Intertrial responses from one compartment to the other were monitored so that on each trial the CS was presented in the compartment occupied by the subject. These intertrial responses had no effect on the time course of training. After completion of the training session, all subjects were returned to their home cages. To increase the probability of avoidance responding during the second training session, subjects that failed to make 3 or more avoidance responses during the 40 original training trials were discarded.

Retraining. After original avoidance training, the subjects were divided into 15 groups of 11 subjects per group. Ten of the 15 groups represented a 2 x 5 factorial design in which the factors were (a) Shocks (0 or 5) and (b) ISI (0.08-, 1-, 2-, 4-, or 24-hrs). The training

procedures for the 0- and 5-shock condition groups are shown in Figure 2. As indicated in this figure, 23-hrs after original training the subjects were placed in the unsignalled shock apparatus and given either 0 or 5 shocks. The interval between shocks was 1-min and each shock was 1-sec in duration. It is important to note that this training experience did not occur until 23-hrs after original avoidance training. Immediately after the final shock or after 5-min in the apparatus, the subjects were returned to their home cages. When the appropriate ISI had elapsed, subjects were placed in the avoidance training apparatus and given 25 retraining trials to measure the retention of the avoidance response. The procedures used in the retraining session were identical to those of original training, except the pretest trials were not presented.

The training procedures for the remaining 5 groups, designated base line control, are also shown in Figure 2. At the end of original training, the subjects in this condition were returned to their home cages for one of the following ISIs: 0.08-, 1-, 2-, 4-, or 23-hrs. When the appropriate ISI had elapsed, subjects were given 25 retraining trials to measure the retention of the avoidance response. The procedures used in the retraining session were identical to those of original training, except the pretest trials were omitted. The base line control condition was a replication of the training procedures that have typically produced U-shaped retention. This condition was included in the present study to provide a retention function against which to compare the retention function after unsignalled shocks.

Figure 2 Time relations between the 0-shock, 5-shock, and base line control groups with respect to original training, retraining, and interpolated shock.


0 - Shock Condition
5 - Shock Condition
Base Line Control Condition
40 Avoidance Training Trials
40 Avoidance Training Trials
40 Avoidance Training Trials
23 hours
23 hours
0.08, 1, 2, 4, 23 hours
ISI
0 Shocks
5 Shocks
25 Retraining Trials
0.08, 1, 2, 4, 24 hours
ISI
0.08, 1, 2, 4, 24 hours
ISI
25 Retraining Trials
25 Retraining Trials

RESULTS

Original Training Phase. The left side of Figures 3, 4, and 5 shows the mean number of avoidance responses in blocks of five trials made during original training by the 5-shock, 0-shock, and base line control groups, respectively. Inspection of each figure indicates that all groups acquired the avoidance response at approximately the same rate and showed an increase in the number of avoidance responses over trials. The mean number of avoidance responses over the 40 training trials for 5-shock, 0-shock, and base line control conditions were 15.6, 15.3, and 15.6, respectively. A 3 x 5 x 8 factorial analysis of variance in which the factors were (a) Condition (0-shock, 5-shock, base line control), (b) ISI (0.08-, 1-, 2-, 4-, or 24-hrs), and (c) Trials (8 blocks of 5 trials) was performed on the data shown in the three figures. The results of this analysis revealed that the only significant effect was Trials ($F = 149.20$, $df = 7/1050$, $p < .01$). Thus, the mean performance levels of the various groups were not significantly different and the rates at which the groups acquired the avoidance response during original training were not significantly different.

Retraining Phase. The right side of Figures 3 and 4 shows the mean number of avoidance responses in blocks of five trials made during the retraining session for each group in the 5-shock and 0-shock condition, respectively.

Figure 3 Mean number of avoidance responses for each 5-shock group as a function of five trial blocks during original training and retraining. The five curves represent the scores of the 0.08-, 1-, 2-, 4-, and 24-hr ISI groups.

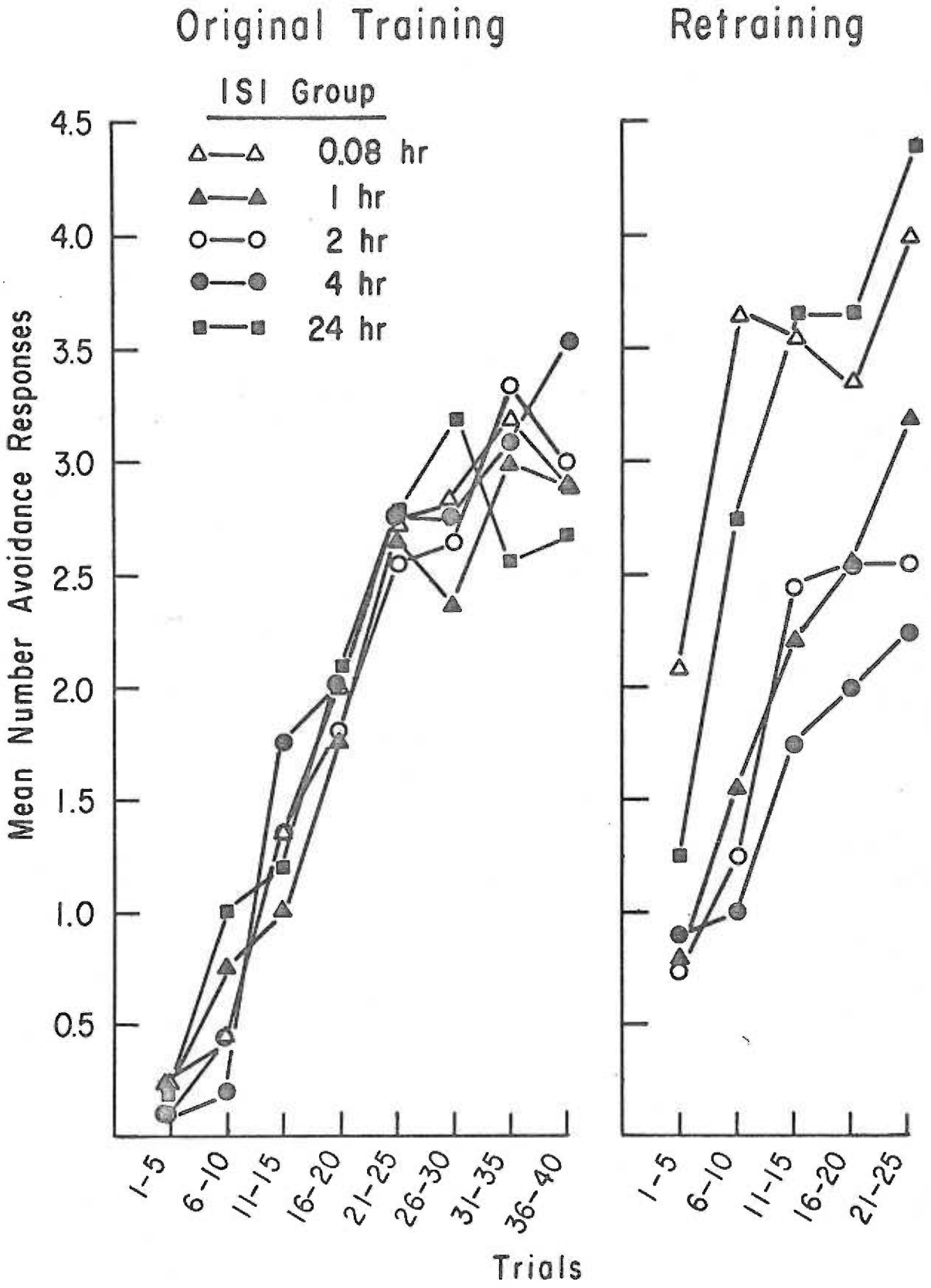

Original Training
Retraining
ISI Group
0.08 hr
1 hr
2 hr
4 hr
24 hr
Mean Number Avoidance Responses
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1-5
6-10
11-15
16-20
21-25
26-30
31-35
36-40
Trials

Figure 4 Mean number of avoidance responses for each 0-shock group as a function of five trial blocks during original training and retraining. The five curves represent the scores of the 0.08-, 1-, 2-, 4-, and 24-hr ISI groups.

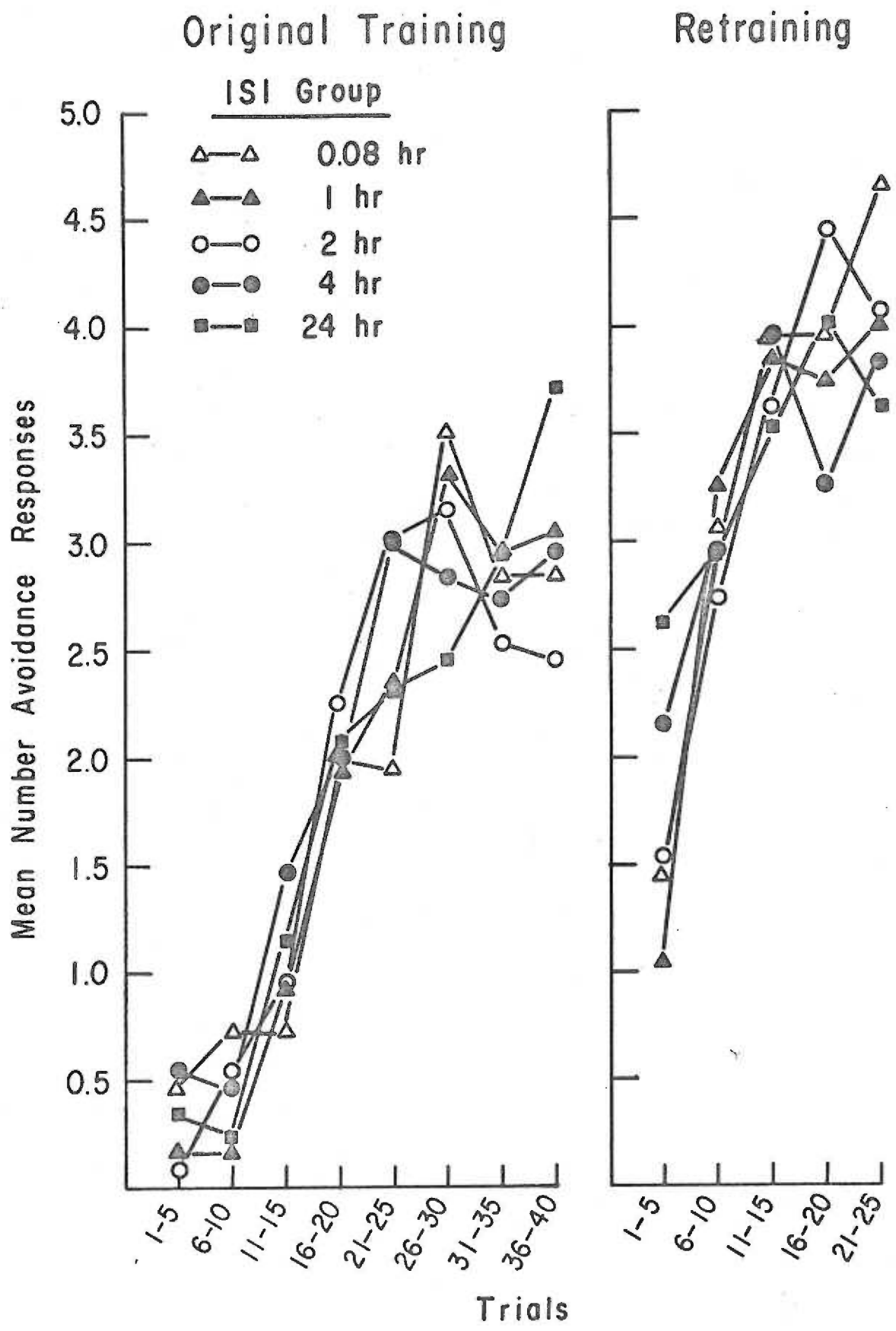
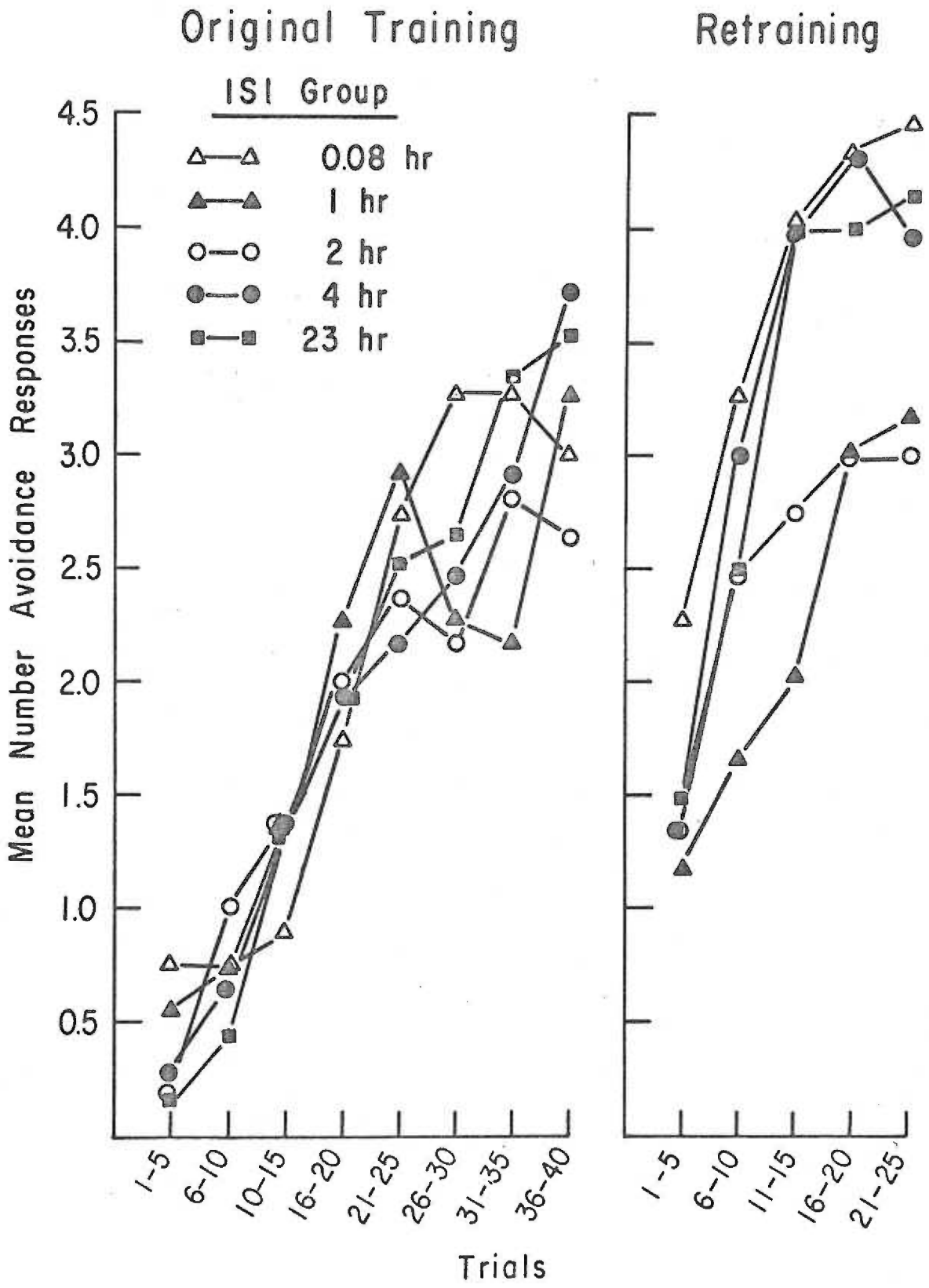

