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Effects of Magnesium Deficiency on Discriminative Avoidance Behavior of Rats

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EFFECTS OF MAGNESIUM DEFICIENCY ON DISCRIMINATIVE AVOIDANCE BEHAVIOR OF RATS

by

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Mahlon B. Dalley

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ABSTRACT

Effects of Magnesium Deficiency on Discriminative Avoidance Behavior of Rats

by

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Utah State University, 1974

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The purpose of this thesis is to determine what effects a dietary magnesium deficiency has on the discriminative avoidance behavior of rats. Three experiments were conducted. Experiment I compared two groups to determine the effects of magnesium deficiency on bar-press discriminative avoidance behavior. The results of Experiment I clearly illustrated that rats fed a diet deficient in magnesium began to lose their discriminative avoidance behavior after approximately five days with a steady decrease in performance over the remaining five days. Experiment II used a single subject design in an attempt to replicate Experiment I and to determine whether or not the magnesium deficiency effect could be reversed. Blood samples of serum magnesium for each rat were taken daily. The results confirmed Experiment I. A magnesium deficiency did cause a decrease in the performance of discriminative bar-press avoidance. Two of the four rats responded to the rehabilitation treatment with

a corresponding increase in avoidance behavior with an increase in serum magnesium levels. The other two rats did not recover avoidance performance with rehabilitation, but did improve with regard to other behavioral measurements. Experiment III again employed two groups of rats in an attempt to determine the effects of a magnesium deficiency upon acquisition of a discriminative shuttlebox avoidance performance. A pilot study to Experiment III showed a clear effect with normal controls displaying statistically more avoidance responses than the experimentals who received subnormal levels of magnesium. The results from Experiment III however showed no statistically significant difference between the controls and experimentals even though there was a statistical difference in serum magnesium concentration.

(87 pages)

INTRODUCTION

Recently a good deal of concern has been expressed over the psychological as well as the physiological problems resulting from malnutrition (Mitchell, 1967; Scrimshaw, 1967; Brožek, 1957). According to Abelson (1969) it seems likely that millions of young children in developing and developed countries are experiencing some degree of retardation because of inadequate nutrition. Brožek (1957) stated that an improper diet brings about alterations in blood chemical constituents, brain metabolism and function, and, finally results in observable behavior abnormalities.

The study of the interaction between nutrition and behavior has been concerned primarily with gross malnutrition or more specifically, protein and/or calorie deficiency (Cravioto and Delicardie, 1970; Barnes, Moore and Pond, 1970; Barnes, 1969; Scrimshaw, 1967; Frankova and Barnes, 1968; Cowley and Griesel, 1964). Few studies have dealt with specific vitamin deficiencies (Gantt, 1957; Weiss, 1957; Sloan and Chow, 1964; Kinsman and Hook, 1971). Still fewer nutritional-psychological studies have dealt adequately with specific mineral deficiencies (Brožek and Vaes, 1961, p. 83). Investigation has begun however on the role of magnesium in the physiological and biochemical functions of men and animals (Shils, 1964; Caddel, 1967; Krehl, 1967; Waker and Parisi, 1968). No research concerning the effects of a dietary magnesium deprivation upon behavior have been published.

Magnesium is an extremely important nutrient influencing the health of both man and animals. Krehl (1967) has indicated that magnesium deficiency is far more prevalent than suspected, "In our opinion, it [magnesium deficiency] can be said to have become one of the common nutritional deficiencies in clinical medicine ... one could anticipate a substantial dietary magnesium deficit for most adults particularly male adults on the customary American diet... ."

In view of these conditions, the present research was designed to determine what effects a dietary magnesium deficiency might have upon a specific behavior.

A relatively simple organism and response were chosen in this work so as to minimize confounding variables and thereby allow for a clear expression of the relationship. Specifically, rats were trained to avoid foot-shock by bar pressing in the presence of a tone-light stimulus compound. Following the establishment of a performance baseline on this schedule, magnesium deficiencies were instituted using an ABA design (A-baseline; B-treatment; A-return to baseline) (Sidman, 1960). Results indicated a clear treatment effect on the dependent variable.

REVIEW OF LITERATURE

Malnutrition and Behavior

Human studies

Field studies and clinical observations have shown malnutrition affects the behavior of humans (Keys, Brožek, Henschel, et al., 1950; Brožek, 1957). Liang, Hiett, Jan, et al. (1967), using intelligence tests, studied the effects of malnutrition in Indonesia and found that children with the lowest I.Q. scores had been malnourished and had shown clinical signs of vitamin A deficiencies when they were between two and four years of age. Pollit and Granoff (1967) have found similar results with marasmic (calorie-deficient) children in Peru, as did Cravioto and Delicardie (1970) with Mexican children.

These field studies are examples of the research being conducted in various parts of the world illustrating the effects of diet upon human behavior. Most of these studies conclude that deficiencies of various nutrients (i.e., protein, vitamins, etc.) clearly have detrimental effects on behavior. Several studies (Cravioto and Delicardie, 1970; Keys, Brožek, Henschel, et al., 1950) show that rehabilitation will reverse the problems created by deficiencies provided the deficiency did not occur during a "critical period" in the child's neurological development.

Field studies with human subjects have provided valuable answers to some questions, but by their very nature, field studies with humans cannot

separate the interacting effects that culture, environment, disease and nutrition have on behavior (Scrimshaw, 1967). Nonhuman subjects, therefore, are very useful in an experimental approach to this problem. With animals, one can restrict to any degree any nutrient he wishes, and, in addition, he can control for other extraneous variables such as culture (MacLeod, 1957).

Animal studies

Animal studies in nutritional-psychology suggest that a deprivation of protein and/or calories has differing effects, depending upon the time at which the deprivation occurs and also on the task that the animal is asked to perform. The predominate procedure incorporated in these studies consists of an instrumental type learning task (i.e., Y or T-mazes), or variations of an operant task (i.e., avoidance conditioning). Examples of these will be discussed below.

Barnes et al. (1966) have shown that rats deprived of food both before and after weaning made more errors on a Y-water maze than controls. A Y-water maze procedure involves placing a rat at the start of a water filled runway. The rat must swim to two arms of the maze then select by some means which of the two has a platform above the water. An incorrect choice places the animal in the arm of the maze without a platform and is counted as an "error." The results of the Barnes et al. (1966) study tended to confirm the hypothesis that malnutrition does affect the behavior of rats by increasing the probability of errors on a discriminative task.

Barnes et al. (1967) extended the previous findings by simulating Kwashiorkor (protein deficiency) in rats by providing lactating females more day-old rats to nurse than they could adequately accommodate. They also restricted food intake to some post-weaned rats. All rats were subsequently rehabilitated. It was found that deprivation of food before the animal was weaned had a more serious effect on the maze task than depriving the animal of food after it was weaned.

Vore and Ottinger (1970) tested adult rats, the offspring of either mothers who were fed normally or restricted in their diet, and found that, as adults, rats deprived of food prenatally and/or post-natally made more errors in learning a T-maze than control rats.

The conclusions from such studies are that food deprivation does harm to an animal's subsequent performance on a Y or T-maze. Another point is that the time in the animal's life at which the deprivation occurs is a critical factor in the degree to which the deprivation affects behavior. The most critical time seems to be during the pre-weaning period.

Barnes et al. (1967) with an avoidance conditioning procedure using pig subjects found that the number of conditioning trials necessary to develop a consistent response was essentially the same for all the animals regardless of previous diet. However, the pigs fed normally in early life extinguished responding after a few trials, whereas the malnourished animals continued to respond. Barnes, Moore and Pond (1970) used a procedure similar to Barnes et al. (1967), but deprived pigs of protein only. The pigs were trained to jump a barrier to avoid

shock. There were no differences between the low-protein pigs and the control pigs in acquisition of the avoidance response; but there was a difference between the deprived and control animals in the amount of time it took to extinguish the response with the low-protein group taking longer.

Leander (1973) using a non-discriminative avoidance procedure showed the effects of manipulating rats' weights on avoidance. The Sidman nondiscriminative avoidance procedure consists of placing a rat in a chamber and giving brief electrical shocks at fixed intervals of time. No external cues are present. A lever press postpones the shock for a specified interval. By manipulating the free feeding weights of rats from 100 percent to 90 percent, 80 percent, 70 percent and back to 100 percent, Leander (1973) discovered that as the weights of the animals decreased responses per minute also decreased, and that the number of shocks delivered per minute increased.

Levitsky (1971) tested rats which had been malnourished in early life, and then rehabilitated on two avoidance tasks. One avoidance task required the animal to jump onto a platform above an electric grid floor to avoid shock. No difference between the malnourished and control animals was found in acquisition, but the previously malnourished animals required longer periods of time to leave the platform. On the other avoidance task, the "Sidman avoidance," which requires the animal to constantly press a lever to insure avoiding shocks, Levitsky (1971) discovered that the previously malnourished animals displayed a greater total number of responses than control animals. The apparent discrepancy between Levitsky's (1970) study and that of Leander's (1973) study can be

partially explained in that Levitsky rehabilitated his animals before the task and Leander gave the task at the time of weight loss.

In these studies it is seen that employing different performance requirements (avoidance as opposed to a T-maze), and testing behavior during or after deficiency, different results can be obtained. There seemed to be no difference in the acquisition of an avoidance task between malnourished and well nourished animals. However, there was a difference in the time it took to extinguish the response with the malnourished animals taking longer. Levitsky (1971) interpreted his results by saying that early protein-calorie malnutrition results in (after rehabilitation) an increase in the sensitivity of an animal to aversive stimulation. Leander (1973) indicated that a weight loss in adult animals resulted in a lower performance level.

Several studies attest to the assumption that a specific vitamin deficiency may have an effect on the behavior of animals. Sloan and Chow (1964) reported that rats fed a diet deficient in pyridoxine (Vitamin B_6) did not perform as well as control rats on three specific behavioral tests. The first two tests involved five minutes of a Sidman avoidance component alternating with five minutes of an escape component. In the avoidance component, the rats were placed in an operant chamber that was provided with a lever and a grid floor that allowed for the delivery of electrical shock. If the animal pressed the lever during the avoidance component, shock was avoided for twelve seconds (called the response-shock interval); if the animal did not press the lever within three seconds (called the shock-shock interval), a brief electrical shock of 1.6 milliamperes was

delivered. In the alternating five minute escape component, shock came on and stayed on until a lever press terminated the shock. Avoiding shock was not possible in this component. Results from this study showed the control subjects better on both acquisition of the avoidance response and acquisition of the escape response. The control rats made more avoidance responses and stayed in shock less time on the escape procedure than the deficient animals.