Original Training
Retraining
ISI Group
0.08 hr
1 hr
2 hr
4 hr
24 hr
Mean Number Avoidance Responses
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1-5
6-10
11-15
16-20
21-25
26-30
31-35
36-40
Trials

Figure 5 Mean number of avoidance responses for each base line control group as a function of five trial blocks during original training and retraining. The five curves represent the scores of the 0.08-, 1-, 2-, 4-, and 23-hr ISI groups.


Original Training
Retraining
ISI Group
0.08 hr
1 hr
2 hr
4 hr
23 hr
Mean Number Avoidance Responses
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1-5
6-10
10-15
16-20
21-25
26-30
31-35
36-40
11-15
Trials

Inspection of Figure 3 reveals that all 5-shock groups showed an increase in avoidance responding over trials, but that the 0.08- and 24-hr ISI groups attained a higher level of responding at the end of retraining than the 1-, 2-, and 4-hr ISI groups. The data in Figure 4 indicate that the 0-shock groups also showed an increase in response rate over trials, but without interpolated shock, all groups attained the same level of responding by the end of retraining. Thus, groups tested for the retention of an avoidance response 1-, 2-, and 4-hrs after interpolated shock made fewer avoidance responses than groups tested after the same intervals without prior shock. These visually apparent findings were tested in an overall analysis of variance comparing the 0- and 5-shock groups. The analysis revealed a highly significant effect of Shocks ($F = 17.79$, $df = 1/100$, $p < .01$), a significant effect of ISI ($F = 3.23$, $df = 4/100$, $p < .05$), and a significant Shocks x ISI interaction ($F = 2.51$, $df = 4/100$, $p < .05$). These results indicate that differences between the 0- and 5-shock groups in the number of avoidance responses during retraining varied as a function of the retraining interval. The analysis also revealed a significant effect of Trials ($F = 64.80$, $df = 4/400$, $p < .01$), which confirms the above observation that a reliable increase in avoidance responding occurred over the 25 retraining trials.

Since the performance differences between the 0- and 5-shock groups during retraining varied as a function of the ISI, individual F tests were used to compare the groups at each interval. The outcome of these tests revealed

a significant difference between the 0- and 5-shock groups in mean performance at the 1- ($F = 6.18$, $df = 1/100$, $p < .01$), 2- ($F = 8.06$, $df = 1/100$, $p < .01$), and 4-hr ($F = 12.22$, $df = 1/100$, $p < .01$) intervals. There was no significant difference in mean number of avoidance responses at the 0.08- ($F = 1$, $df = 1/100$, $p > .05$) and 24-hr ($F = 1.12$, $df = 1/100$, $p > .05$) intervals.

Figure 6 summarizes the data from Figures 3 and 4 to show the mean number of avoidance responses collapsed across the 25 retraining trials for each ISI group in the 0- and 5-shock conditions. Inspection of this figure indicates that the significant difference in avoidance performance found between the 0- and 5-shock group at the 1-, 2-, and 4-hr intervals resulted from a performance decrement in the 5-shock group. Whereas the 0-shock groups showed little change in avoidance responding as a function of the retention interval, the 5-shock groups showed a substantial decrease in mean number of avoidance responses between the 0.08- and 4-hr intervals, followed by an increase between the 4- and 24-hr intervals. Individual F tests comparing the mean performance levels of the 5-shock groups are shown in Table 1. The outcomes of these tests reveal that the performance levels of the 1-, 2-, and 4-hr ISI groups were significantly below that of the 0.08- and 24-hr ISI groups. There were no significant differences between the 1-, 2-, and 4-hr groups, or between the 0.08- and 24-hr groups.

Looking at just the last 5 trials of retraining (21-25), Figures 3 and 4 show that the performance levels of the 2- and 4-hr ISI groups given 5 shocks

Figure 6 Mean number of avoidance responses during retraining for each group in the 0-shock and 5-shock conditions as a function of the intersession interval.

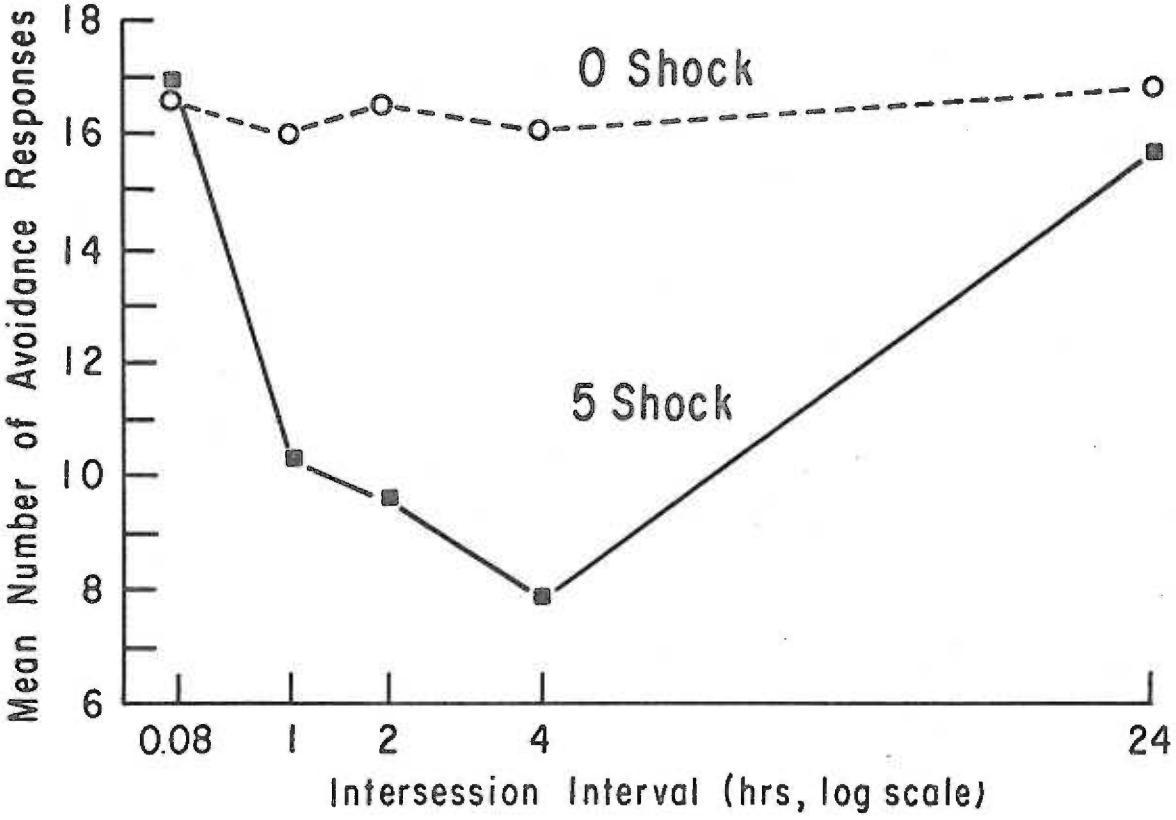

Mean Number of Avoidance Responses
18
16
14
12
10
8
6
0 Shock
5 Shock
0.08
1
2
4
24
Intersession Interval (hrs, log scale)

Table 1 Results of individual F tests on avoidance responses for the 5-shock groups collapsed across subjects and trials. Tabulated are the computed F values and the level of significance attained (** = $p < .01$; * = $p < .05$).

Table 1

Individual Comparisons

5-shock group	0.08-hr	1-hr ISI	2-hr ISI	4-hr ISI	24-hr ISI
0.08-hr ISI	-	6.53*	8.04**	12.01**	1.00
1-hr ISI	-	-	1.00	1.00	4.27*
2-hr ISI	-	-	-	1.00	5.50*
4-hr ISI	-	-	-	-	8.85**
24-hr ISI	-	-	-	-	-

** = $p < .01$ * = $p < .05$ df for all pairs = 1/50

were substantially below the levels of the same ISI groups in the 0-shock condition. A factorial analysis of variance comparing the 0- and 5-shock groups over the last 5 trials of retraining revealed a significant effect of Shocks ($F = 5.87$, $df = 1/100$, $p < .05$) and a significant Shocks x ISI interaction ($F = 2.48$, $df = 4/100$, $p < .05$). Individual F tests indicated that a significant performance difference existed between the 0- and 5-shock groups at the 2- ($F = 6.23$, $df = 1/100$, $p < .05$) and 4-hr ($F = 6.23$, $df = 1/100$, $p < .05$) intervals. Additional F tests revealed significant differences in performance over the last 5 retraining trials between the 5-shock groups retrained 0.08- and 24-hrs and those retrained 2- and 4-hrs after shock (2- vs. 0.08-hr, $F = 5.51$, $df = 1/100$, $p < .05$; 2-vs. 24-hr, $F = 8.62$, $df = 1/100$, $p < .01$; 4- vs. 0.08-hr, $F = 7.78$, $df = 1/100$, $p < .01$; 4- vs. 24-hr, $F = 11.40$, $df = 1/100$, $p < .01$). There were no significant differences between the 0-shock groups in performance over the last 5 retraining trials.

The regression of the mean avoidance scores on retention interval for the 5-shock groups was tested and found to depart significantly from linearity ($p < .01$). This analysis revealed that retention of the avoidance response was a curvilinear function of the retention interval with a significant quadratic component ($p < .01$). The regression analysis of the 0-shock groups indicated that retention of the avoidance response was a significant linear function of the retention interval ($p < .01$).

These results demonstrate that the retention of an avoidance response in groups given 5 unsignalled shocks 23-hrs after original training is a

U-shaped function of time after shock. When shocks are not interpolated between original training and retraining, retention of an avoidance response is a constant linear function. Taken together, these results indicate that experience with shock 23-hrs after the acquisition of an avoidance response is sufficient to produce a U-shaped retention function for that response.

The right side of Figure 5 shows the mean number of avoidance responses during retraining for each group in the base line control condition. Inspection of this figure indicates that all groups showed an increase in response rate over trials, but that the 0.08-, 4-, and 23-hr ISI groups attained a higher avoidance rate by the end of retraining than the 1- and 2-hr ISI groups. An analysis of variance revealed a highly significant effect of ISI ($F = 5.38$, $df = 4/50$, $p < .01$), which indicates that retraining performance varied as a function of the retraining interval. The analysis also revealed a significant effect of Trials ($F = 35.49$, $df = 4/200$, $p < .01$), confirming the above observation that an increase in response rate over trials was present. None of the remaining sources of variation were significant.

Figure 7 summarizes the data in Figure 5 to show the mean number of avoidance responses collapsed across the 25 retraining trials for each ISI group in the base line control condition. This figure indicates that a decrement in performance occurred at the 1- and 2-hr intervals compared to the remaining intervals. Individual F tests comparing the base line control groups at each ISI confirmed the above observation. The outcome of these tests, shown in Table 2, revealed a significant decrease in the mean number of avoidance responses between 0.08- and 1-hr after original training, followed

Figure 7 Mean number of avoidance responses during retraining for each group in the base line control condition as a function of the intersession interval.

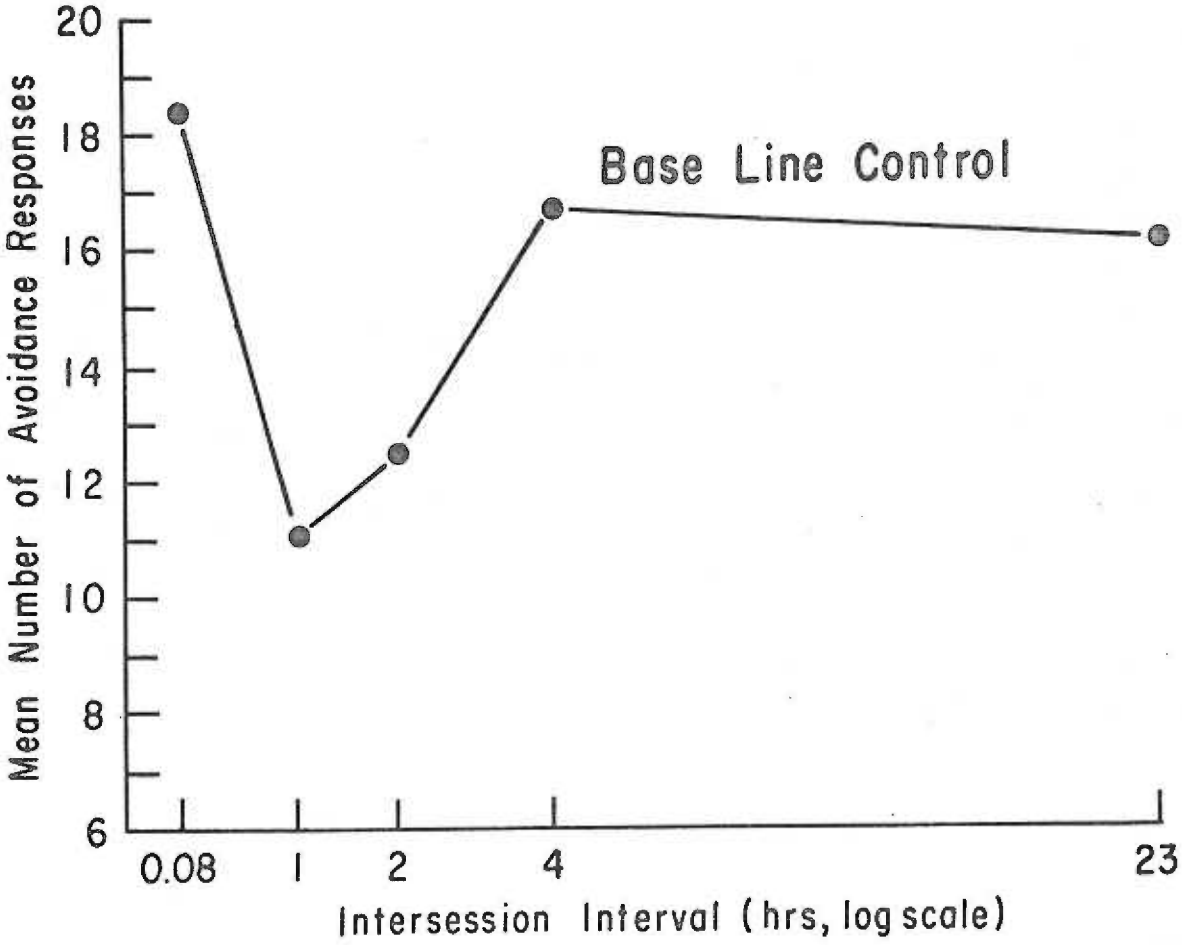

Mean Number of Avoidance Responses
20
18
16
14
12
10
8
6
Base Line Control
0.08
1
2
4
23
Intersession Interval (hrs, log scale)

Table 2 Results of individual F tests on avoidance responses for the base line control groups collapsed across subjects and trials. Tabulated are the computed F values and the level of significance attained (** = $p < .01$; * = $p < .05$).

Table 2

Individual Comparisons

base line control group	0.08-hr ISI	1-hr ISI	2-hr ISI	4-hr ISI	23-hr ISI
0.08-hr ISI	-	15.37**	10.15**	1.00	1.00
1-hr ISI	-	-	1.00	9.23**	7.53**
2-hr ISI	-	-	-	5.30*	4.04*
4-hr ISI	-	-	-	-	1.00
23-hr ISI	-	-	-	-	-

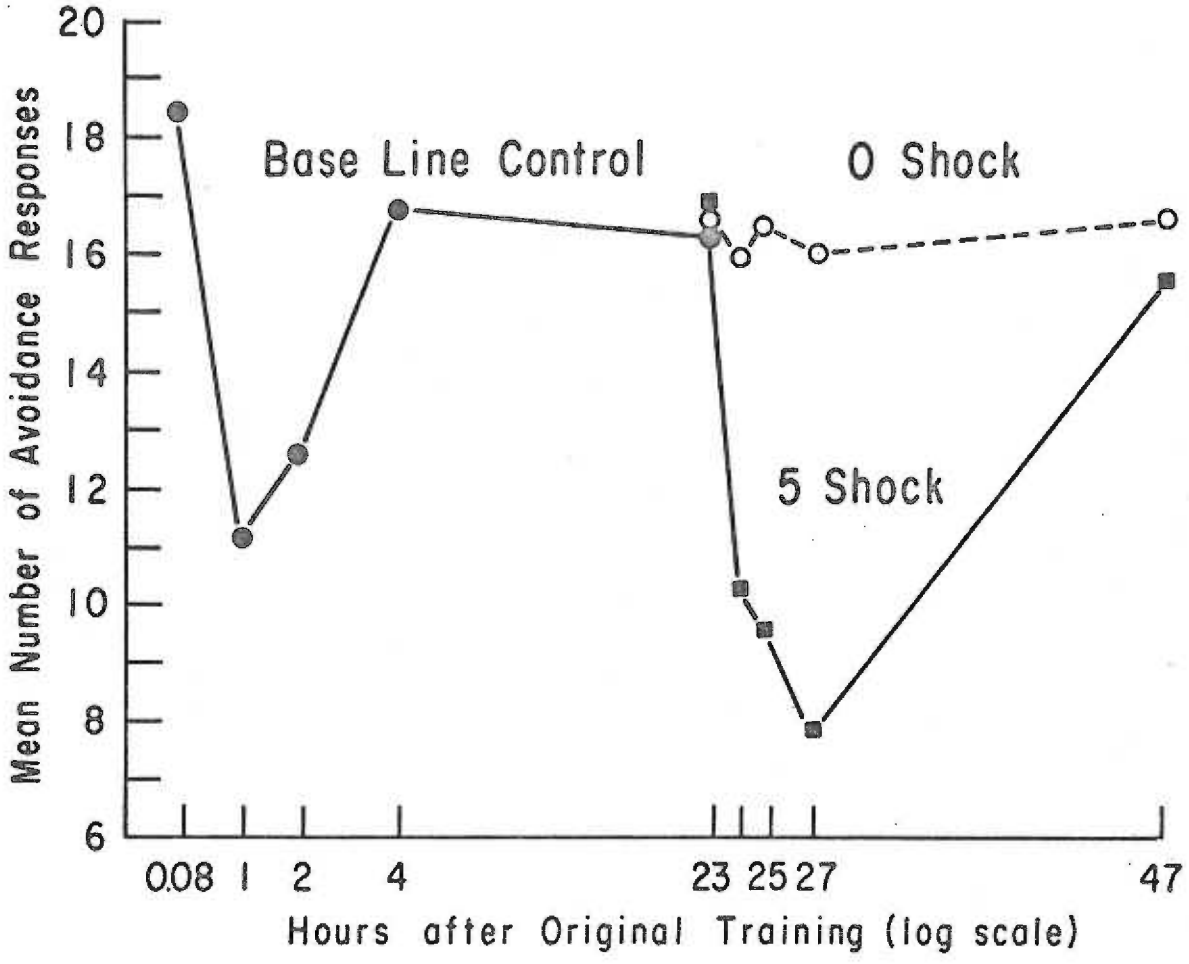
** = $p < .01$ * = $p < .05$ df for all pairs = 1/50

by a significant increase between the 2- and 4- intervals. There were no significant differences between the 1- and 2-hr ISI groups, nor between the 0.08-, 4-, and 23-hr groups. A regression analysis indicated that avoidance performance during retraining was a curvilinear function with significant quadratic ($p < .01$) and cubic ($p < .01$) components.

Figure 8 summarizes the data from Figures 6 and 7 to show the mean number of avoidance responses during retraining for each group as a function of hours after original training. Inspection of this figure clearly shows that the critical variable controlling the retention of the learned avoidance response was time since shock, not time since original training. Moreover, the retention function after original training differed considerably from the retention function after interpolated shock. First, a decrement in performance occurred only at the 1- and 2-hr retention intervals in the base line control condition, but a decrement continued through the 4-hr interval after interpolated shock. Second, the interval of minimum retention after interpolated shock shifted from that observed after original training. However, differences in the mean number of avoidance responses were not found between the 1- and 2-hr ISI groups in the base line control condition or the 1-, 2-, and 4-hr ISI groups in the 5-shock condition.

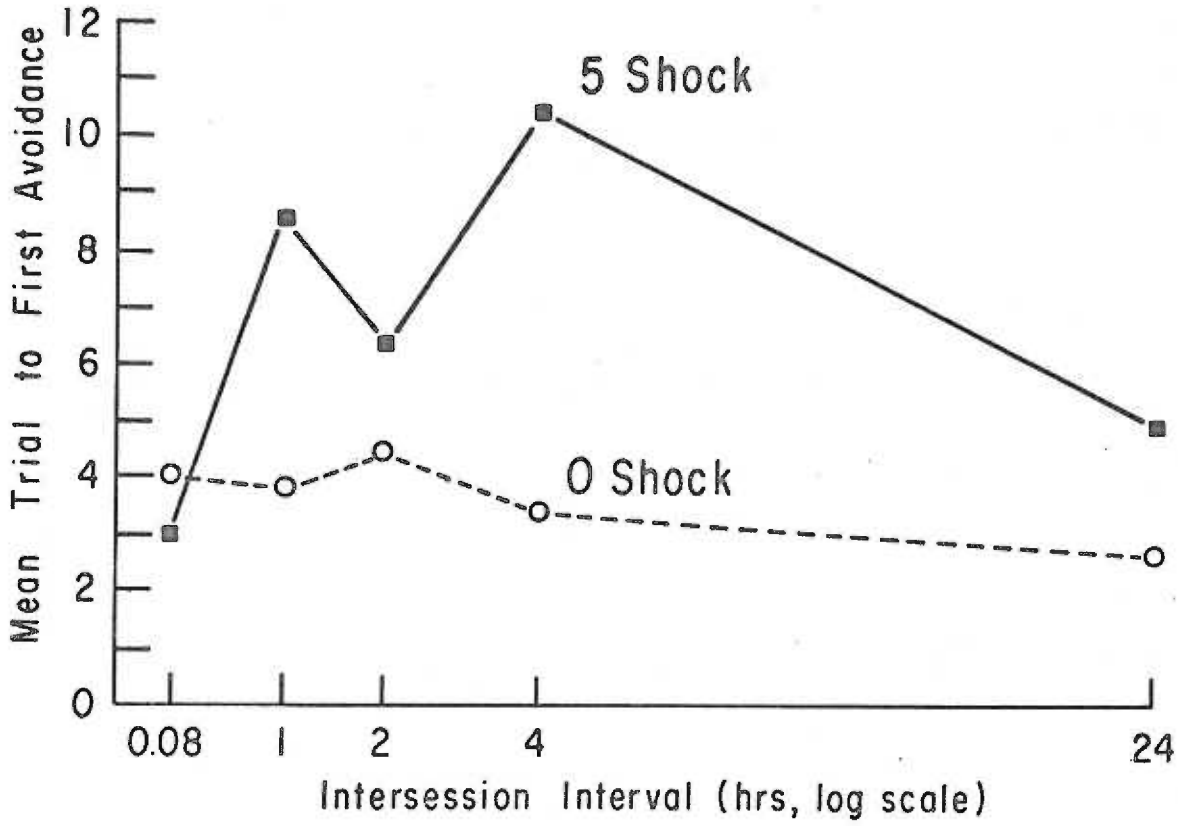
Several interpretations of the U-shaped retention function implicitly assume that avoidance performance is impaired during the early trials of retraining at intermediate intervals (e.g., Brush and Levine, 1966; Kamin, 1957).