Sloan and Chow's (1964) third test of behavior involved the acquisition of a lever press using water as the reinforcer. Both the controls and deficient animals were deprived of water; the deficient animals were also deprived of vitamin B_6. The results indicated that the control animals acquired the lever press using water as the reinforcer in less time than did the pyridoxine deficient rats.

The results from various studies on animal behavior as it relates to nutrition allow one to conclude that malnutrition, protein and/or calorie deficiencies, and some vitamin deficiencies, do affect the behavior of animals as measured by such tasks as Y-mazes, T-mazes, avoidance tasks, and acquisition of lever presses with positive reinforcers. It is also seen that deprivations may have differing effects on an animal's behavior depending upon the task, the time the deprivation occurs, and whether the animal has been rehabilitated or not.

Discriminative Avoidance Behavior

Rationale for use of discriminative behavior

To study the relationship between magnesium deprivation and the behavior of an organism, an adequate and objective measure of the organism's behavior before and/or during deprivation is appropriate. The use of a single subject ABA design and such parameters as escaping or avoiding shock by pressing a lever or moving to another compartment provides such a measure (Sidman, 1960). Other measurements of behavior using schedules of positive reinforcement were not felt to be applicable because such measurements require the deprivation of food or water. These deficiencies might produce unwanted and interacting effects on the magnesium deficiency. Conditioned emotional response is another program, but it also requires working for appetitive rewards.

There are two types of avoidance procedures most frequently used in the lab: free-operant avoidance (Sidman, 1966) and discriminative avoidance (Hoffman, 1966). The distinction between these two types of procedure is that discriminative avoidance provides an exteroceptive stimulus and cancels a scheduled shock; in effect avoids the shock. If the shock is delivered, however, it stays present until the animal responds and thereby terminates the shock (escape) (Sidman, 1966).

Either type of avoidance procedure is appropriate in the study of a nutritional deficiency. But for this particular research, the discriminative avoidance schedule was used, for one reason, because Guthrie (1971) suggested that a mag-

nesium deficiency might reveal itself more readily in a condition of "stress." Several studies attest to the assumption that the discriminative avoidance paradigm generates a condition of "emotionality" which suppresses other ongoing behaviors during the presentation of the exteroceptive stimulus prior to shock (Hoffman and Fleshler, 1962a). The discriminative avoidance schedule in this research was used to generate a condition of "stress." Another reason for the use of the discriminative avoidance behavior was to gather data on both avoidance and escape behavior. Within the free-operant avoidance procedure brief electrical shocks are given which cannot be terminated by the animal. The discriminative avoidance procedure, on the other hand, does provide for the measurement of responses that both avoid shock and escape shock.

Discriminative avoidance procedures

The discriminative avoidance procedure represents merging operant conditioning techniques with the methods of classical conditioning. A neutral stimulus (i.e., tone or light) is scheduled to precede each occurrence of an aversive event (i.e., electrical shock). This stimulus serves, in effect, as a warning stimulus; and is sometimes referred to as a "conditional stimulus" because of its repeated pairings with aversive event. If the animal emits a response during the conditional stimulus presentation, the aversive event does not occur. If no response is emitted during this warning period, the aversive event and the conditional stimulus are continuously presented until the animal does emit a response which then terminates both the aversive event and the conditional

stimulus. This procedure is repeated on a trial by trial process until the animal comes to emit a response during the warning stimulus and therefore avoids the aversive event. The behavior is then said to be discriminated in the sense that it is under the control of the exteroceptive stimulus (Hoffman, 1966).

Problems encountered with discriminative avoidance behavior

Problems with the acquisition and subsequent maintenance of discriminative avoidance have been noted in the literature. Hoffman, Fleshler and Chorney (1961), for example, reported an experiment in which many subjects failed to reach efficient avoidance even after fifty sessions. Meyer, Cho and Wisenmann (1960) described unsuccessful attempts to develop avoidance behavior in their animals. Coons, Anderson and Myers (1960) reported that some animals that had learned to avoid well, eventually lost the avoidance response with no changes within the parameters of the experiment.

A problem encountered with a lever press response has been the phenomenon of lever holding (Hoffman, 1966). This occurs when the animal does not let go of the lever after making a response, and continues holding the lever down throughout the interval between trials. Three techniques have been used successfully to eliminate, or reduce this problem. One technique is to place shock on the lever at the same time shock is delivered to the grid floor (Myers, 1959). Another technique is to make the manipulandum relatively inaccessible to the animal, thus making it extremely hard for the animal to hold the response

(Sidman, 1953). In another technique, Feldman and Bremner (1963) arranged for grid shock to be delivered whenever the animal did not release the lever within a period of time after either an avoidance or escape response was emitted; thus, in effect punishing lever holding behavior.

Another problem that interferes with avoidance performance is the phenomenon of freezing (Hoffman, 1966; Bolles, 1970). Several investigators have reported that at the onset of the warning stimulus some animals will freeze in their tracks and very slowly emit the avoidance response, other animals will go to the lever and simply hover over it until shock is presented; thus, failing to avoid shock (Myers, 1959; Hoffman and Fleshler, 1962a). Hoffman (1966) suggested that this phenomenon might represent conflicting response tendencies. The source of this conflict might possibly occur when the animal begins to execute the avoidance response and before the response is made, shock is delivered; the animal then is, in effect, being punished for an approach to the very behavior that would permit avoidance of the shock. Hoffman (1966) suggested that this theory of conflicting response tendencies could explain loss of avoidance behavior and other failures of avoidance behavior that have been reported. Bolles (1970), on the other hand, suggested that the freezing behavior is a natural reaction which is a part of the animals species specific defense reaction (SSDR) that involves fleeing, freezing, and fighting.

Feldman and Bremner (1963) devised a technique which seems to reduce the problem of freezing. The technique, in effect, is to punish freezing behavior. During the warning stimulus, the experimenter delivers a sequence of

shocks if the animal freezes. As soon as the animal approaches the lever, the series of shocks are terminated unless the animal freezes again.

Nakamura and Anderson (1962) reported differences in avoidance behavior between strains of rats. The general findings were that females were superior to males and Long-Evans hooded rats were superior to Sprague-Dawley albinoes.

Variables affecting discriminative avoidance behavior

The general data as to how long the interstimulus interval should be (the time the warning stimulus precedes the aversive event) is somewhat inconclusive. Most of the work with bar-press discriminative avoidance has involved intervals around five seconds, but as Hoffman (1966) concluded, "whether or not this is an optimal value is not yet clear." Kamin (1963), in a study on the effects of the intersession interval, concluded that intersession intervals of less or greater than one hour did not usually produce a reduction in the rate of improvement within the session.

Myers (1959) reported that a tone as the conditional stimulus was superior to a buzzer because animals will escape from a buzzer sound that never was paired with shock, but animals will not escape from a pure tone that was never paired with shock.

Theories of discriminative avoidance behavior

One of the earliest theories on avoidance behavior was conceived by Tolman (1932). According to this theory the animal, after repeated pairings between the warning stimulus and aversive event, begins to anticipate the aversive event. According to this theory, the avoidance response is a reaction involving foresight. A major problem with this theory is that the introduction of the term foresight creates more problems and questions in defining foresight than it does in explaining avoidance behavior.

Sidman (1953) and Dinsmoor (1954) have attempted to explain avoidance behavior in terms of punishment of all behavior other than the avoidance response. In this theory, all behavior except avoidance behavior is likely to be followed and subsequently punished by an aversive event. What occurs is that avoidance behavior acts to terminate all other behavior which is aversive by its association with the aversive stimulus. The probability of behavior associated with an avoidance response occurring is increased; the probability of behavior associated with the aversive stimulus is decreased. Sidman (1953) used this theory, mainly to describe free-operant avoidance behavior, but it does have implications in an analysis of discriminative avoidance behavior.

A theory called the "dual-process" or the conditional stimulus termination theory has received a great deal of attention in explaining the acquisition and maintenance of the discriminated avoidance behavior. This theory states that the avoidance response is motivated by a conditioned emotional reaction

which develops when the conditioned stimulus is paired with the aversive stimulus on the trials when the animal fails to avoid. The theory maintains that reinforcement occurs when the avoidance response terminates the warning signal and this termination leads to a reduction in emotionality within the animal (Hoffman, 1966).

Bolles (1970) has recently attacked the "dual process" theory and presented a theory of discriminative avoidance behavior based upon the concept of species-specific defense reactions (SSDR). Bolles (1970) claimed that each specie of animal has a set of reactions in which to handle aversive events. These reactions include fleeing, freezing and fighting. He further maintained that any novel stimulus can elicit these reactions. With this basic concept of SSDR, Bolles felt that the difference found in acquisition of avoidance behavior can be explained. The reason bar-press discriminative avoidance behavior is learned slowly and uncertainly is that the response is not a SSDR. For an avoidance response to be rapidly learned in a given situation, as seen in wheel turning, the response must be an effective SSDR in that situation, and when rapid learning does occur, it is primarily due to the suppression of ineffective SSDRs. Bolles' (1970) basic objection to the "dual process" theory lies in the fact that rapid learning of an avoidance response can take place without extensive pairings of the conditional stimulus and the unconditional stimulus. In fact, Bolles maintained that the so called conditional stimulus, the termination of which reinforces the animal, functions as a discriminative stimulus which when terminated tells the animal, in effect, that shock is not going to occur. This

theory, then, puts avoidance behavior in the realm of discrimination learning and out of reinforcement phenomenon.

As can be seen by the review, the discriminative avoidance schedule can provide a trial by trial measurement of the specific behavior of an organism. It has also been pointed out that several precautions must be taken into account (i.e., lever holding, freezing etc.) when using this schedule. It is also noted that several theories have been formulated in which to explain the formulation and maintenance of avoidance behavior. Both the "dual process" theory and Bolles' SSDR theory seem to be the best suited theories at this time for explaining the discriminative avoidance behavior.

Dietary Magnesium

Physiological and biochemical functions

Intercellular magnesium acts as a catalyst to several hundred biological reactions, a major portion taking place in the mitochondria (Guthrie, 1971). All the enzymes that catalyze the transfer of phosphate from adenosine triphosphate (ATP) to a phosphate receptor, or from a phyosphorylated compound to adenosine diphosphate (ADP) are activated by magnesium (Aikawa, 1963). Magnesium increases the activity of ATP. Skou (1965) reported that the ATP-magnesium complex is the active enzymatic substrate involved in the transfer. Since reactions involving ATP and ADP are fundamental and widespread, magnesium therefore influences all life processes (Krehl, 1967).

Magnesium is also essential in carbohydrate metabolism when sugar enters the glycolytic pathway. A lack of magnesium would inhibit at least seven reactions essential to the glycolytic pathway of converting sugar into pyruvic acid (Aikawa, 1963).