Figure 8 Mean number of avoidance responses during retraining for each group in the 0-shock, 5-shock, and base line control conditions as a function of hours after original training (23-hrs = 0.08-hr ISI in the 0- and 5-shock conditions; 24-hrs = 1-hr ISI; 25-hrs = 2-hr ISI; 27-hrs = 4-hr ISI; and 47-hrs = 24-hr ISI).


Mean Number of Avoidance Responses
20
18
16
14
12
10
8
6
Base Line Control
0 Shock
5 Shock
0.08
1
2
4
23
25
27
47
Hours after Original Training (log scale)

Therefore, it was thought possible that the 0- and 5-shock groups retrained after intermediate intervals would differ in terms of the mean number of trials required to make the first avoidance response. Figure 9 shows the trial data for the 0-shock and 5-shock groups as a function of ISI. Inspection of this figure indicates that the subjects in the 5-shock condition generally required more trials to make their first avoidance response during the retraining session than subjects in the 0-shock condition. A factorial analysis of variance on the data from Figure 9 revealed a highly reliable effect of Shocks ($F = 10.40$, $df = 1/100$, $p < .01$), confirming the above observation. Since the effect of ISI ($F = 2.08$, $df = 4/100$, $0.05 < p < .10$) and the Shocks x ISI interaction ($F = 2.16$, $df = 4/100$, $0.05 < p < .10$) approached significance, individual F tests comparing the groups at each ISI were performed. The outcomes revealed a significant difference between the 0- and 5-shock groups in mean trial to first avoidance at the 1- ($F = 4.78$, $df = 1/100$, $p < .05$) and 4-hr ($F = 11.93$, $df = 1/100$, $p < .01$) intervals. These results indicate that one contributing factor to the poor performance of the 5-shock groups at these intervals was the subject's inability to begin avoiding.

Figure 9 Mean trial to first avoidance response during retraining for the 0-shock and 5-shock groups as a function of the intersession interval.


Mean Trial to First Avoidance
12
10
8
6
4
2
0
5 Shock
0 Shock
0.08
1
2
4
24
Intersession Interval (hrs, log scale)

DISCUSSION

The principal finding of Experiment I was that when 5 shocks are interpolated between original training and retraining, the retention of an avoidance response is a curvilinear function of time after the shocks. In contrast, when 0 shocks are given between original training and retraining, retention performance is a constant linear function of time. The curvilinear retention function was characterized by a significant decrement in avoidance responding at the 1-, 2-, and 4-hr intervals. Retention at these intermediate intervals was significantly poorer than that of groups retrained 0.08- and 24-hrs after interpolated shock. Whereas all of the groups that had been given 5 interpolated shocks showed the same rate of increase in avoidance responding over trials, the groups tested after intermediate intervals failed to increase to the same performance level as that attained by the 0.08- and 24-hr ISI groups. These data suggest that the process(es) that mediate the poor performance after intermediate intervals remain active at least through 25 retraining trials. Since retraining was terminated after 25 trials, it is not possible to determine how many trials the 1-, 2-, and 4-hr ISI groups would have required to reach the performance level of the 0.08- and 24-hr ISI groups. However, the retraining curves of the intermediate interval groups indicate that performance may have reached an asymptote, at least temporarily, and that a considerable number of additional trials would have been necessary.

The retention performance of the 0-shock and 5-shock groups differed in a number of ways. First, the avoidance response rates at the end of retraining were grossly different at the 2- and 4-hr retention intervals. Whereas the 0- and 5-shock groups retrained after these intervals showed the same rate of increase in avoidance responding over trials, the 0-shock groups attained significantly higher performance levels by the end of retraining. Second, the groups retrained 1- and 4-hrs after 5 interpolated shocks required more trials to perform their first avoidance response than the 0-shock groups retrained after the same intervals. Thus the process(es) responsible for the performance decrements at intermediate intervals were probably present at the beginning of the retraining session and did not develop as a consequence of the retraining experience.

The retraining performance of the base line control groups was similar to that previously reported in a number of studies (e.g., Kamin, 1957; Denny, 1958; Brush, 1964). Retention after original training was a curvilinear function of the retraining interval, with a substantial decrease in mean number of avoidance responses present at the 1- and 2-hr retention intervals.

The critical variable controlling retention of a learned avoidance response was time since shock, not time since original training. Moreover, the effect of shock, whether given during original training or interpolated between original training and retraining, was to impair rather than facilitate retention performance after intermediate intervals. Apparently this debilitating

effect is peculiar to shock, since U-shaped retention follows only those conditioning procedures that use shock to motivate behavior, and not conditioning procedures that use appetitive motivators (cf. Skinner, 1950; Brush, 1971).

Although the retention performance of the base line control groups that had been given only original training and that of the groups given 5 interpolated shocks were found to be a U-shaped function of the retraining interval, the two functions differed in a number of ways. First, the retention function after interpolated shock was quadratic, whereas the function after original training had both a quadratic and a cubic component. Second, significant differences in terms of trials to the first retraining avoidance response were found between the groups given interpolated shock but not between the groups given only original training. Third, performance decrements were present at the 1-, 2-, and 4-hr retention intervals after interpolated shock, but only at the 1- and 2-hr intervals after original training. These differences in retraining performance may have resulted from the fact that the shocks during original training could be escaped, whereas the interpolated shocks were inescapable. Weiss, Stone, and Harrell (1970) reported that when rats were exposed to escapable shock, alterations in brain chemistry were present 40-min later and that these alterations did not occur if the shocks were inescapable. These data indicate that whether or not an animal has the opportunity to control its exposure to shock has a significant effect on the physiological responses resulting from that experience. Comparable differences in physiological activity could have

affected retention performance after the escapable and inescapable shocks in the present study.

The finding that shocks interpolated between the acquisition of an avoidance response and a retraining session produce a U-shaped retention function is relevant to several current theories of retention. One interpretation of the curvilinear retention function is based on the combined effect of adrenal activity and olfactory cues. King (1969) proposed that avoidance performance immediately after acquisition (5- to 10-min) is good because of high adrenal activity. As adrenal activity gradually decreases between 0 and 2-hrs after acquisition, avoidance performance becomes impaired. After 6-hrs the cue value of olfactory material generated during original training begins to increase, and avoidance performance improves. Such an interpretation, however, cannot account for the results of the present experiment without a number of additional assumptions. It will be recalled that the retraining session of the 1-hr ISI group that had received interpolated shock occurred 24-hrs after original training. According to King's theory, the high cue value of olfactory material in the shuttleboxes at this interval should have been sufficient to produce good avoidance performance, even though the effect of the interpolated shock was to reduce the activity of the adrenal system. The performance level of this group, however, was significantly lower than that of a control group that did not receive interpolated shock. Either the relationship between adrenal activity and olfactory cue value is more complicated than King has proposed or

none exists that directly relates to retention after interpolated shock.

A second theory, proposed by Brush and Levine (1966), interprets the curvilinear retention function as the direct consequence of a decrease in plasma corticosterone observed at intermediate intervals in rats that had received fear conditioning. According to this hypothesis, the low plasma corticosterone levels interfered with the rat's ability to cope with the stress of retraining. However, Brush and Levine also found that a decrease in plasma corticosterone was not accompanied by a decrement in avoidance performance in rats given unsignalled shocks before avoidance training. To account for this apparent discrepancy, they suggested that both low levels of plasma corticosterone and fear conditioning are necessary for the decrement in avoidance performance at intermediate intervals. In contrast, the present study found that unsignalled shocks given after avoidance training led to a decrement in the retention of an avoidance response at intermediate intervals. Taken together, the results of Brush and Levine's study and those of the present study suggest that unsignalled shocks affect the stimulus-response associations involved in the occurrence of an avoidance response, rather than the motivation to perform that response. Moreover, unsignalled shocks apparently cannot impair the formation of these stimulus-response associations, but can disrupt the retention of these associations.

The theoretical position that best accounts for U-shaped retention function with the interpolated shock procedure is the state-dependent hypothesis (Klein and Spear, 1970a). According to this hypothesis, the critical effect

of the interpolated shock is to initiate internal physiological reactions similar to those produced by shock during original training. In both cases, the internal state is different at the intermediate intervals. Thus, retraining at intermediate intervals after interpolated shock results in poor avoidance performance because many of the internal cues to which the avoidance response was conditioned are absent.

The state-dependent hypothesis can also account for the fact that unsignalled shocks do not affect avoidance performance when presented at various times before original training (Brush and Levine, 1966). In this case, the avoidance response is simply conditioned to the internal physiological cues present at the time of original learning, and therefore no differences in performance would be expected. On the other hand, once the avoidance response has become conditioned to specific internal cues, a change in these cues results in poor retention of that response.

The nature of the internal physiological reactions and the mechanisms by which they change over time are not specified in detail by the state-dependent hypothesis. However, there are several physiological reactions to stress that demonstrate the properties necessary to mediate the U-shaped retention function. Gold, Altschuler, Kleban, Lawton, and Miller (1969) reported that 2-hrs after escape training, subjects showed an increase in brain protein and a decrease in nonprotein nitrogen of the brain. Weiss, Stone, and Harrell (1970) demonstrated an increase (10%) in the norepinephrine concentration in the

brain of dogs 40-min after exposure to escapable or avoidable shocks. In addition, as we have already seen, Brush and Levine (1966) found that plasma corticosterone levels change after avoidance training. Finally, several studies have shown that corticosterone inhibits ACTH-produced central excitability and emotionality at intermediate intervals following stress (Brodish and Long, 1956; Smelik, 1963a, 1963b; Weiss, McEwan, Silva, and Kalkut, 1969).

EXPERIMENT II

The results of Experiment I provide evidence for the hypothesis that shock, per se, may elicit internal cues that could mediate the retention of an avoidance response. However, these results did not reveal the source of these shock-produced cues or the way in which they may differ at the intermediate intervals. One physiological reaction to shock that could provide different internal cues as a function of time since shock is the hypothalamic-pituitary-adrenal response. It is well documented that this system is refractory during the first few hours after stress, then gradually recovers within approximately 24-hrs. These changes in the responsiveness of the hypothalamic-pituitary-adrenal system follow the same time course as the retention function after avoidance training. Moreover, curvilinear retention function has been reported after conditioning procedures that employ stressful stimuli but not after appetitive conditioning procedures. Since the hypothalamic-pituitary-adrenal system is presumably more active during the stressful paradigm, this difference in activity may be responsible for the different retention functions that follow the two conditioning procedures.

Previous studies on the role of the hypothalamic-pituitary-adrenal system in the retention of an avoidance response have failed to find a direct relationship between hypothalamic-pituitary-adrenal activity and the U-shaped retention function (e.g., Marquis and Suboski, 1969; Snider, Marquis, Black, and Suboski, 1971). One explanation for these negative findings may have

been the experimental procedures employed. It will be recalled that according to Snider et al. (1971), adrenalectomy does not eliminate the U-shaped retention function after avoidance training. However, surgical procedures such as adrenalectomy actually may exaggerate those physiological responses that mediate the U-shaped retention function. For example, Hodges and Jones (1964) reported that in response to stress adrenalectomized rats release considerably greater quantities of ACTH than intact rats.