Magnesium influences protein synthesis by affecting the arrangements of the protein-synthesizing organelles of the cell, the ribosomes, and by facilitating the attachment of ribonucleic acid (RNA) to the ribosome. This makes magnesium necessary for the activation of amino acids, for the synthesis, degradation and stability of deoxyribonucleic acid (DNA) (Guthrie, 1971).

Magnesium also provides the proper environment in the extracellular fluid of nerve cells to promote the conduction of nerve impulses and to allow normal muscular contraction. The composition of the interstitial fluid must represent a certain balance between elements that tend to stimulate muscular contraction such as calcium, and those that exert a relaxing effect such as sodium, potassium and magnesium (Guthrie, 1971).

Magnesium deficiency

When discussing the effects of a dietary magnesium deficiency, it must be realized that there are many other nutrients that interact with magnesium; and that a decrease in magnesium levels can be affected by other nutrients through decreasing or increasing their level. Krehl (1967) reported that a magnesium deficiency is readily produced by increasing the protein intake of an animal having a marginal magnesium intake. It is also reported (Guthrie, 1971; Krehl,

1967) that the amount of magnesium absorbed in the body decreases as fat, calcium and vitamin D increase in the body. Krehl (1967) explained that the reason the absorption of magnesium decreases when calcium absorption increases might be that they both share a common transport mechanism across the intestinal membrane with possible preference for calcium in this mechanism.

The close interaction between calcium and magnesium is seen in their antagonistic roles in conduction of nerve impulses and normal muscular contraction. Magnesium is a relaxor substance and calcium is a stimulant substance. The overall effect of a decrease in either nutrient leads to increased neural excitability and enhanced neuromuscular transmission (Wacker and Parisi, 1968). Low concentrations of magnesium lower the excitability thresholds of the presynaptic nerve and of the muscular membrane; and since magnesium acts as an inhibitor of the release of acetycholine from motor-nerve terminals in pharmacological doses, the low magnesium concentration possibly increases the liberation of the acetylcholine (Wacker and Parisi, 1968; Krehl, 1967).

In adult rats, a decrease in magnesium also results in a decrease in potassium and an increase in sodium. Evidence suggests that with sufficient magnesium depletion the pumping mechanism that maintains normal Na^- and K^+ gradients falter (Wacker and Parisi, 1968). (For a more extensive review of elements that interact with or against magnesium, see Aikawa, 1963, pp. 87-98).

In rats, low magnesium level, leading to magnesium tetany, has the physical signs of uncontrolled neuromuscular activity diagnosed as tremors becoming increasingly more serious until convulsive seizures occur in the more severe deprivations (Guthrie, 1971).

Aikawa (1963) reported that with a diet containing 1.8 ppm of magnesium (a low level), there was acute hyperemia of the skin, loss of hair, convulsions and many fatalities within eleven days. The onset of symptoms is very rapid, being detectable within a few days after the animals are placed on a low magnesium diet. The first phase of the deficiency, lasting about two weeks, is characterized by vasodilation (reddening of the ears and feet), hyperemia and hperexcitability. The second phase is marked by the development of malnutrition, cachexia and renal damages. During this period, the calcium content in the heart and muscle increases by 50 to 100 percent, and in the kidney the calcium level may be as much as fifteen times its normal value. During prolonged deprivation, the body content of magnesium is reduced to about two-thirds of the normal value. Krehl (1967) reported that magnesium deficiency in rats takes about eight to ten days for the marked peripheral vasodilation. This vasodilation may fade and reoccur depending on the activity and stimulation of the animal. As the deficiency proceeds, animals become more susceptible to any noise or stimulation which startles them, and this precipitates severe convulsive seizures which may result in death.

In human studies, Vallee, Wacker and Ulmer (1960) claimed discovery of human magnesium deficiency tetany. The physical manifestations are close

to those seen in magnesium deficient animals. The administration of magnesium sulfate promptly and completely reversed the symptoms. The authors claimed the causes of the deficiency may be dietary restrictions, malnutrition or malabsorption, or increased excretion of magnesium.

Magnesium deficiency in humans may develop as part of a general nutritional deficiency (i.e., Kwashiorkor) or severe alcoholic cirrhosis (Aikawa, 1963). Guthrie (1971) said that the problem of magnesium deficiency seems very acute with alcoholics since alcohol increases the rate of magnesium excretion and may partially explain the loss of neuromuscular control diagnosed as magnesium tetany in alcoholics. Krehl (1967) maintained that alcohol may adversely affect the renal capacity to reabsorb magnesium which may contribute, along with dietary inadequacies, to the severe hypomagnemia of the alcoholic.

Body content, absorption, excretion and requirements

In human adults, magnesium content of the body is about 21 to 28 grams; 50 to 60 percent is concentrated in bone, the remainder being almost equally distributed between muscle and the nonmuscular soft tissue (Guthrie, 1971; Krehl, 1967; Wacker and Parisi, 1968). The serum level of magnesium for an average human is 2.5 milligrams/100 milliliters. Values of serum magnesium for different age groups and sex are not significantly different, indicating a mechanism in the body responsible for maintenance of stable

magnesium levels (Raut and Viswanathan, 1972). (For a complete survey of magnesium content in the human body, see Aikawa, 1963, pp. 62-64; also Raut and Viswanathan, 1972). Altman (1961) reported that the normal level of magnesium in rats' blood serum is around 16 milligrams/liters or 1.6 milligrams/100 milliliters.

The factors controlling magnesium absorption are not fully understood, but it seems that the gastrointestinal mucosa absorbs the magnesium ion selectively and variably. Only a small proportion of the magnesium exposed to the surface of the mucosa is absorbed. The magnesium enters the extracellular fluid compartment from the intestinal mucosa. A small portion of the extracellular magnesium is absorbed into plasma proteins and may serve as the most readily available pool of magnesium supplying the needs of the soft tissues. The bone store of magnesium functions to maintain the usual concentration of magnesium in soft tissue and extracellular fluid. The younger the animal, the more rapid the uptakes of magnesium by bone. More than half the total bone store is released before the serum magnesium concentration decreases. In a study by Watchoun and McCance (1937) rats partially deficient in magnesium were normal or only slightly subnormal in amounts of magnesium in the organs, but the blood, bone and teeth became permanently deficient in magnesium and calcification of the kidney was found. Apparently bone magnesium represents a store which can be mobilized under conditions of deficiency to supply the needs of soft tissues.

Approximately two-thirds of ingested magnesium is excreted in the feces. Of the amount of magnesium absorbed in the body, approximately one-third or less is excreted in the urine (Aikawa, 1963).

Even though the minimum daily requirement for magnesium has not been established for humans, the average daily recommended (ADR) intake of magnesium is around 300 to 350 milligrams for women and 350 to 400 milligrams for men (Krehl, 1967). According to the Food and Nutrition Board of the National Council, the typical American diet provides about 120 milligrams of magnesium per 1000 Kcal, a level that will barely provide the recommended intake (Guthrie, 1971). Guthrie (1971) further maintained that the reason we do not see magnesium deficiency symptoms in the American population can be explained by the fact that the population experiences a slight deficit that becomes significant only when conditions of stress are present. It has also been pointed out (Guthrie, 1971) that alcoholics as a group possess the most serious deficiency of magnesium in this country because of the depletion of minerals that is experienced with drinking alcohol and by the poor diets that are maintained by this group. Krehl (1967) said that it is apparent that children suffering from Kwashiorkor (protein deficiency), persons maintained for long periods on magnesium free fluids, and persons suffering prolonged losses because of nausea or diarrhea are also subject to magnesium deficiencies.

Guthrie (1971) provided a general account of foods high in magnesium content. The high chlorophyll content of green leafy vegetables accounts for their relative position as a good food source for magnesium. Oriental diets

with emphasis on rice, soybeans, and fish provide a good source of magnesium. Diets high in pork, beef, milk products, eggs and fruits provide a relatively poor source of magnesium.

This review has shown that magnesium must be considered of vital importance in health and maintenance of man and animal. Since there is no research that reports on magnesium deficiency as it affects avoidance behavior, and since some level of magnesium deficiency is suspected to affect many people, magnesium deficiency as it affects an animal's behavior appears worthy of investigation. It is especially important in terms of avoidance since periods of stress maximize the use of magnesium by the body.

STATEMENT OF THE PROBLEM

It has been shown that general malnutrition, specific protein deficiencies, specific calorie deficiencies and certain specific vitamin deficiencies affect both animal and human behaviors. It has also been illustrated that a variety of tests can be used to measure behavior. These tests include intelligence tests for humans; and Y and T-mazes, positive reinforcement schedules, avoidance and escape schedules for animals. The conditions as to the measurement of behavior have also varied from testing of acquisition of specific behaviors both during certain deprivations and/or after rehabilitation from the deficiencies, to testing an animal's already learned behavior during deprivation and after rehabilitation.

The use of an analysis of behavior design and such parameters as escaping and avoiding shock by pressing a lever, or moving to another compartment, provide a measurement as to the study of the functional relationship between a specific nutrient deprivation and the behavior of an organism. One objective measure of an animal's behavior is discriminative avoidance behavior. With this schedule, one can observe an animal's behavior on a trial-by-trial process of both avoiding and escaping an aversive stimulus.

Magnesium has been shown to be an extremely important mineral in the physiology and behavior of humans and animals. Yet no research has reported on the effects of magnesium deprivation upon the avoidance behavior of animals.

The objectives of this research were as follows:

1. To determine if a magnesium deficient diet affects ongoing avoidance behavior.
2. If there is an effect on avoidance behavior; to determine if this effect is reversible.
3. To determine if a magnesium deficient diet affects acquisition of an avoidance task.

To this end in Experiment I, rats underwent discriminative avoidance training, then were provided a magnesium deficient diet. In Experiment II, rats stable on an avoidance schedule were given a magnesium deficient diet and effects noted then returned to the original basal diet and stability re-established, then given a second magnesium deficient diet series. In Experiment III, rats were given a magnesium deficient diet, then original avoidance training.

METHODS AND RESULTS

Diet Composition

The diet formula used for all subjects in all three experiments was obtained from Nalder et al. (1972) and is listed in Appendix A. The control diet, given to all the control animals, was made by combining all ingredients listed. The magnesium deficient diet, given to all experimental animals, was the same as the control diet except that $MgCO_3$ was omitted.

Experiment I

Subjects

Male Sprague-Dawley rats from Simonsen Laboratories, Gilroy, California, served as subjects for Experiments I and II. The weights on arrival varied from 40 to 60 grams. All rats were housed in individual stainless steel cages with free access to distilled water and either the control diet or the magnesium deficient diet throughout the experiments. For experiment I, 22 rats were used.