The purpose of Experiment II was to investigate the relationship between the hypothalamic-pituitary-adrenal system and U-shaped retention by means of a procedure that does not interfere with the subjects' normal physiological state. The study was designed to determine the plasma corticosterone level produced in intact rats by three different stress procedures at two time intervals after original avoidance training. The stress procedures represented various degrees of stress to which subjects would be exposed during a retraining session. Time intervals of 1- and 24-hrs were selected on the basis of data from Experiment I, which showed that after a 1-hr retention interval, performance of an avoidance response was significantly poorer than after a 24-hr interval.

According to the state-dependent hypothesis, (Klein and Spear, 1970a), the hypothalamic-pituitary-adrenal system could mediate the U-shaped retention function if plasma corticosterone is released in different quantities at the 1- and 24-hr intervals, since different levels of plasma corticosterone may

provide different internal stimulus cues at the two retention intervals. The results of this study will also provide direct evidence on the hypothalamic-pituitary-adrenal interpretation of the curvilinear retention function (Brush and Levine, 1966). This hypothesis states that the low levels of plasma corticosterone at intermediate intervals after avoidance training reflect a refractory state of the hypothalamic-pituitary-adrenal system. When retraining occurs at these intervals, the reduced responsiveness of the system impairs the subject's ability to cope with the stress of retraining. According to this hypothesis, then, exposure to stress 1-hr after avoidance training should result in significantly lower levels of plasma corticosterone than would be produced 24-hrs after training.

METHOD

Subjects

The subjects were 90 experimentally naive, male, Sprague-Dawley albino rats obtained from Simonsen Laboratories (Gilroy, California). All subjects were 50 to 60 days old and weighed between 200 and 220 grams at the onset of experimentation. Upon arrival at the laboratory 10 days before testing, all subjects were placed in individual cages under conditions of ad libitum food and water and continuous illumination.

Apparatus

Subjects were given avoidance training in the same automatic shuttleboxes described in Experiment I. Unsignalled shock was also administered in the shuttleboxes, but the 6-watt incandescent light and white noise CS were not delivered before the onset of each shock. The fan noise and all other components of the automatic shuttleboxes during unsignalled shock remained unchanged.

Procedure

Original Avoidance Training. Eighty of the 90 subjects received the following avoidance training. Each subject was placed in the shuttlebox and given a 2-min interval to acclimate to the apparatus. After this interval, 10 pretest trials were given during which only the CS was presented. Each

trial was separated by a 2-min interval and the CS terminated when the subject ran to the opposite compartment or after 45-sec. If during the pretest trials a subject responded during the first 5-sec of the CS on more than 5 trials out of the 10 or on 3 of the last 5, it was discarded.

Immediately after the 10 pretest trials and without removing the subject from the apparatus, 40 active avoidance training trials were presented, using a 5-sec CS-US interval and a 1-min intertrial interval. During each trial, a subject could avoid the shock by running to the opposite compartment of the shuttlebox during the 5-sec CS-US interval; the same response occurring after 5-sec escaped the shock and simultaneously terminated the CS. Intertrial responses from one compartment to the other, which occurred when neither the CS nor US were operating, were monitored so that during each trial the CS was presented in the compartment occupied by the subject. These intertrial responses had no effect on the time course of training. After completion of a single training session, all subjects were returned to their home cage. So that the training procedures of Experiment I and II would be identical, subjects that failed to make 3 or more avoidance responses during the 40 avoidance training trials were discarded.

Stress Session. After completion of original avoidance training, the subjects were assigned to one of eight groups, with 10 subjects per group. Six of the 8 were experimental groups representing a 2 x 3 factorial design in which the factors were (a) ISI (1 or 24-hrs) and (b) Shocks (0, 1, or 5). The subjects assigned to an experimental group were treated as follows.

After the appropriate ISI, subjects were removed from their home cage and placed back in the same shuttlebox in which they had received original training. After an acclimation period of 1-min, the appropriate number of shocks were presented, each separated in time by one minute. The shocks were terminated by a running response to the opposite compartment. Subjects given 0 shocks were simply placed in the shuttlebox for 8-min; subjects given 1 shock received that shock 1-min after placement in the box and were undisturbed for the next 7-min; subjects given 5 shocks received the first shock 1-min after placement in the box, the remaining shocks at one minute intervals thereafter, and were undisturbed for the final 3-min. It should be noted that regardless of the number (0, 1, 5) of shocks, all subjects remained in the shuttlebox for exactly 8-min so that exposure to apparatus cues would be constant for all groups. After 8-min each subject was removed from the shuttlebox and anesthetized with ether for 45-sec; then a blood sample was taken to be later analyzed for plasma corticosterone concentration. The entire sequence -- placement in the shuttlebox, administration of 0, 1, or 5 shocks, removal from the box, and withdrawal of blood -- took approximately 10-min.

The remaining two groups that had received original avoidance training were unstressed control groups included to determine plasma corticosterone levels after original training when no additional stress is introduced. After an ISI of either 1- or 24-hrs, each subject was removed from its home

cage and anesthetized with ether for 45-sec; then a blood sample was taken for plasma corticosterone determination.

One additional group of 10 subjects, designated Basal Control, was employed to determine the level of plasma corticosterone in experimentally naive rats. These subjects were not given original avoidance training, but after the 10-day confinement to their home cage, they were removed and anesthetized with ether for 45-sec; then a blood sample was collected for plasma corticosterone determination.

Collection of plasma samples. Blood was collected from the anesthetized subjects via direct cardiac puncture. The rat was placed ventral side up, and a 2 in incision was made in the skin beginning 1/2 in anterior to the sternum and proceeding posteriorly. With a hemostat clamped to the exposed tip of the sternum, the rib cage was raised to expose the chest cavity. After the diaphram had been cut and the heart exposed, a 5 cc heparinized syringe supplied with a 20 ga, 1 in disposable needle was used to puncture the heart and withdraw approximately 4 cc of whole blood. The blood was immediately transferred to a 12 ml pyrex centrifuge tube, which had been kept on ice, and then centrifuged for 20-min at 1500 rpm. The entire collection process required less than 75-sec from the onset of anesthesia. After centrifugation, four 0.5 cc aliquots of the plasma were taken with a 1 ml serological pipette, and each was transferred to a separate 4 ml pyrex test tube and frozen for future corticosterone determination.

Determination of plasma corticosterone. A competitive protein binding assay was used to determine the amount of corticosterone present in the plasma samples. Duplicate values were obtained on each sample and the assay was performed over a 5 week period, starting about 3 weeks after completion of the experimental training. Appendix C outlines the procedural details of the assay.

RESULTS

Original training phase. Figures 10 and 11 show the mean number of avoidance responses in blocks of five trials made during original training by the unstressed control and experimental groups, respectively. Inspection of each figure reveals that all groups showed an increase in the number of avoidance responses over trials, but that the rate of change in performance fluctuated between groups. These visually apparent findings were tested in a 4 x 2 x 8 factorial analysis of variance in which the factors were (a) Degree of Stress (unstressed control, 0 shocks, 1 shock, 5 shocks), (b) ISI (1- or 24-hrs), and (c) Trials (8 blocks of five trials). The outcome of this analysis confirmed the above observations by revealing a significant effect of Trials ($F = 52.98$, $df = 7/504$, $p < .01$) and a significant ISI x Trials interaction ($F = 4.95$, $df = 7/504$, $p < .01$). It should be noted that the procedures during original training were the same for all groups and thus the significant ISI x Trials interaction can probably be attributed to sampling error. In no case should these minor differences between groups have differentially affected the plasma corticosterone levels that resulted from the subsequent stress sessions. None of the remaining sources of variation were significant.

Figure 12 shows the mean plasma corticosterone levels of the three control groups. The points labeled Control 1 hr and Control 24 hr indicate the mean levels of plasma corticosterone in the unstressed control groups.

Figure 10 Mean number of avoidance responses during original training for the two unstressed control groups as a function of five trial blocks.

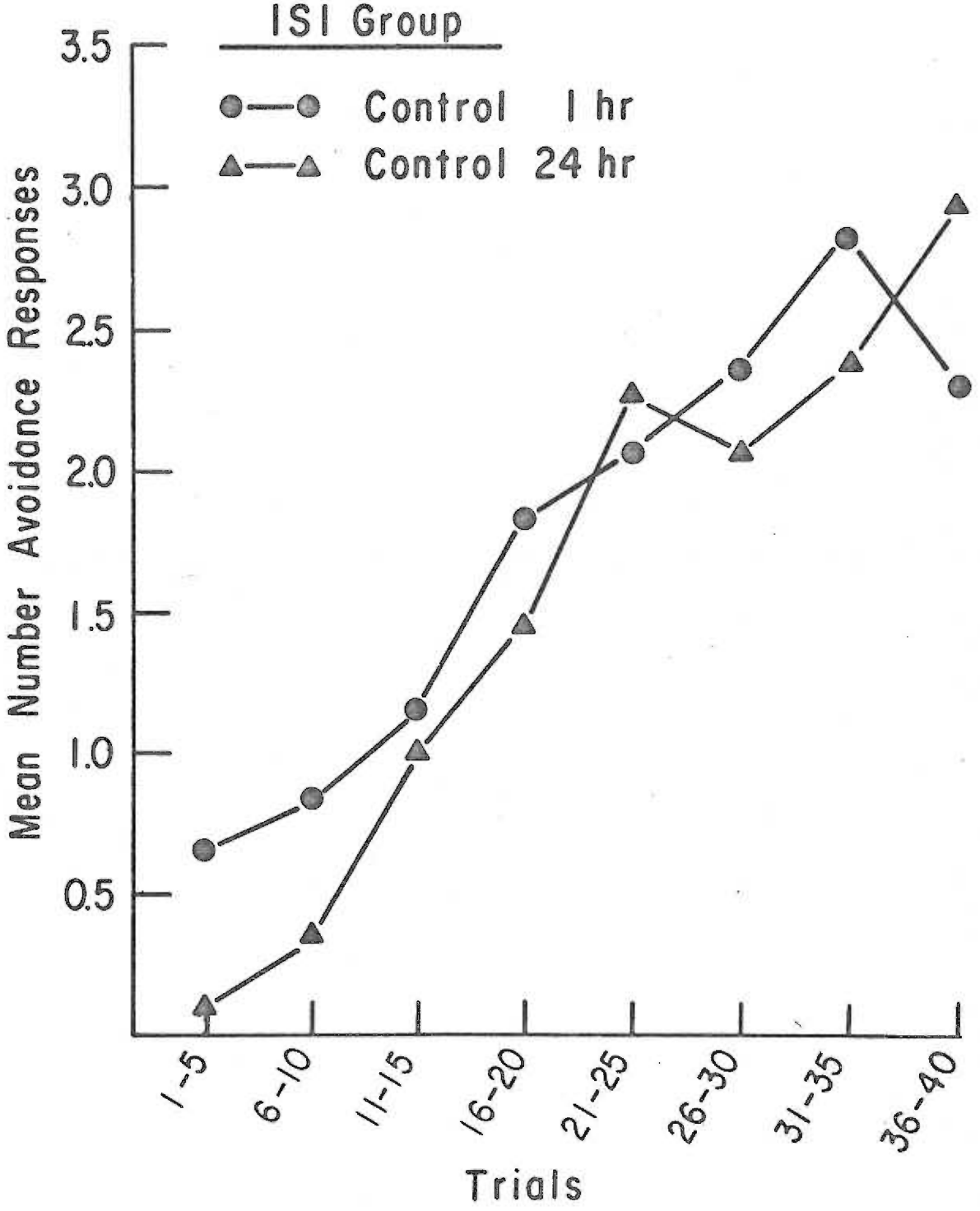

ISI Group
Control 1 hr
Control 24 hr
Mean Number Avoidance Responses
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1-5
6-10
11-15
16-20
21-25
26-30
31-35
36-40
Trials

Figure 11 Mean number of avoidance responses during original training for the six experimental groups as a function of five trial blocks.