Apparatus

For Experiments I and II, which used a lever press as the response, a chamber measuring 12 inches long by 10 1/4 inches wide by 8 inches high was employed. Within this chamber, a response bar protruded from the center of

one wall 5/8 of an inch and 3 1/8 inches above the floor. A force of 23 grams was sufficient to displace this lever and to operate the contact switch. On the same wall immediately to the left of the lever was a red light used as a conditional stimulus (CS). In conjunction with the red warning light a Malory speaker (model SC628) which at 2 to 28 volts produces an 80 db tone also acted as a CS. The floor of this chamber was parallel stainless steel rods measuring 1/8 of an inch in diameter and spaced 1/2 of an inch apart. An 0.5 to 2.0 milliamperes scrambled electrical shock could be delivered to the animals' feet via the rods. Outside the chamber an 115 volt light was turned on at the beginning of the session to provide illumination inside the chamber. It was turned off at the end of the session. The entire experimental chamber was located in a modified refrigerator shell. Mechanical relays, timers, counters, a shock scrambler, a cumulative recording device, etc. programmed the discriminative avoidance schedule and recorded the data.

Procedure

A discriminative avoidance schedule was programmed for each subject. An intertrial interval (ITI) of 30 seconds terminated with the onset of the interstimulus interval (ISI) which coexisted with the CS (red light and tone). A response occurring during the 8 second ISI terminated the CS and reset the ITI for 30 seconds and shock was avoided. If no response occurred during the 8 second ISI a scrambled electrical shock of 0.6 milliamperes was delivered through the grid bars to the subjects' feet. The shock and CS were continually presented until

the subject emitted a response at which time shock and the CS were terminated and another ITI of 30 seconds was again started. Any response made during the ISI, which in effect postponed shock, was recorded as an avoidance response. Any response made after the ISI, which in effect terminated shock, was recorded as an escape response. Basic data collected over the experiment were total responses per session, avoidance responses per session, escape responses per session and the total time in seconds that the animal was in the presence of electrical shock within a session.

Rats in Experiment I were given 3-hour daily sessions. The procedure was to use 4 to 6 rats, then after about 20 days, another 4 to 6 rats were started. This procedure continued until 22 rats were run. Since these subjects were also being used for biochemical analysis and had to be killed approximately a week after magnesium deficiency began, it was only possible to use the rats about 17 to 20 days, with the first 0 to 10 days being used for training avoidance and escape. The baseline period (BL) in this paper will refer to the consecutive daily sessions prior to the introduction of the magnesium deficient diet which was usually instituted around the 6th to 10th session. After the BL behavior had stabilized (three days of approximately the same number of avoidance responses per session), a magnesium deficient diet was given to half of the rats (the experimental group) chosen randomly. The other rats were maintained as before with no change in their diet (the control group). Because food intake might have presented a problem, each control rat was assigned on the basis of similar BL avoidance or escape performance to one of the experimental rats as a pair-fed

partner. This simply meant that the pair-fed control rat whose behavior was similar to its pair-mate was given the same amount in grams of control diet as the magnesium deficient rat consumed the previous day.

Results

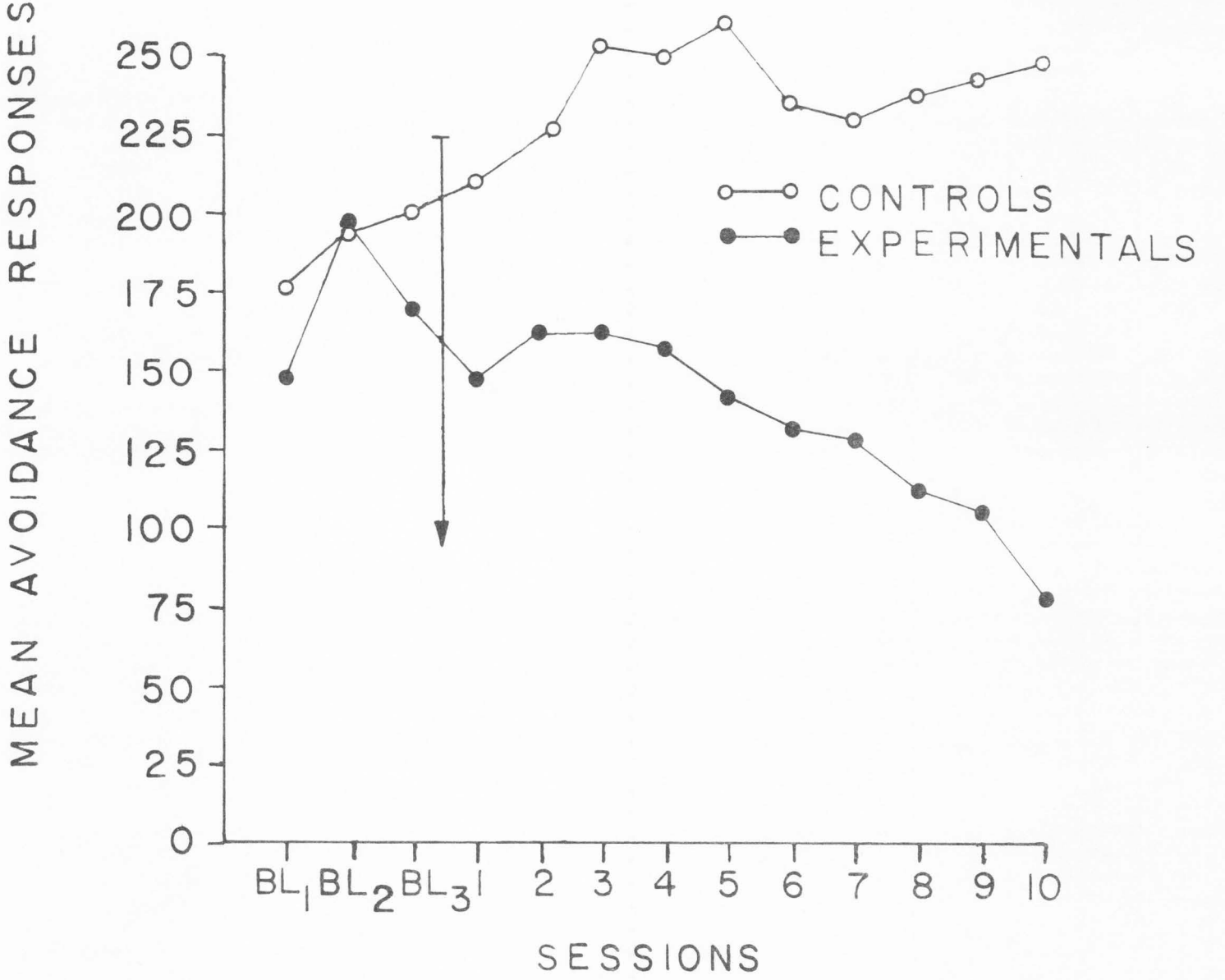
Out of the 22 rats used for Experiment I, 10 were avoiding shock at an average of 75 or more avoidance responses per session during the 3-day BL period (Figures 1 and 2). This minimum avoidance response level represented an approximate mean of 25 percent of the total responses per session for the 3-day BL period.

Eight of the 22 rats failed to acquire the avoidance response to criterion level, but continued to escape shock at a high rate throughout the experiment (Figure 3).

The remaining 4 rats represented unstable BL performance by either beginning to avoid shock during the 3-day BL period or began to systematically decrease their avoidance responses before introduction of the magnesium deficient diet to the experimental animals.

The main concern of Experiment I was with the 10 animals that were avoiding shock at the minimum criterion level. Half of these rats (5) were the experimental group; half (5) were their pair-fed control partners. Figure 1 illustrates the effect that the magnesium deficient diet had on the day-by-day mean avoidance responses of the experimental animals as compared to the day-by-day mean avoidance responses of the control rats. Both groups of rats

Figure 1. Mean avoidance responses for each daily session for the control and experimental rats which were avoiding shock at least 75 times per session. BL_1, BL_2, and BL_3 are the baseline sessions. The arrow represents the day the magnesium deficient diet began to be fed to the experimentals. Controls, N = 5; experimentals, N = 5.


MEAN AVOIDANCE RESPONSES
250
225
200
175
150
125
100
75
50
25
0
CONTROLS
EXPERIMENTALS
BL1 BL2 BL3 1 2 3 4 5 6 7 8 9 10
SESSIONS

were avoiding shock at approximately the same level during the BL period. The experimentals did not decrease their avoidance responses until the fifth day of magnesium deficiency. A steady decrease was noted for the remaining five days. The controls, on the other hand, continued to increase in their number of avoidance responses over the ten day period.

Figure 2 illustrates that the control animals were avoiding shock at an average of 191 $\pm$ 13 (SE) times per session during the three day BL period. The experimental animals, which had not as yet been introduced to the magnesium deficient diet were avoiding shock for this three day BL period on the average 172 $\pm$ 17 (SE) times per session. Using a paired T-test (Steele and Tori, 1961), no statistical difference in avoidance responses was found between these two groups during this period. The two bars at the right of Figure 2 indicate that the control animals for the last three days of the experiment had increased their avoidance responses to an average of 243 $\pm$ 9 (SE) per session. The experimental animals had decreased from their BL average to an average for the last three days of 99 $\pm$ 17 (SE) avoidances per session. The paired T-test for the last three days of the experiment gave a statistical difference ($P < .001$) between the two groups.

Figure 3 shows the results from the eight rats which never did avoid at criterion level, but were escaping shock at a consistently high level. The mean number of escapes for the experimental rats and their pair-fed control mates are plotted for each day. As can be seen, no difference between the two groups was noted in escape behavior throughout the entire experiment. Both groups

Figure 2. Mean avoidance responses and standard error of the mean for the controls and experimentals which were avoiding shock at least 75 times per session. The two bars to the left show the mean avoidances and standard error for the three days prior to the introduction of the magnesium deficient diet (BL_1, BL_2 and BL_3). N = 15. The two bars to the right show the mean avoidance responses and standard error for the last three sessions of the experiment (Sessions 8, 9, and 10). N = 15.

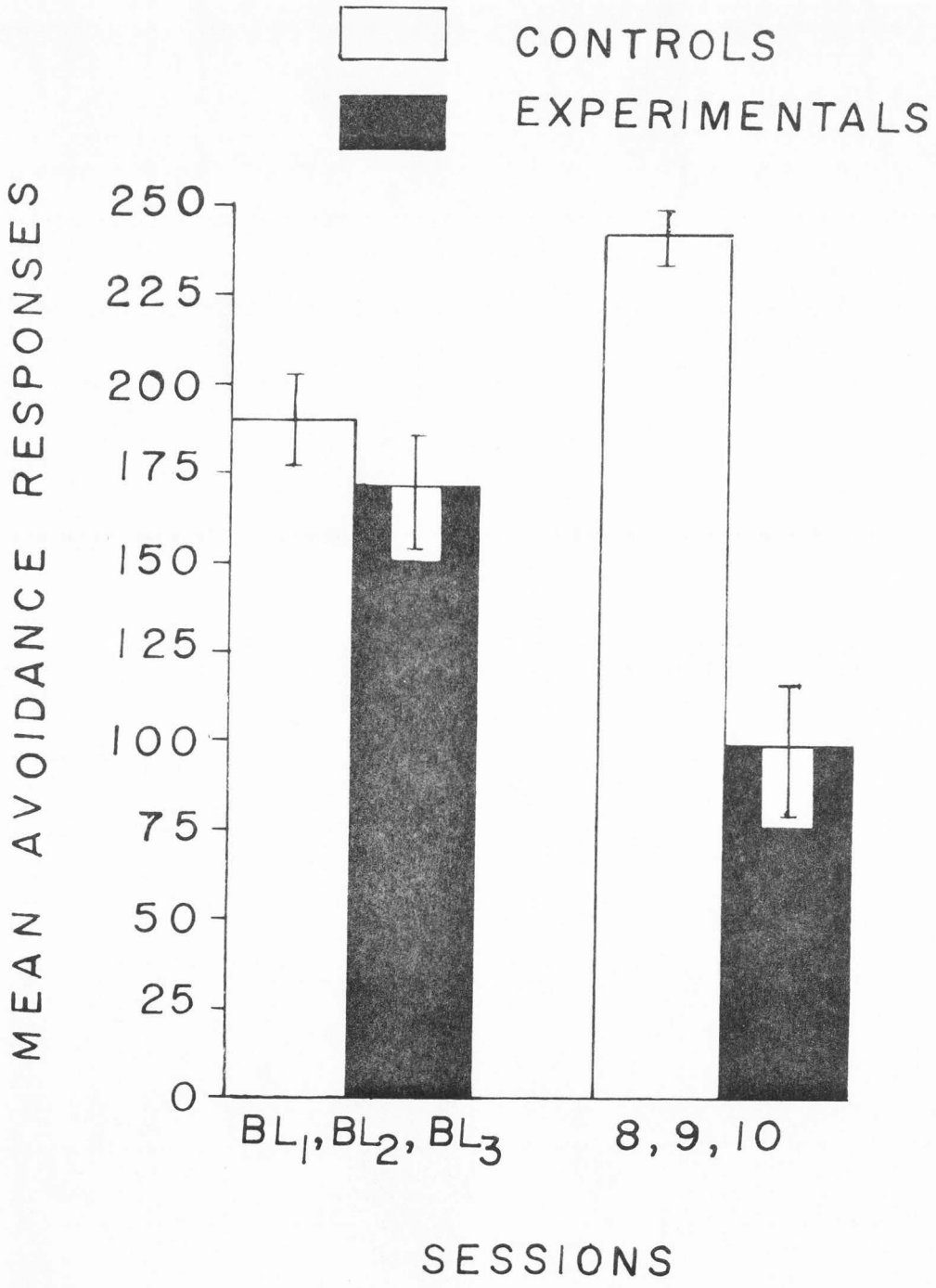
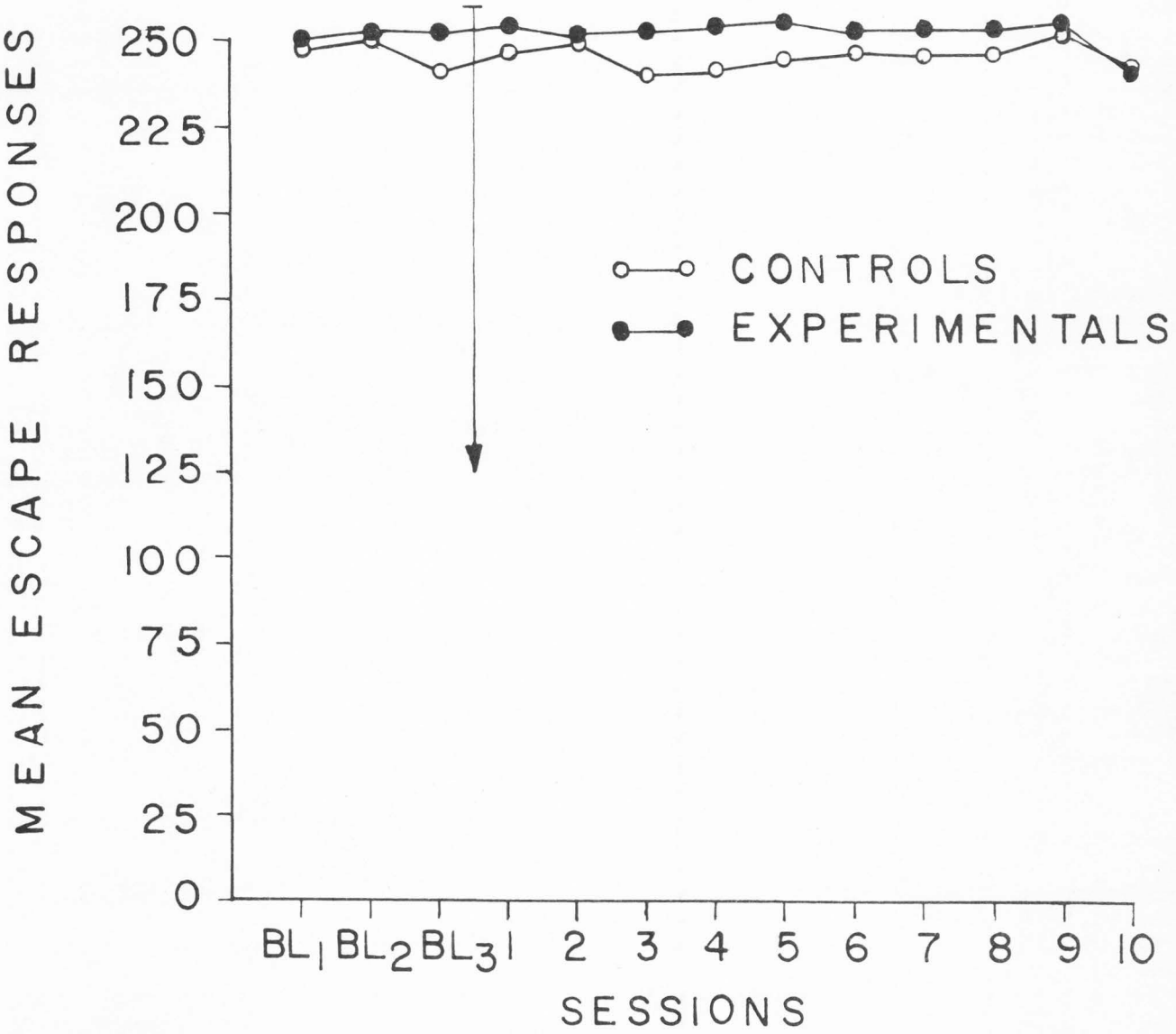

CONTROLS
EXPERIMENTALS
MEAN AVOIDANCE RESPONSES
250
225
200
175
150
125
100
75
50
25
0
BL1, BL2, BL3
8, 9, 10
SESSIONS

Figure 3. Mean escape responses for each daily session for the controls and experimental rats which were not avoiding shock at the criterion level. BL_1, BL_2 and BL_3 are the baseline sessions. The arrow indicates the day the magnesium deficient diet began to be fed to the experimentals. Controls, N = 4; experimentals, N = 4.


MEAN ESCAPE RESPONSES
250
225
200
175
150
125
100
75
50
25
0
CONTROLS
EXPERIMENTALS
BL1 BL2 BL3 1 2 3 4 5 6 7 8 9 10
SESSIONS

continued to escape shock at an average of 240 to 250 times.

Experiment II

Subjects

Four male Sprague-Dawley rats served as subjects throughout Experiment II, A-1, A-2, A-3 and A-5. (See page 26 for further details.)

Apparatus

The same chamber, same response, same shock intensities and the same CS were used as in Experiment I (pages 26-28). Daily session time was shortened to one hour with a five minute warm-up period during which responses were not recorded. The ITI time was reduced to 20 seconds. The ISI stayed at 8 seconds.

Procedure

Since the purpose of this experiment was to replicate Experiment I, and also to rehabilitate the animals, a single subject design was employed using only four subjects; thus it was possible to follow the day-by-day performance of each animal. The procedure was first to train the four animals to avoid shock by bar-pressing (10 to 16 sessions). Then they were given a magnesium deficient diet (4 to 8 sessions). Then their original diet was reintroduced (7 to 13 sessions), thus determining the effects of rehabilitation. Rats A-1 and A-2

both received an additional magnesium deficiency phase (A-1, 4 sessions; A-2, 7 sessions), and an additional rehabilitation period (A-1, 9 sessions; A-2, 2 sessions).

Two added features in Experiment II provided information additional to that of Experiment I. One was to take blood samples for every session from the four rats to determine the serum magnesium levels. Special precautions were taken in the cleaning of cages, food dishes, water containers and test tubes by giving each an acid wash to reduce the chances that any magnesium might be available to the rats. The procedure for the determination of serum magnesium is given in Appendix B. The second feature was to record response latency. Response latency was the length of time in seconds, from the onset of the CS, to either the avoidance response or the escape response. Total avoidance responses, total escape responses and total time in shock were measured as in Experiment I.