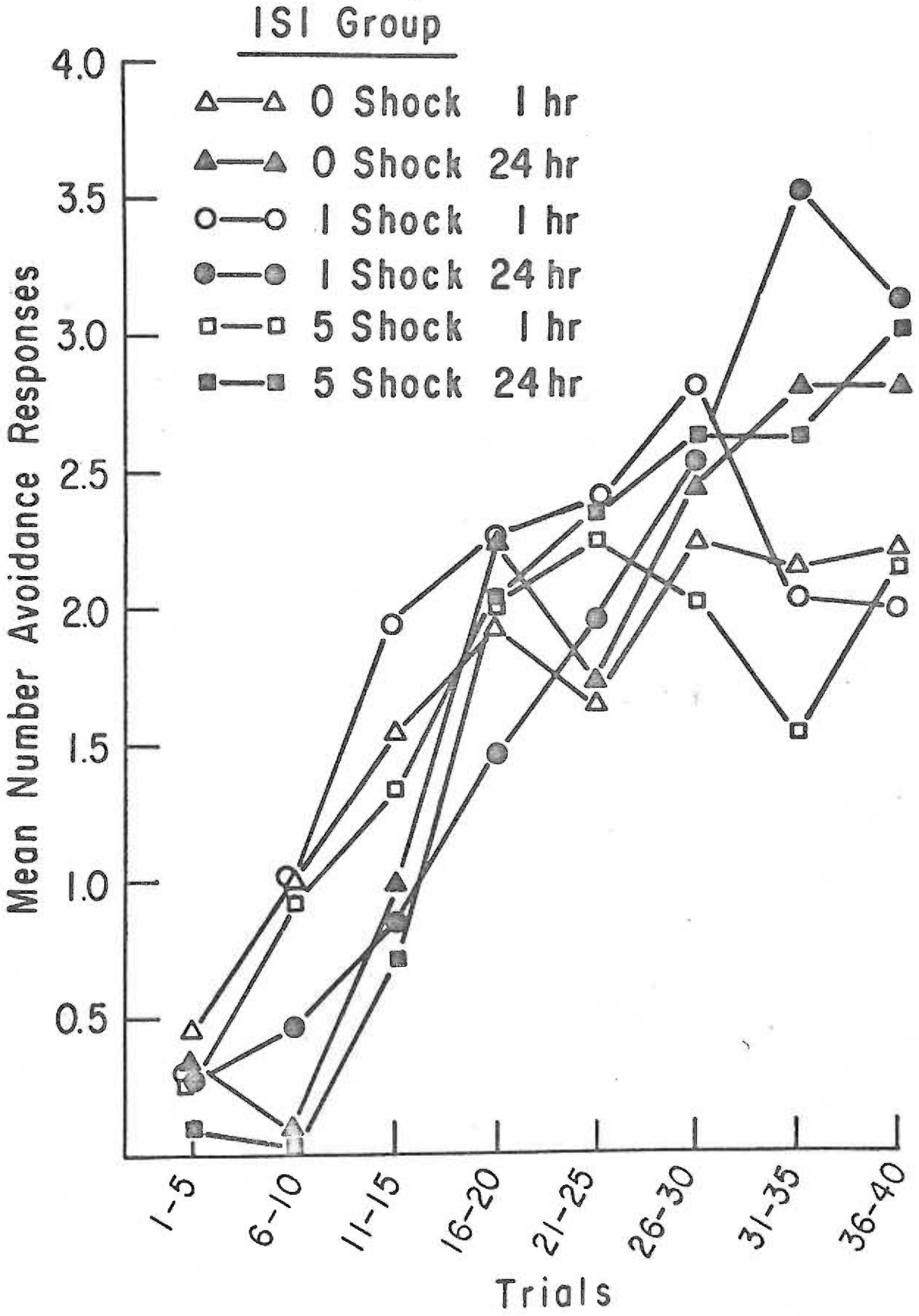
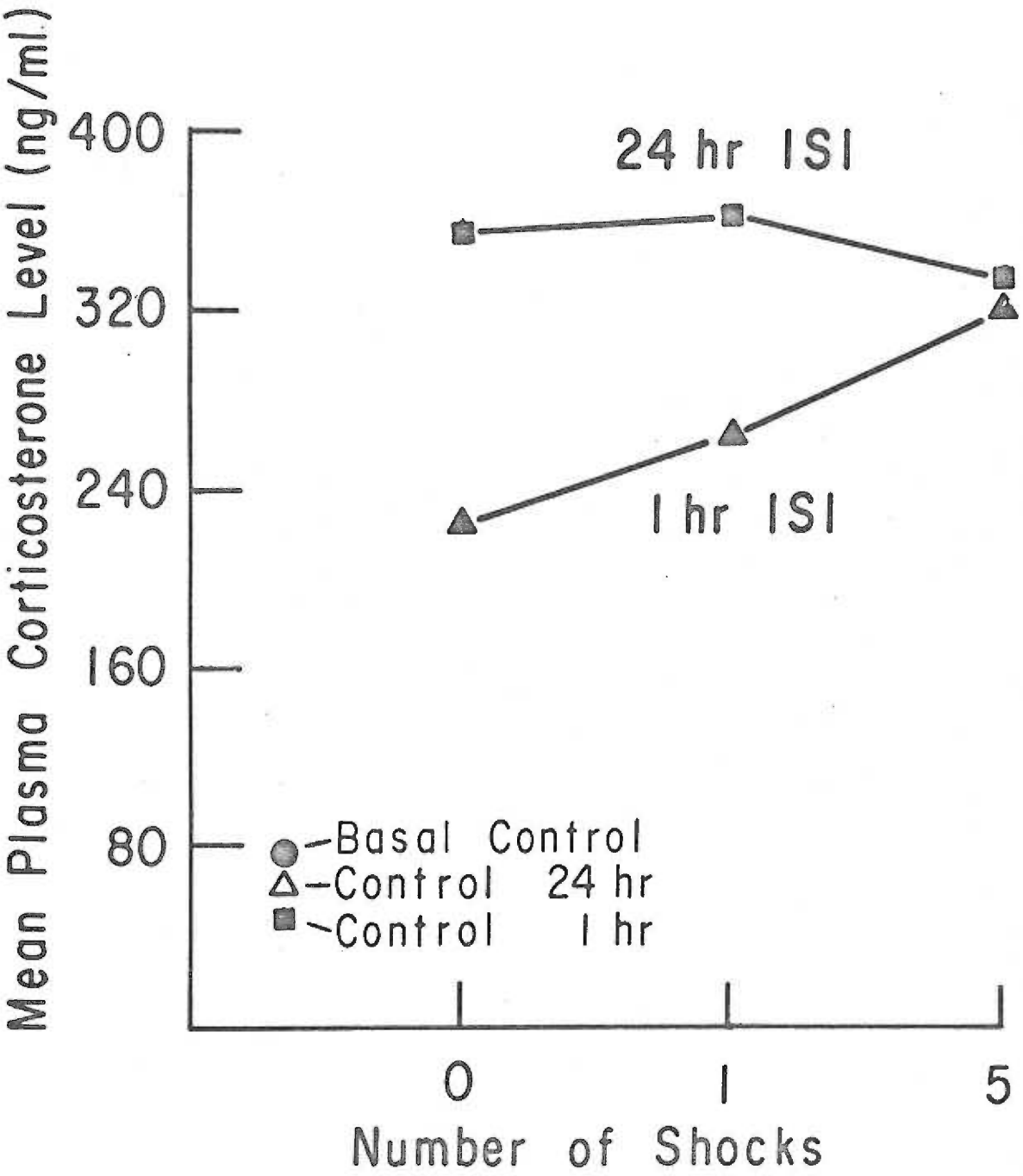

ISI Group
0 Shock 1 hr
0 Shock 24 hr
1 Shock 1 hr
1 Shock 24 hr
5 Shock 1 hr
5 Shock 24 hr
Mean Number Avoidance Responses
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1-5
6-10
11-15
16-20
21-25
26-30
31-35
36-40
Trials

Figure 12 Mean plasma corticosterone levels of the experimental groups as a function of the number of shocks during the stress session. The points labeled Control 1 hr and Control 24 hr show the mean plasma corticosterone levels of the unstressed control groups at 1- and 24-hrs after avoidance training, respectively. The point labeled Basal Control shows the mean plasma corticosterone level of the experimentally naive group.


Mean Plasma Corticosterone Level (ng/ml)
400
320
240
160
80
24 hr ISI
1 hr ISI
Basal Control
Control 24 hr
Control 1 hr
0
1
5
Number of Shocks

It will be recalled that these groups were employed to determine the plasma corticosterone levels at 1- and 24-hrs after avoidance training. The point labeled Basal Control shows the mean plasma corticosterone level of the experimentally naive group. Inspection of this figure indicates that the mean levels of plasma corticosterone in the control groups were approximately the same. A one-way analysis of variance on these data agreed with the above observation by revealing no differences between the three groups ($F = 1.14$, $df = 2/27$, $p > .05$). Therefore, the data from the three control groups were combined to form a single control group to simplify the statistical analyses of the remaining experimental data.

Stress session phase. Figure 12 also shows the mean plasma corticosterone levels of the six experimental groups as a function of the number of shocks. This figure indicates that pronounced elevations in plasma corticosterone over the unstressed control levels resulted from the stress session, regardless of the interval between original training and the stress or the number of shocks presented. These visually apparent findings were tested in an overall analysis of variance modified for a factorial experiment with a single control group (Winer, 1962). The outcome revealed a highly significant difference between the combined control group and all other groups ($F = 39.44$, $df = 1/80$, $p < .01$). In addition, t tests using the pooled within-cell variance as the error term were performed on the mean plasma corticosterone levels of the experimental groups versus the single control group. The results

of these analyses, as shown in Table 3, revealed that a significant difference in plasma corticosterone was present between each experimental group and the control group.

A comparison of the mean plasma corticosterone levels attained by the 1- and 24-hr ISI groups indicates that, regardless of the number of shocks, the 24-hr group attained a higher level than the 1-hr group. A factorial analysis of variance on these data revealed a significant effect of ISI ($F = 6.31$, $df = 1/80$, $p < .05$), which confirmed the above observation that the level of plasma corticosterone was higher in the 24-hr ISI groups than in the 1-hr ISI groups. The effect of Shocks ($F = 1$, $df = 2/80$, $p > .05$) and the ISI x Shocks interaction ($F = 1.12$, $df = 2/80$, $p > .05$) were not significant, which indicates that the plasma corticosterone levels of the 1- and 24-hr ISI groups did not differ as a function of the number of shocks.

Figure 12 also shows that as the number of shocks during the stress session was increased from 0 to 5, differences in plasma corticosterone level between the 1- and 24-hr ISI groups became progressively smaller. Since this observation suggested that differences in plasma corticosterone between the 1- and 24-hr ISI groups may not have been present at each shock level, individual t tests using the pooled within-cell variance as the error term were performed on each pair of groups. The results of these tests indicated that the mean level of plasma corticosterone in the 24-hr ISI group given 0 shocks was significantly higher than the 1-hr group given 0 shocks

Table 3 Results of individual t tests on mean plasma corticosterone levels for each experimental group compared with the single control group. Tabulated data in each cell represent the computed t values and the level of significance attained between the control group and the designated experimental group (** = $p < .01$).

Table 3

ISI	Number of Shocks: 0	1	5
1-hr	3.47**	4.44**	5.90**
24-hr	6.70**	6.84**	6.23**

** = $p < .01$ df = 80

($t = 1.84$, $df = 80$, $p < .05$). However, no significant differences were found between the ISI groups given 1 or 5 shocks ($t = 1.45$, $df = 80$, $p > .05$; $t = 0.23$, $df = 80$, $p > .05$, respectively). The outcomes of these tests probably resulted from the continued increase in plasma corticosterone in the 1-hr ISI groups as the number of shocks increased, and the stable level of plasma corticosterone across shocks in the 24-hr ISI groups.

Since the stress session shocks were terminated by a running response to the opposite compartment of the shuttlebox, the 1- and 24-hr ISI groups may have escaped the shocks at different latencies. If this was the case, the duration of shock would be unequal for the ISI groups at each shock level and could have contributed to the plasma corticosterone values obtained. Inspection of the escape latencies, however, indicated that there were no differences between the 1- and 24-hr groups given 1 or 5 shocks. The mean total duration of shock received by the 1- and 24-hr ISI groups given 1 shock was 0.93 and 0.86-sec, respectively. For the 1- and 24-hr ISI groups given 5 shocks, the mean total duration was 6.05 and 6.35-sec, respectively. Individual t tests revealed that these differences were not significant.

It was thought possible that a relationship existed between the number of avoidance responses a subject made during original training and that subject's corticosterone level after the stress session. To test this relationship, a Pearson Product-Moment correlation test was carried out to compare each subject's total number of avoidance responses during original training with

its plasma corticosterone level. The results revealed that none of the groups displayed a significant relationship between number of avoidance responses and level of plasma corticosterone. The correlation coefficients were: Control - 1-hr ISI, $r = +0.17$, $p > .05$; Control - 24-hr ISI, $r = +0.22$, $p > .05$; Experimental - 0 shocks - 1-hr ISI, $r = +0.24$, $p > .05$; Experimental - 0 shocks - 24-hr ISI, $r = +0.45$, $p > .05$; Experimental - 1 shock - 1-hr ISI, $r = -0.31$, $p > .05$; Experimental - 1 shock - 24-hr ISI, $r = +0.08$, $p > .05$; Experimental - 5 shocks - 1-hr ISI, $r = -0.60$, $p > .05$; Experimental - 5 shocks - 24-hr ISI, $r = -0.37$, $p > .05$.