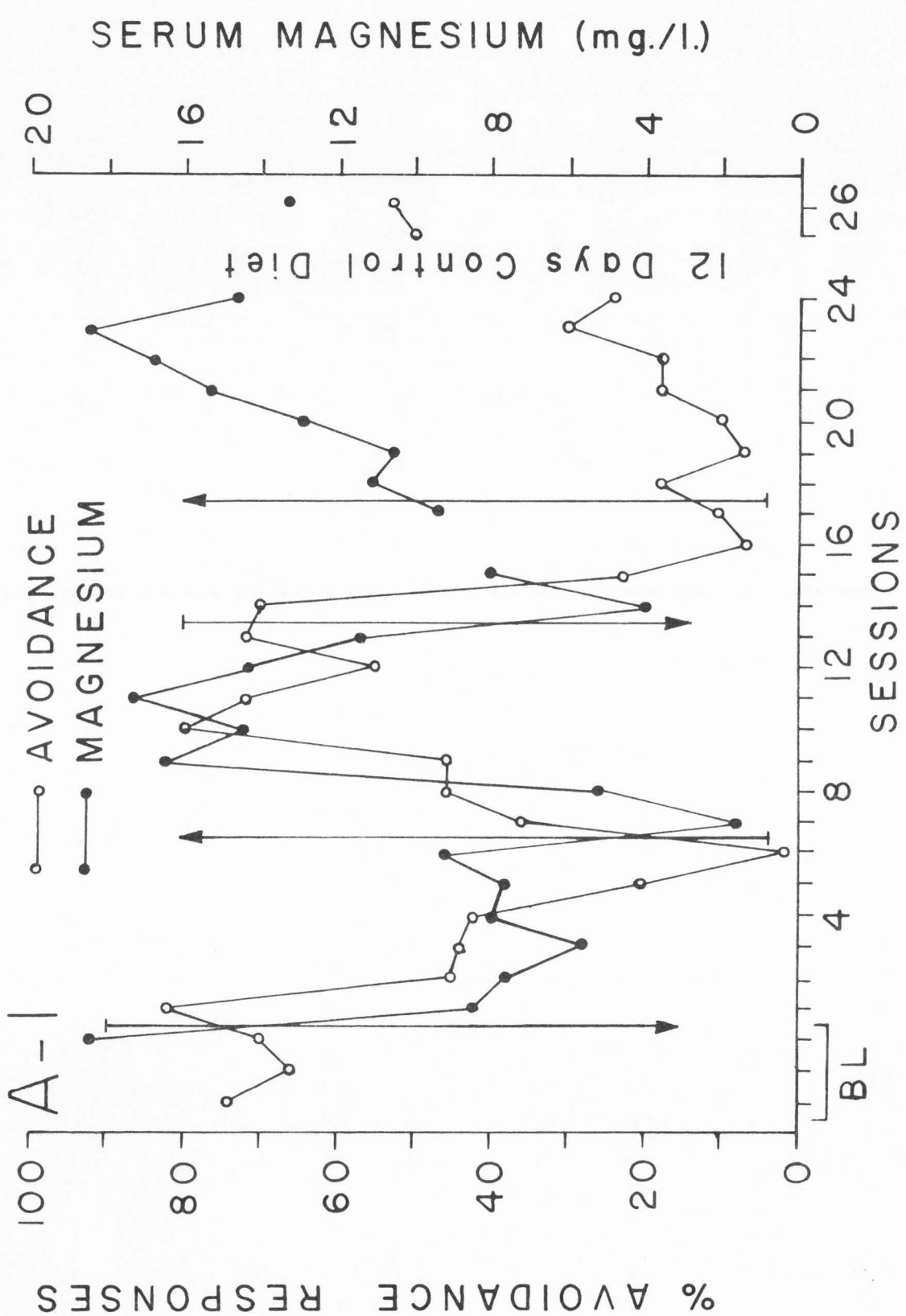
The procedure for training the animals to avoid shock by bar-pressing in Experiment II also differed from that of Experiment I. In Experiment I, animals were placed in the chamber, the program initiated, and avoidance was either learned or not. The problem with this procedure was that many of the rats never acquired the avoidance response. For Experiment II, a procedure similar to that reported by Feldman and Bremner (1963) was instituted. This consisted of punishing freezing behavior during the presentation of the CS by delivering a sequence of brief shocks until the animal moved towards the lever. This procedure proved successful in that all rats learned the avoidance response.

Results

Figures 4, 5, 6, and 7 are individual daily records of the avoidance responses for the four subjects and corresponding concentration of serum magnesium. It should be noted that percent avoidances represented on the left ordinate of each figure were used instead of total avoidances. Percent avoidance was the number of avoidances made per session divided by the number of avoidances plus escape responses made per session. Thus both the percent avoidance and the amount of serum magnesium of each animal for each day can be seen. To the right of each figure a twelve day period is represented in which each rat was given a control diet, but no avoidance sessions were run. This is followed by two additional sessions of the avoidance task and one day of the serum magnesium concentration determination. It should be noted that the normal concentration of magnesium in the serum of rats is 16 milligrams/liter (Ditman, 1963).

Rat A-1 (Figure 4) clearly illustrates the effect that low concentration of blood serum magnesium has upon the discriminative bar-press avoidance behavior. Rat A-1 was fed the magnesium deficient diet twice and rehabilitated twice. One day after both deficiencies, the magnesium level dropped considerably; but it took two days of low magnesium levels before a noticeable drop in avoidance behavior occurred. It took approximately three days of rehabilitation before normal magnesium levels were reached in both rehabilitations. Four days were needed for the first rehabilitation before normal avoidance behavior was reached. The avoidance behavior for the second rehabilitation never did reach BL level, but an increase was noted over the last three weeks.

Figure 4. Percent avoidance responses for rat A-1 and corresponding concentration of serum magnesium for each session. Arrows represent, if pointing down, introduction to the magnesium deficient diet, if pointing up, re-introduction to the control diet. Breaks in the magnesium line indicate that magnesium determinations could not be made for that session. BL refers to the three day baseline period.


SERUM MAGNESIUM (mg./l.)
20
16
12
8
4
0
26
12 Days Control Diet
24
20
16
12
8
4
BL
SESSIONS
AVOIDANCE
MAGNESIUM
A-1
100
80
60
40
20
0
% AVOIDANCE RESPONSES

This same pattern of behavior is seen in Figure 5 with rat A-2. The BL period for this animal is extended to nine days corresponding to A-1's three day BL period and A-1's six subsequent days after. The only difference between A-1 And A-2 was the time it took for rehabilitation. On the first day of rehabilitation for A-2, the magnesium concentration had reached normal levels. Except for this, the pattern of magnesium deficiency and rehabilitation remained approximately the same for A-1 and A-2. A decrease in magnesium concentration corresponded with a decrease in avoidance responses, and an increase in magnesium concentration corresponded with an increase in avoidance response.

Figure 6 represents rat A-3's avoidance behavior and magnesium concentration. The low concentrations of magnesium did produce a decrease in avoidance behavior. The effect of rehabilitation is less certain. On the first day of rehabilitation, A-3's avoidance behavior surpassed its BL level. The subsequent days, even with normal concentrations of magnesium, showed that the avoidance behavior steadily declined and stayed at a very low level throughout the experiment.

Figure 7 illustrates A-5's performance which is similar to that of A-3, a decrease in avoidance behavior with the magnesium deficiency. Rat A-5, though, never exhibited any increase in avoidance behavior after rehabilitation.

Figure 5. Avoidance responses of rat A-2 and corresponding concentration of serum magnesium for each session. Arrows represent, if pointing down, introduction to the magnesium deficient diet, if pointing up, re-introduction to the control diet. Breaks in the magnesium line indicate that magnesium determinations could not be made for that session. BL refers to the nine day baseline period.

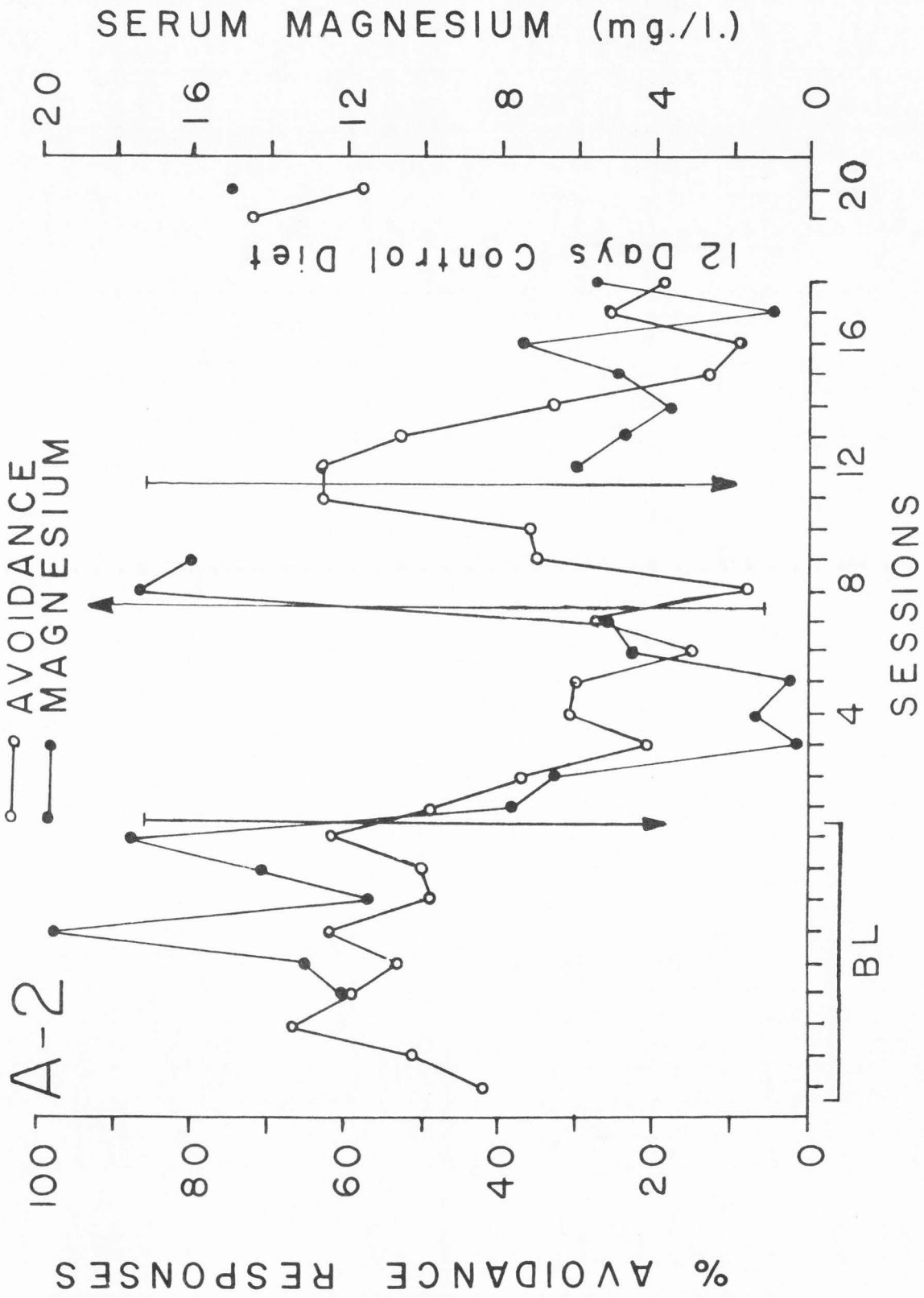

SERUM MAGNESIUM (mg./l.)
20
16
12
8
4
0
20
12 Days Control Diet
AVOIDANCE
MAGNESIUM
A-2
100
80
60
40
20
0
BL
4
8
12
16
SESSIONS
% AVOIDANCE RESPONSES

Figure 6. Avoidance responses of rat A-3 and corresponding concentration of serum magnesium for each session. Arrows represent, if pointing down, introduction to the magnesium deficient diet, if pointing up, reintroduction to the control diet. Breaks in the magnesium line indicate that magnesium determinations could not be made for that session. BL refers to the three day baseline period.