DISCUSSION

The principal finding of Experiment II was that the level of plasma corticosterone that resulted from a stress at 1- or 24-hrs after avoidance training varied with the stress procedure employed. When the stress consisted solely of removing the subject from its home cage and placing it in the original training apparatus for 8-min (0 shocks), a significantly greater amount of plasma corticosterone was released 24-hrs after avoidance training than 1-hr after training. However, when 1 or 5 shocks were included in the stress procedure, plasma corticosterone differences between the 1- and 24-hr ISI groups were no longer present. It will be recalled that the 24-hr ISI groups attained the same plasma corticosterone level, regardless of whether 0, 1, or 5 shocks were presented. These results suggest that the 0 shock procedure was sufficient to result in a maximum release of endogenous corticosterone 24-hrs after avoidance training. The 1 and 5 shock procedures, although presumably more stressful than the 0 shock procedure, could therefore not produce further elevations in plasma corticosterone.

A comparison of the 1-hr ISI groups, on the other hand, revealed that the level of plasma corticosterone increased as the number of shocks increased, with the 5 shock group attaining a higher level of plasma corticosterone than the 0 shock group. Apparently, the experience of simply being placed in the avoidance training apparatus 1-hr after training did not stimulate maximum

corticosterone release. Therefore, the additional stress afforded by 1 or 5 shocks resulted in greater releases of plasma corticosterone. Evidently, a group stressed 1-hr after avoidance training requires more stimulation than a group stressed 24-hrs after training to reach maximum corticosterone production.

The results also revealed that each of the stress procedures (0, 1, or 5 shocks) was sufficient to produce a significant elevation in plasma corticosterone over the unstressed control levels. In the 0 shock groups, for instance, handling the subjects and exposing them to the "fear eliciting" cues of the original training apparatus produced a marked increase in hypothalamic-pituitary-adrenal activity, regardless of the interval between original training and the stress experience. These data are in contrast to those reported by Kitay, Holub, and Jailer (1959), which showed that 24-hrs after an initial stress there was a marked inhibition of ACTH release to the stress of ether anesthesia. Similar results have also been reported by Knigge, Penrod, and Schindler (1959) and Henkin and Knigge (1963). Although there may have been some suppression of the steroid response to the stress procedure in the present study, the response was by no means diminished or abolished to the degree reported by Kitay et al. Insofar as the stress procedures employed in the present study differ from those used by Kitay et al., direct comparisons are difficult. It does appear, however, that ACTH can be released in considerable quantities upon further stimulation, regardless of whether a 1- or 24-hr interval has elapsed since its initial activation.

The finding that the latencies to escape shock of the 1- and 24-hr ISI groups were similar ruled out the possibility that a difference in exposure to shock contributed to the plasma corticosterone levels. The data which indicated that 1- and 24-hr ISI groups that experience comparable exposure to shock attain a similar level of plasma corticosterone are consistent with the data reported by Brush and Levine (1966). They found that when plasma corticosterone levels were adjusted to control for total duration of shock during an active avoidance retraining session, the 1- and 24-hr ISI groups had similar plasma corticosterone levels at the end of retraining.

The finding that groups stressed 1- or 24-hrs after original avoidance training escape shock at the same speed provides indirect evidence against the hypothalamic-pituitary-adrenal hypothesis (Brush and Levine, 1966). It will be recalled that this hypothesis proposed that avoidance performance is impaired 1-hr after original training due to an inability on the part of the subjects to behaviorally cope with the stress of a second training session. To the degree that escape latency data provide an assessment of "coping" behavior, the results of the present study suggest that the 1- and 24-hr groups are equally capable of coping with stress.

Data indicating that the plasma corticosterone levels of the unstressed control groups given avoidance training 1- or 24-hr prior to blood collection are equal and at the same level as an untrained group agree with the results of previous experiments (Brush and Levine, 1966; Levine and Brush, 1967).

However, the experiments differed in the actual steroid values reported. In general, the plasma corticosterone values reported by Levine and Brush were 2 to 4 times higher than those reported in the present experiment. For example, they reported that the mean plasma corticosterone level of untrained rats was approximately 180 ng/ml, whereas the present study reported a mean value of 76 ng/ml in a comparable group of rats. Moreover, Levine and Brush found that 1-hr after avoidance training the level of plasma corticosterone was 170 ng/ml, but the present study found a concentration of only 42 ng/ml. Approximately the same difference in steroid levels was reported 24-hrs after avoidance training. One procedural difference between the two studies that undoubtly contributed to the discrepant values was the assay procedure employed to measure corticosterone concentrations in the plasma. In the Levine and Brush study, corticosterone concentrations were determined by a microfluorometric method (Glick, Von Redlich, and Levine, 1964), whereas the present study used a competitive protein binding (CPB) assay procedure. Several differences in the assay procedures could have resulted in the magnitude and direction of the different steroid values. First, the microfluorometric method did not remove interfering fluorescent materials that undoubtly would contribute to the values obtained. The CPB method, however, subjected the plasma sample to chromatographic separations after extraction to remove those compounds that compete with the corticosterone during the binding stage of the assay. Second, the volume of plasma used in

the microfluorometric method was 10 times greater than the volume used in the CPB method. Thus, the plasma analyzed by the microfluorometric method contained 10 times the contamination as the CPB analyzed plasma. Since these assay differences contribute to an overestimation of plasma corticosterone by the microfluorometric procedure, it seems reasonable to conclude that the values obtained by the CPB assay represent a more accurate estimation of the physiological concentrations of plasma corticosterone in the rat.

Apparently, the type of stress used has a direct influence on the time course of the plasma corticosterone levels after that stress. Hodges and Jones (1964) reported that 1-hr after a sham adrenalectomy operation the level of plasma corticosterone was significantly higher than after a 24-hr interval. Moreover, it was not until 4-hrs after stress that plasma corticosterone decreased to a preoperative level, or that level found 24-hr after stress. In contrast, the present experiment found that 1-hr after avoidance training plasma corticosterone was at the same level as after a 24-hr interval. Insofar as there are no means to compare the degree of stress produced under the two procedures, interpretation of these differences is difficult. It does appear, however, that corticosterone values obtained after different stress procedures may be quite different, and therefore statements based on a single procedure may have limited generality.

GENERAL DISCUSSION

The present study investigated the role of a number of variables in the retention of aversively motivated behaviors. The results revealed that the critical variable controlling the retention of an avoidance response was time since shock, not time since acquisition of the response. When a series of 5 shocks were interpolated between the original learning of an avoidance response and later retention tests, performance was a curvilinear function of time since shock. This retention function was characterized by a significant decrease in avoidance performance between a 0.08- and 4-hr retention interval test, followed by an increase between a 4- and 24-hr interval test. Basically, the retention function after interpolated shock was similar to the function found after original training without interpolated shock.

The present study also revealed that significant elevations in plasma corticosterone resulted from a stress procedure, regardless of whether the interval between original training and the stress was 1- or 24-hrs, or whether 0, 1, or 5 shocks were presented as part of the stress. When subjects were placed back in the training apparatus and given no shocks, a 24-hr interval group attained a significantly higher level of plasma corticosterone than a 1-hr interval group. However, when at least one shock was presented as part of the stress procedure, there was no difference in the amount of corticosterone released by a 1- and 24-hr group.

The results of the present studies suggest that plasma corticosterone does not influence the retention performance of an avoidance response. This follows from a comparison of the data in Experiments I and II. The results of Experiment I revealed that a group retrained 1-hr after original training made significantly fewer avoidance responses than a group retrained 24-hrs after training, and that the subjects in both groups had received at least one shock by the second trial of retraining. Therefore, the finding in Experiment II that experience with a single shock results in equivalent amounts of plasma corticosterone at 1- and 24-hrs after avoidance training suggests that the avoidance performance differences between the 1- and 24-hr ISI groups in Experiment I occurred when plasma corticosterone levels were approximately the same. While it could be suggested that the performance differences between the groups were present early in retraining, before plasma corticosterone levels were known, the data indicated that this was not the case. No significant difference between the 1- and 24-hr ISI groups was found in mean number of avoidance responses during the first 10 trials of retraining.

Insofar as the procedures during the stress session in Experiment II differed from the retraining procedures in Experiment I, direct comparisons are difficult. It does appear, however, that the pituitary-adrenal system responds to a moderately strong stress in a comparable fashion at both 1- and 24-hrs after initial stress. These data are consistent with the results of previous studies which indicated that the U-shaped retention function occurs

independently of hypothalamic-pituitary-adrenal activity (e.g., Marquis and Suboski, 1969; Snider, Marquis, Black, and Suboski, 1971).

This paper follows others (Bower, 1967; Underwood, 1969; Spear, 1971) in assuming that the property of behavior singularly referred to as "a memory" actually consists of a number of separate and independent memories. The implications of the above assumption merit at least brief discussion. If, indeed, the memory of an event consists of several interrelated, yet independent memories, it is inappropriate to conceptualize the behavior of animals as representing "complete forgetting". When results indicate that an animal has forgotten a recently acquired response, we almost certainly are viewing a consequence of incomplete measurement during the retention test. The obvious point is that when a correct response occurs during a retention test, we cannot be certain that all the memories associated with learning have been aroused; nor can we be sure that none of the memories have been aroused when the animal makes an incorrect response or none at all. Perhaps methods that allow direct neutral measurement will soon permit assessment and identification of the memories aroused during a retention test, even though the animal has overtly behaved as if no prior learning had been given at all.

At the risk of overgeneralization, the usual experimental design to demonstrate the U-shaped nature of retention has closely followed the paradigm conceived by Kamin (1957). A single training session consisting of approximately 25 trials is presented to subjects, followed by retraining sessions at

intervals of 5-min, 1- and 24-hrs. Performance at these three intervals, then, has almost universally provided the three points that describe retention as U-shaped. Many theoretical interpretations of the U-shaped function have incorporated processes to account for the performance at each interval (e.g., King, 1969; Kamin, 1957). However, the basic feature of the U-shaped retention function is the persistent failure to demonstrate good retention at the 1-hr interval. Retention 5-min after training might best be considered as simply an extension in the number of trials of original training, with the 5-min interval between sessions representing either a longer intertrial interval or a "time-out" period. It is well known that performance after a "time-out" suffers little from the imposed delay and, under certain conditions, is often superior to the performance before the delay. Likewise, the retention after a 24-hr interval is consistent with our present knowledge of the normal forgetting (or extinction) of a learned response over time. The only behavior, then, that a theoretical interpretation of the U-shaped function must explain is the decrement in performance at the intermediate interval (1-hr). Performance at this interval is contrary to our present knowledge of the normal course of forgetting.

The foregoing considerations apply as well to an interpretation of the present finding that U-shaped retention follows interpolated shock. Nevertheless, the fact that interpolated shock does result in a recurrence of the performance decrement after intermediate intervals documents the reality of the phenomenon and emphasizes the need for a viable explanation of the mechanisms involved.

SUMMARY AND CONCLUSIONS

Two experiments were conducted to determine the role of different variables in retention of an active avoidance response. Experiment I investigated the effect of unsignalled shock presented 23-hrs after acquisition of an avoidance response. The original training session consisted of 40 active avoidance trials and the retention test was 25 active avoidance trials. Five groups of rats were tested at intervals of either 0.08-, 1-, 2-, 4-, or 24-hrs after the unsignalled shock. In addition, five groups were tested after the same retention intervals but without prior unsignalled shock. Finally, five groups were tested for retention of the avoidance response at intervals of 0.08-, 1-, 2-, 4-, and 23-hrs after original training.

The principal findings were:

(1) Retention of an avoidance response after unsignalled shock was a curvilinear function of the retention interval.

(2) The mean number of avoidance responses made by groups given unsignalled shock 1-, 2-, or 4-hrs before the retention test were significantly lower than the means of groups tested after the same intervals but without unsignalled shock before the retention test.

(3) Groups tested 1- and 4-hrs after unsignalled shock required more trials to make their first avoidance response than groups tested at the same intervals but without unsignalled shock.

(4) Retention of an avoidance response after original training was a curvilinear function of the retention interval.

(5) The critical variable controlling the performance of an avoidance response was time since shock, not time since acquisition.

(6) The results of Experiment I were consistent with an interpretation of the U-shaped retention function based on state-dependent learning, but not with alternative interpretations.