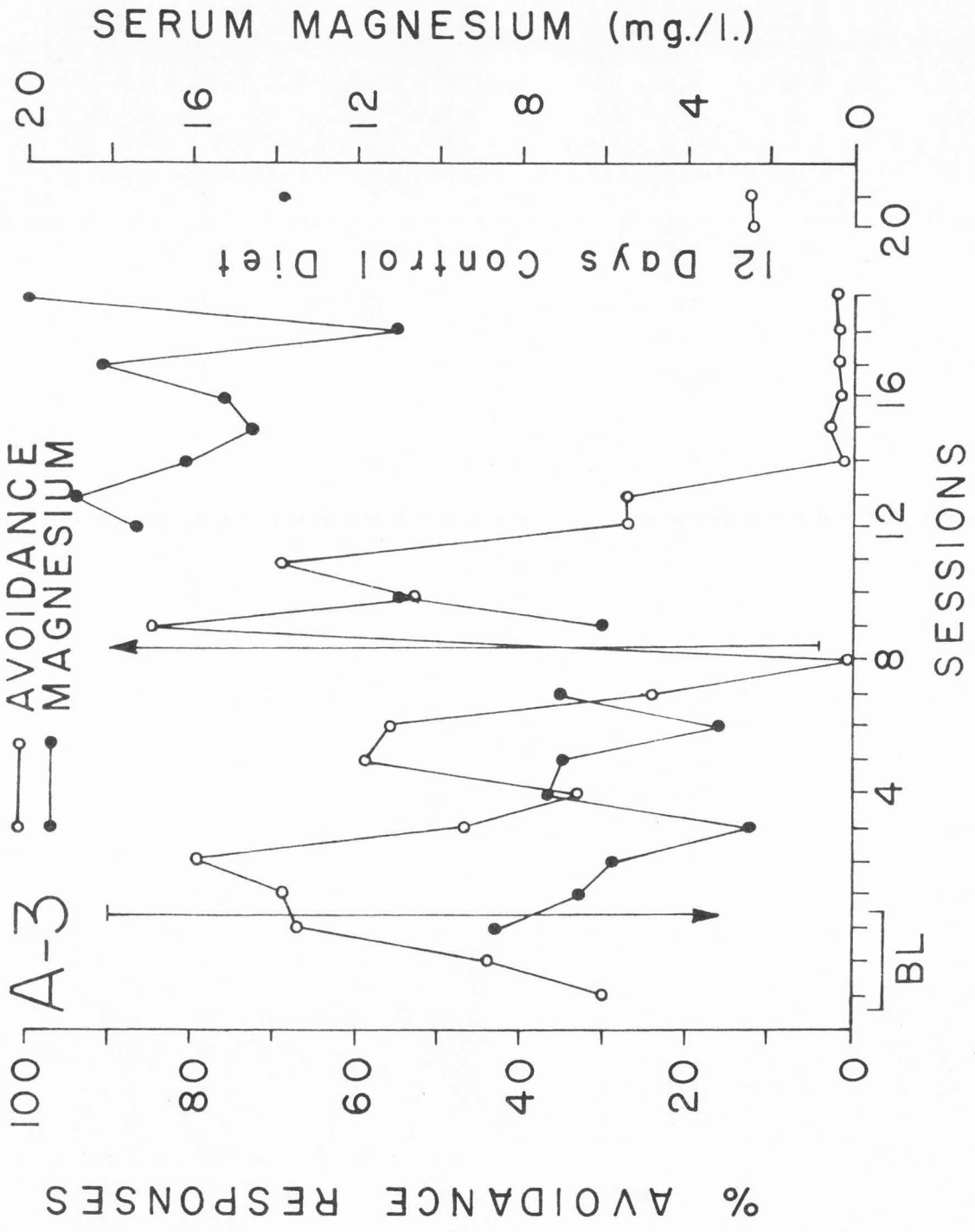

SERUM MAGNESIUM (mg./l.)
20
16
12
8
4
0
12 Days Control Diet
20
A-3
AVOIDANCE
MAGNESIUM
100
80
60
40
20
0
BL
4
8
12
16
SESSIONS
% AVOIDANCE RESPONSES

Figure 7. Percent avoidance responses of rat A-5 and corresponding concentration of serum magnesium for each session. Arrows represent, if pointing down, introduction to the magnesium deficient diet, if pointing up, reintroduction to the control diet. Breaks in the magnesium line indicate that magnesium determinations could not be made for that session. BL refers to the eleven day baseline period.

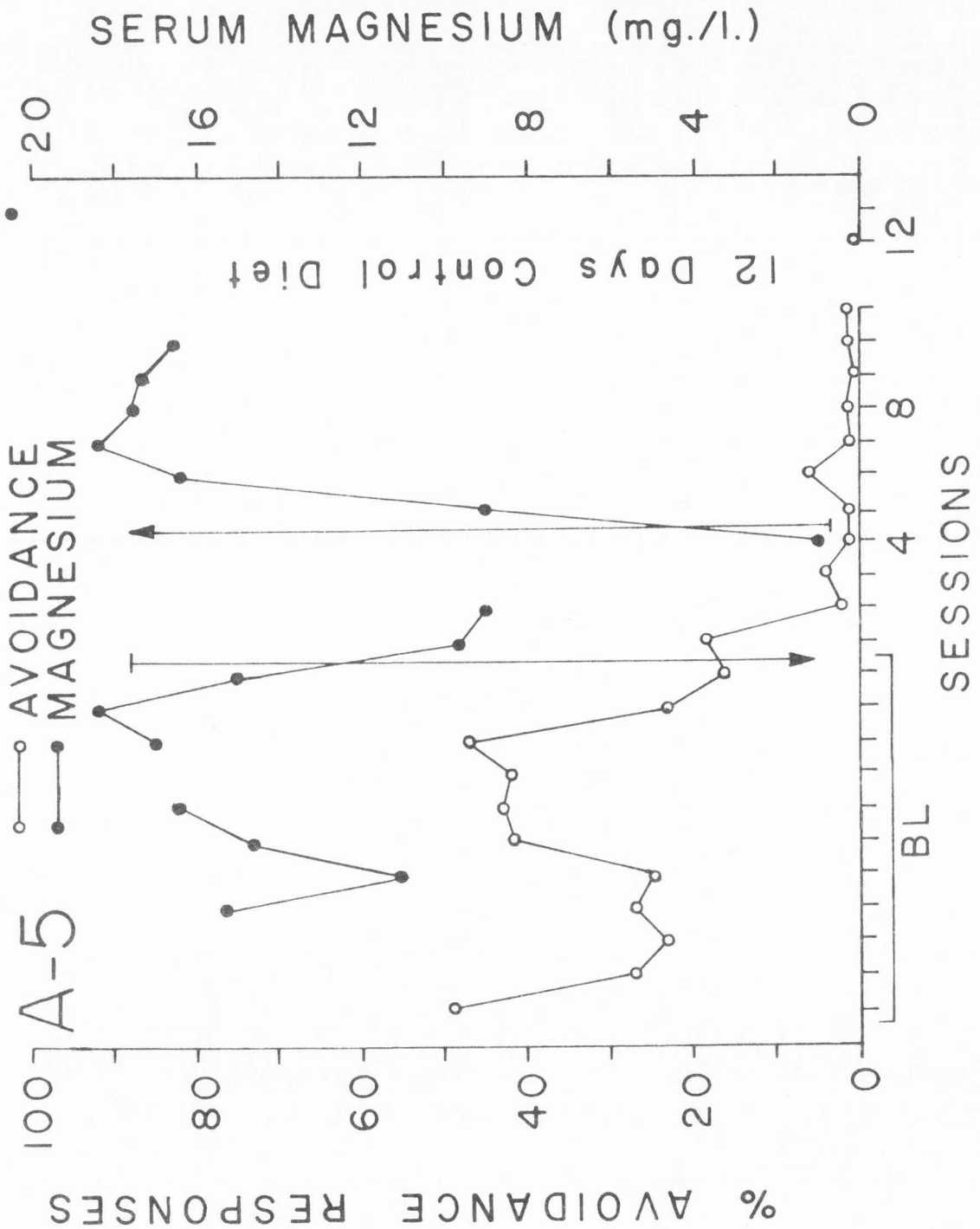

SERUM MAGNESIUM (mg./l.)
20
16
12
8
4
0
12
12 Days Control Diet
A-5
AVOIDANCE
MAGNESIUM
100
80
60
40
20
0
BL
4
8
SESSIONS
% AVOIDANCE RESPONSES

Figure 8a shows the effects that magnesium deficiency had on all four rats' latencies. Latency was the period of time in seconds from the onset of the CS to either an avoidance response or an escape response. The general pattern of all four rats is a longer latency with magnesium deficiency and shorter latencies after rehabilitation. One notable finding was that with a deficiency those animals that were previously avoiding did not stop responding, but began to escape rather than avoid. This is shown by the shift, after deficiency, to mean latency times terminating after the onset of shock. In effect, then, after deficiencies, the rats did continue to bar-press even though it was escape responses.

Figure 8b shows the effects of deficiency and rehabilitation on the escape latency time of A-3 and A-5. The escape latency is the period of time between the onset of shock and the escape response. As can be seen, magnesium deficiency did increase this time. After rehabilitation both animals displayed shorter escape latency times than during deficiency. This effect is seen in spite of the fact that avoidance behavior never did return to normal for the two rats.

Figure 9 illustrates the effects of deficiency and rehabilitation on the total time in shock for each rat. Three subjects displayed more total time in shock after deficiency, and less total time in shock after rehabilitation. A-2 was the exception, but this may be explained by the fact that A-2 had only four sessions of rehabilitation treatment and the data of Figure 9 is the mean of the last three days. The total time in shock for A-2 for the last session was 77

Figure 8a. Mean latency times for the last three days for rats A-1, A-2, A-3 and A-5 under each condition: BL-baseline, D_1-first deficiency, R_1-first rehabilitation, D_2-second deficiency, R_2-second rehabilitation. The dotted line at 8 seconds represents the onset of shock.

8b. Mean escape latency time for the last three days for rats A-3 and A-5 under each condition: BL-baseline, D-deficiency, R-rehabilitation.