Experiment II investigated the possibility that plasma corticosterone is released in different quantities at 1- and 24-hrs after avoidance conditioning, and thereby provides different internal stimulus cues at each interval. Six groups of rats were given an avoidance conditioning session consisting of 40 active avoidance trials. A stress session consisting of either 0, 1, or 5 shocks followed conditioning at intervals of 1- or 24-hrs. Plasma corticosterone was measured 10-min after the onset of each stress procedure. In addition, two groups were employed to determine plasma corticosterone levels 1- and 24-hrs after avoidance training when stress was not introduced before measurement. The plasma corticosterone level of an experimentally naive group was also measured to determine the basal level.

The principal findings were:

(1) Substantial elevations in plasma corticosterone over unstressed control levels resulted from stress after avoidance conditioning,

regardless of whether the interval between conditioning and the stress was 1- or 24-hrs, or whether 0, 1, or 5 shocks were part of the stress.

(2) In general, plasma corticosterone levels after stress were higher 24-hrs after avoidance training than 1-hr after training. However, individual comparisons revealed that a significant difference in plasma corticosterone level was present only between the 1- and 24-hr ISI groups given 0 shocks.

(3) The mean plasma corticosterone level of an unstressed group measured 1-hr after avoidance training, 24-hrs after training, or an experimentally naive group were approximately the same.

(4) Taken together, the results of Experiment I and II indicated that plasma corticosterone does not directly influence the retention performance of an avoidance response.

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APPENDIX A

Experiment I

Tabulated Raw Data

Note: Raw data tables employ the following abbreviations:

OT = Original avoidance training session

RT = Retraining avoidance session

ISI = Intersession Interval

Base Line Control Groups

Raw Data

Avoidance Responses

	0.08-hr ISI		1-hr ISI		2-hr ISI		4-hr ISI		23-hr ISI	
	OT	RT	OT	RT	OT	RT	OT	RT	OT	RT
Subject 1	23	22	32	16	8	12	7	20	25	17
Subje ct 2	29	22	11	11	16	12	27	20	20	16
Subject 3	23	22	13	3	15	6	32	23	12	19
Subject 4	16	15	10	5	16	14	7	13	9	13
Subject 5	14	22	25	20	4	5	13	16	23	18
Subject 6	7	16	16	8	18	10	24	19	13	15
Subject 7	26	18	22	12	6	5	11	13	5	9
Subject 8	19	19	13	6	16	16	6	13	23	16
Subject 9	9	19	15	15	20	21	14	20	22	18
Subject 10	3	15	7	16	25	17	16	15	9	18
Subject 11	11	13	3	11	16	20	17	13	15	20
Mean	16.4	18.5	15.2	11.2	14.6	12.6	15.8	16.8	16.0	16.3
Median	16.0	19.0	13.0	11.0	16.0	12.0	14.0	16.0	15.0	17.0

O-shock Groups

Raw Data

Avoidance Responses

	0.08-hr ISI		1-hr ISI		2-hr ISI		4-hr ISI		24-hr ISI	
	OT	RT	OT	RT	OT	RT	OT	RT	OT	RT
Subject 1	26	21	21	18	18	13	6	6	13	12
Subject 2	7	16	23	20	21	22	10	18	11	11
Subject 3	7	23	3	10	7	22	12	14	3	10
Subject 4	27	21	3	18	8	18	9	18	22	22
Subject 5	22	18	22	18	31	13	21	18	23	23
Subject 6	16	4	6	14	10	19	20	11	30	23
Subject 7	5	16	17	11	13	10	31	24	18	19
Subject 8	10	18	11	11	21	17	12	15	10	18
Subject 9	12	15	17	20	6	13	19	20	13	13
Subject 10	17	11	24	22	14	16	21	18	10	15
Subject 11	16	19	18	14	16	18	14	15	16	18
Mean	15.0	16.6	15.0	16.0	15.0	16.5	15.9	16.1	15.4	16.7
Median	16.0	18.0	17.0	18.0	14.0	17.0	14.0	18.0	13.0	18.0

5-shock Groups

Raw Data

Avoidance Responses

	0.08-hr ISI		1-hr ISI		2-hr ISI		4-hr ISI		24-hr ISI	
	OT	RT	OT	RT	OT	RT	OT	RT	OT	RT
Subject 1	18	14	16	10	6	0	8	6	10	14
Subject 2	9	13	9	16	20	14	18	9	23	18
Subject 3	7	18	5	0	20	13	9	4	26	22
Subject 4	6	20	7	15	10	1	29	22	5	18
Subject 5	23	22	20	7	21	12	12	6	4	13
Subject 6	14	8	18	18	23	18	16	17	22	13
Subject 7	17	22	17	12	12	4	8	0	12	14
Subject 8	27	20	10	7	13	17	23	10	19	8
Subject 9	22	20	23	4	18	22	16	11	18	17
Subject 10	21	19	15	19	6	1	12	2	16	16
Subject 11	7	10	21	5	18	3	24	0	25	19
Mean	15.6	16.9	14.6	10.3	15.2	9.6	15.9	7.9	16.4	15.6
Median	17.0	19.0	16.0	10.0	18.0	12.0	16.0	6.0	18.0	16.0

APPENDIX B

Experiment II

Tabulated Raw Data

NOTE: Raw data tables employ the following abbreviations:

OT = Number of avoidance responses during original training

B = Measured level of plasma corticosterone (ng/ml)

ISI = Intersession Interval

Control Data

	Basal Control		Control-1-hr ISI		Control-24-hr ISI	
	OT	B	OT	B	OT	B
Subject 1	-	15	6	43	19	147
Subject 2	-	27	12	34	4	16
Subject 3	-	150	28	34	22	79
Subject 4	-	35	3	73	4	6
Subject 5	-	26	25	12	11	20
Subject 6	-	67	5	66	14	66
Subject 7	-	38	4	5	21	32
Subject 8	-	111	27	76	14	146
Subject 9	-	113	29	25	3	93
Subject 10	-	138	15	60	25	33
Mean		72.0	15.4	42.8	13.7	63.8
Median		52.5	13.5	38.5	14.0	49.5

Experimental Data

1-hr ISI groups

	0 shocks		1 shock		5 shocks	
	OT	B	OT	B	OT	B
Subject 1	6	47	9	532	10	294
Subject 2	3	314	27	246	15	276
Subject 3	25	271	10	140	22	45
Subject 4	8	183	4	246	3	539
Subject 5	26	289	16	316	25	288
Subject 6	18	163	15	478	6	500
Subject 7	12	260	18	94	13	85
Subject 8	18	284	6	218	22	485
Subject 9	-	-	31	104	20	100
Subject 10	-	-	-	-	4	591
Mean	14.5	226.4	15.1	263.8	14.0	320.3
Median	15.0	265.5	15.0	246.0	14.0	291.0

Experimental Data

24-hr ISI Groups

	0 shocks		1 shock		5 shocks	
	OT	B	OT	B	OT	B
Subject 1	9	433	24	212	23	182
Subject 2	8	205	23	478	3	364
Subject 3	3	522	4	340	20	470
Subject 4	16	371	14	312	26	185
Subject 5	21	416	6	94	11	173
Subject 6	28	265	12	568	21	294
Subject 7	21	155	29	288	10	264
Subject 8	18	412	9	312	7	448
Subject 9	12	401	14	610	24	430
Subject 10	12	377	21	406	5	545
Mean	14.8	355.7	15.6	362.0	15.0	335.5
Median	14.0	389.0	14.0	326.0	15.5	329.0

APPENDIX C

COMPETITIVE PROTEIN BINDING ASSAY FOR CORTICOSTERONE

Materials and Reagents

1. ^{3}H-B-CBG: 250 λ (6.5 uc/ml S. A. = 50 C/mmole made up in ethanol)

   1, 2- ^{3}H-corticosterone is added to a 100 ml volumetric flask and dried under purified nitrogen. Some cold deionized water is added to the flask before 2.5 ml of dog (male) plasma is added. The remainder of the water is then added. The solution is stored in the refrigerator until used. (Stable for 1 week)

2. Corticosterone Standard:

   a) Stock Solution: 10 mg compound B is dissolved in 10 ml cold redistilled ethanol. This solution is prepared in an ice bath giving a 1 mg/ml solution.

   b) Working Standard Solution: 3 λ of the stock solution is added to a 10 ml volumetric flask. Cold redistilled ethanol is added giving a working standard of 0.3 mg/λ .

3. Florisil: purified by making a slurry with deionized water and shaking. The water is decanted and the procedure repeated until the water is clear. The florisil is dried at 100° C overnight and stored in a capped bottle under

desiccation. 80 mg florisil is weighed on a Mettler balance and stored in capsules in a desiccator overnight before using.

4. Triton Solution: 5 g Omnifluor + 1000 ml Toluene + 500 ml Triton X-100.
5. Scintillation Fluid: 4 g Omnifluor + 1000 ml Toluene.
6. Standard Curve: 0, 0.3, 1.2, 2.1, 4.2, 8.4, and 9.9 ng STD B is added to assay tubes (rinsed in ether) in triplicate (STD B is kept on ice during the measuring procedure). Tubes are dried under purified nitrogen.
7. Isatin Dye: 7.5 mg Isatin powder + 10 ml Ethanol.
8. 4% methanol/methylene chloride: 4 ml methanol + 96 ml methylene chloride (made up at room temperature).
9. Solvent system chloroform:methanol, 95:5 v/v.
10. Chloroform:methanol, 1:1 v/v.
11. Chloroform: double distilled and kept in a dark location.
12. Anhydrous ether: used directly from freshly opened containers.
13. Plates: Eastman chromagram sheet, 6060 Silica Gel with fluorescent indicator (20 x 20-cm).

Procedure

Extraction

1. Add 5000 CPM ^{3}H Corticosterone (high specific gravity) to a 12 ml citric acid tube per series of samples and to duplicate scintillation vials. Dry under nitrogen. Store scintillation vials in refrigerator.
2. 10 λ plasma plus 1/2 ml of .9% saline is added to 12 ml citric acid tubes. A plasma blank and recovery blank (adrenalectomized-ovariectomized plasma) are used for each series of samples. Throughout the extraction procedure the samples are kept in an ice bath.
3. The samples are extracted with 4 ml chloroform by shaking vigorously for 2-min on a Vortex shaker. After shaking, the samples are centrifuged at 1500 RPM for 5-min, then returned to the ice bath and the upper layer is aspirated off and discarded.
4. Deionized water (1/2 ml) is added to each sample, and after hand shaking for 2-min, the samples are centrifuged for 5-min at 1500 RPM. The top layer is aspirated off and discarded.
5. The residues are dried under nitrogen and concentrated at the tip of the tube with chloroform. If the samples are to be stored, a few drops of redistilled ethanol are added to the tip of each tube.

Elution

1. After the drying and concentrating procedure or the drying of ethanol, the samples are applied to the origin of the plate over the isatin dye in chloroform:methanol following a 4, 3, 2 drop procedure. The samples are separated by 0.6 ml lanes to prevent contamination and cross-over of steroid during development of plate.
2. After the samples are applied to the plate, the plate is developed in a saturated tank in the solvent system chloroform:methanol. The plate is removed from the tank when the solvent reaches 1-cm from the top of the plate.
3. The mobility of the corticosterone is directly proportional to that of the dye. An area 3-cm wide is removed from the plate by cutting out that section at points 1.5-cm above and below the center of the dye. Each section is attached by one corner to a clip located below a 10 ml syringe.
4. The columns are eluted with 6 ml of 4% methanol/methylene chloride into culture tubes (12 ml) which have been rinsed with anhydrous ether. The recoveries are eluted into vials, dried, and scintillation fluid is added. They are counted for 20-min. The samples are dried under purified nitrogen and concentrated at the tip with chloroform.
5. The samples are now ready for the protein-binding assay. If the samples are to be stored, a few drops of redistilled ethanol are added to the tip of each tube. The tubes are dried under purified nitrogen before using.

Assay (Corticosteroid Binding Globulin System)

1. Add 1 ml ^{3}H-B-CBG (cold) to each assay tube --both samples and standard curve.
2. Tubes are placed in a 45^{o} C water bath for 5 min.
3. Each tube is shaken on a Vortex shaker for a few seconds and placed in an ice bath for 10-min.
4. Add 80 mg florisil to each tube and immediately shake on Vortex for 30-sec.
5. 30-sec later a 500 λ aliquot is taken from the tube and placed in a vial containing 10 ml Triton scintillation fluid and enough deionized water to produce a clear solution when in the counter.
6. All vials are counted for 20-min in liquid scintillation spectrometer (Packard Model 3375).