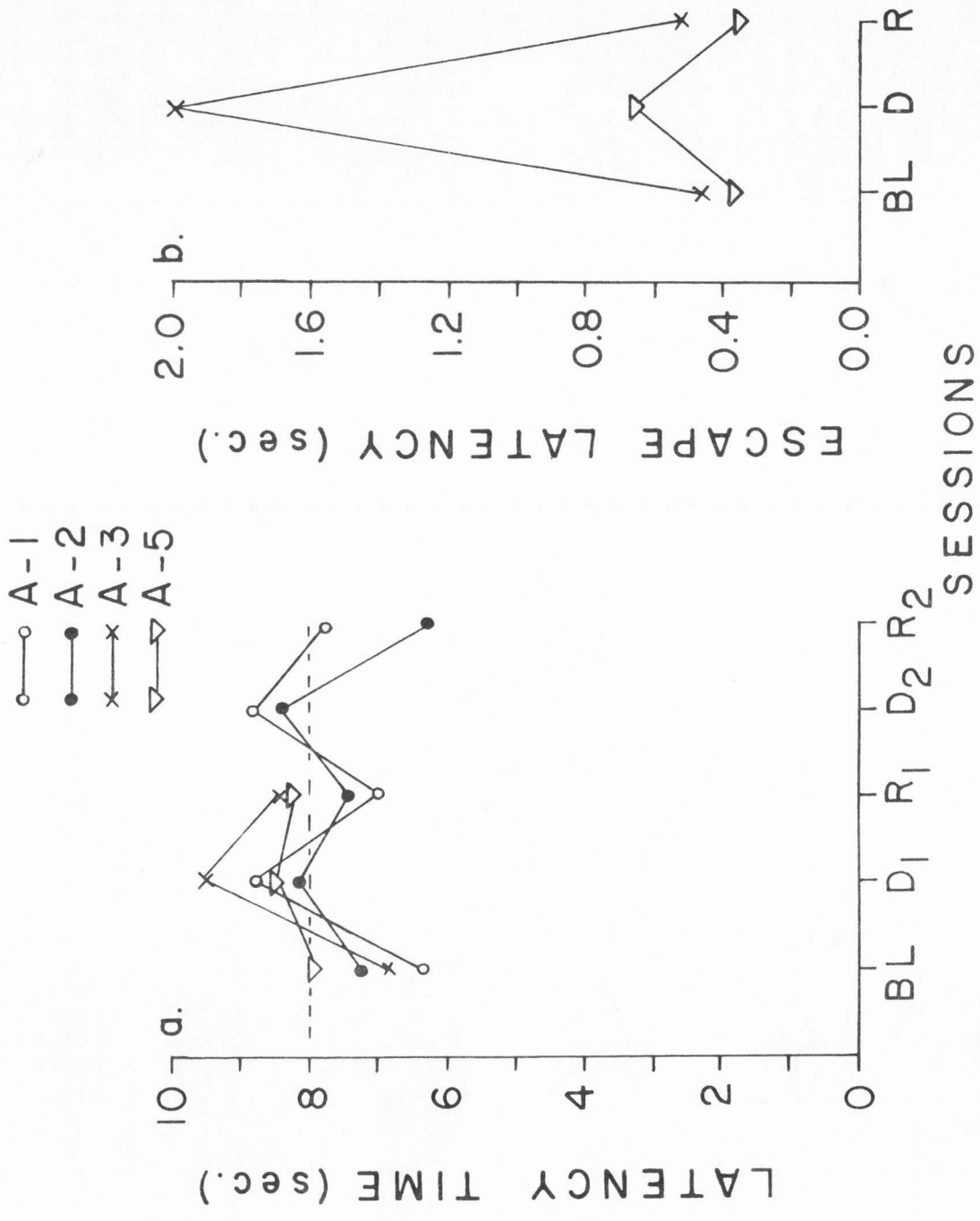
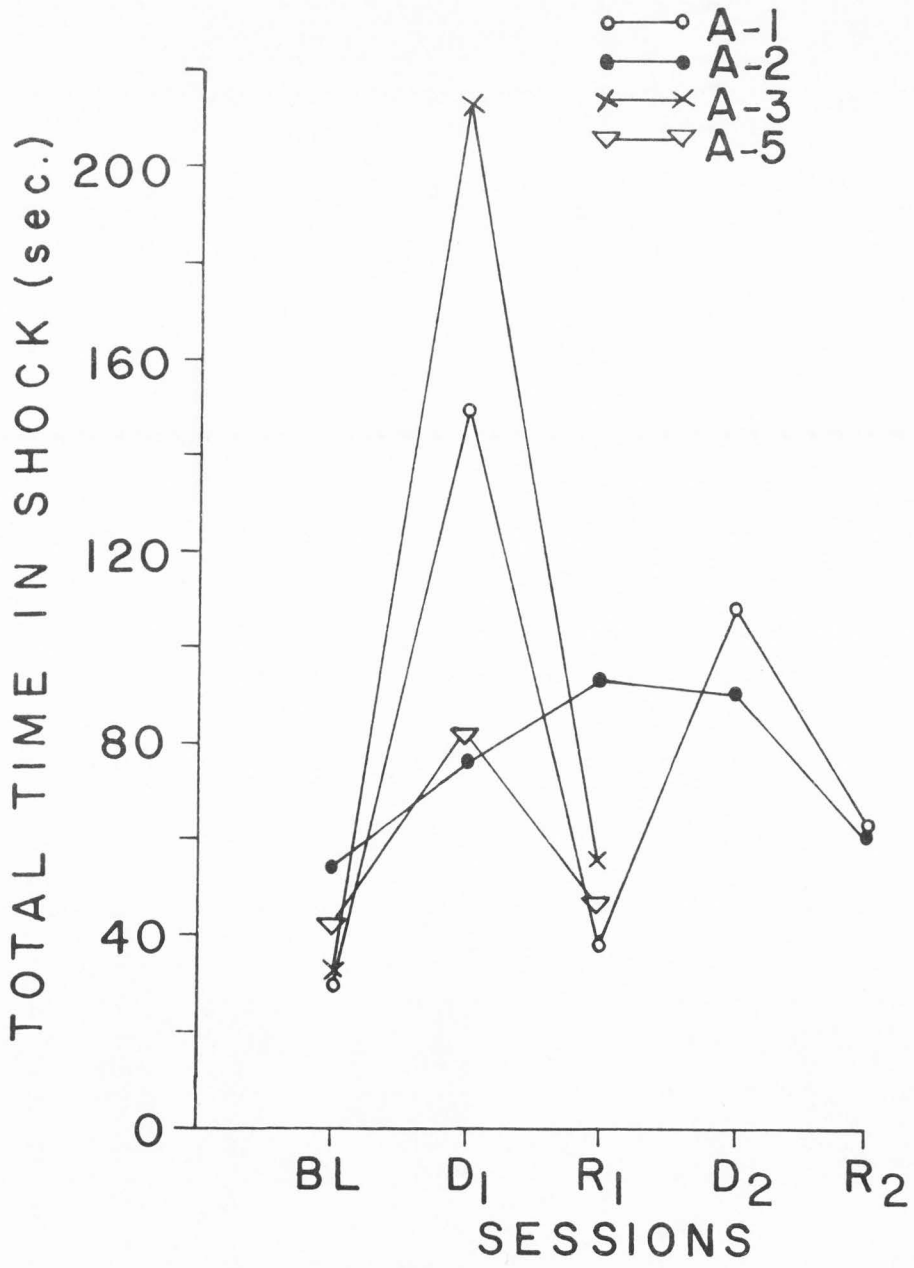

A-1
A-2
A-3
A-5
a.
LATENCY TIME (sec.)
10
8
6
4
2
0
BL
D1
R1
D2
R2
SESSIONS
b.
ESCAPE LATENCY (sec.)
2.0
1.6
1.2
0.8
0.4
0.0
BL
D
R

Figure 9. Mean total time in shock for the last three days for rats A-1, A-2, A-3 and A-5 under each condition: BL-baseline, D_1-first deficiency, R_1-first rehabilitation, D_2-second deficiency, R_2-second rehabilitation.


A-1
A-2
A-3
A-5
TOTAL TIME IN SHOCK (sec.)
200
160
120
80
40
0
BL
D1
R1
D2
R2
SESSIONS

seconds, a definite decrease in total time in shock over the four day period. It should be noted, that even for subjects A-3 and A-5 who did not show an avoidance effect after re-introduction of their original diet, there was a profound effect after rehabilitation in terms of total time in shock.

Experiment III

Subjects

Two tests were conducted in Experiment III. The first was a preliminary study in which six female Sprague-Dawley rats (three experimentals, three controls) ranging in weight from 140 to 160 grams were employed. For the main study in Experiment III, twelve male Sprague-Dawley rats (six experimentals and six controls) varying in weight from 74 to 99 grams served.

Apparatus

The objective of Experiment III was to determine effects of magnesium deprivation on the acquisition of a discriminative avoidance task rather than the effects on an already learned avoidance behavior. A shuttlebox chamber was used instead of the bar-press chamber. It has been suggested (Bolles, 1970) that the acquisition of shuttle avoidance is acquired more quickly than that of bar-press avoidance.

The shuttlebox consisted of two identical chambers connected in the center by a square opening 4 x 4 inches, placed 1 and 15/16 inches above the grid

floor. On the end walls opposite this opening were three horizontal red lights, each 1 and 1/2 inches in diameter placed 4 and 1/8 inches above the floor that served along with a Malory speaker as the CS. The movement of a rat from one chamber, via the square opening, to the other chamber interrupted a beam of light from a photo-cell and was counted as a response. The floors of both chambers were parallel stainless rods 5/15 inches in diameter spaced 1/2 inch apart capable of delivering 0.5 to 2.0 milliamperes of electrical shock. The length of the shuttlebox was 20 inches, the width 10 and 1/2 inches, the height from grid floor to ceiling was 9 and 14/16 inches. Mechanical relays, timers, counters, a shock scrambler, etc. programmed the discriminative avoidance schedule and recorded the data.

Procedure

Two tests were conducted for Experiment III. The first was designed as a pilot study, the second was Experiment III. A group design was used in both experiments to determine if magnesium deficiency would influence the acquisition of avoidance behavior in the shuttlebox. The working hypothesis was that a statistical difference ($P < .01$) using a T-test (Steele and Tori, 1961) would be found in the acquisition of avoidance behavior and in the concentration of serum magnesium between the control and experimental rats.

In both experiments, forty trials were run each day. The ITI was 20 seconds, the ISI, 10 seconds. The CS were the three red lights plus the tone. The procedure for these studies was basically the same as that used for the

bar-press discriminative avoidance. The rat was placed in one of the two chambers, the 20 second ITI was followed by the onset of the 10 second ISI and if a response (the movement of the animal from one chamber to the other) occurred during the ISI, shock was avoided. If the response did not occur, shock was delivered and remained present until the animal escaped by moving to the other compartment. Shock intensity was always 1.0 milliamperes.

For the pilot study, three rats were given a magnesium deficient diet for seven days; three others were maintained on the control diet. On the eighth and ninth day (sessions 1 and 2) all rats were given forty trials in the shuttlebox. Blood samples were taken from all subjects immediately after sessions one and two to determine serum magnesium (see Appendix B).

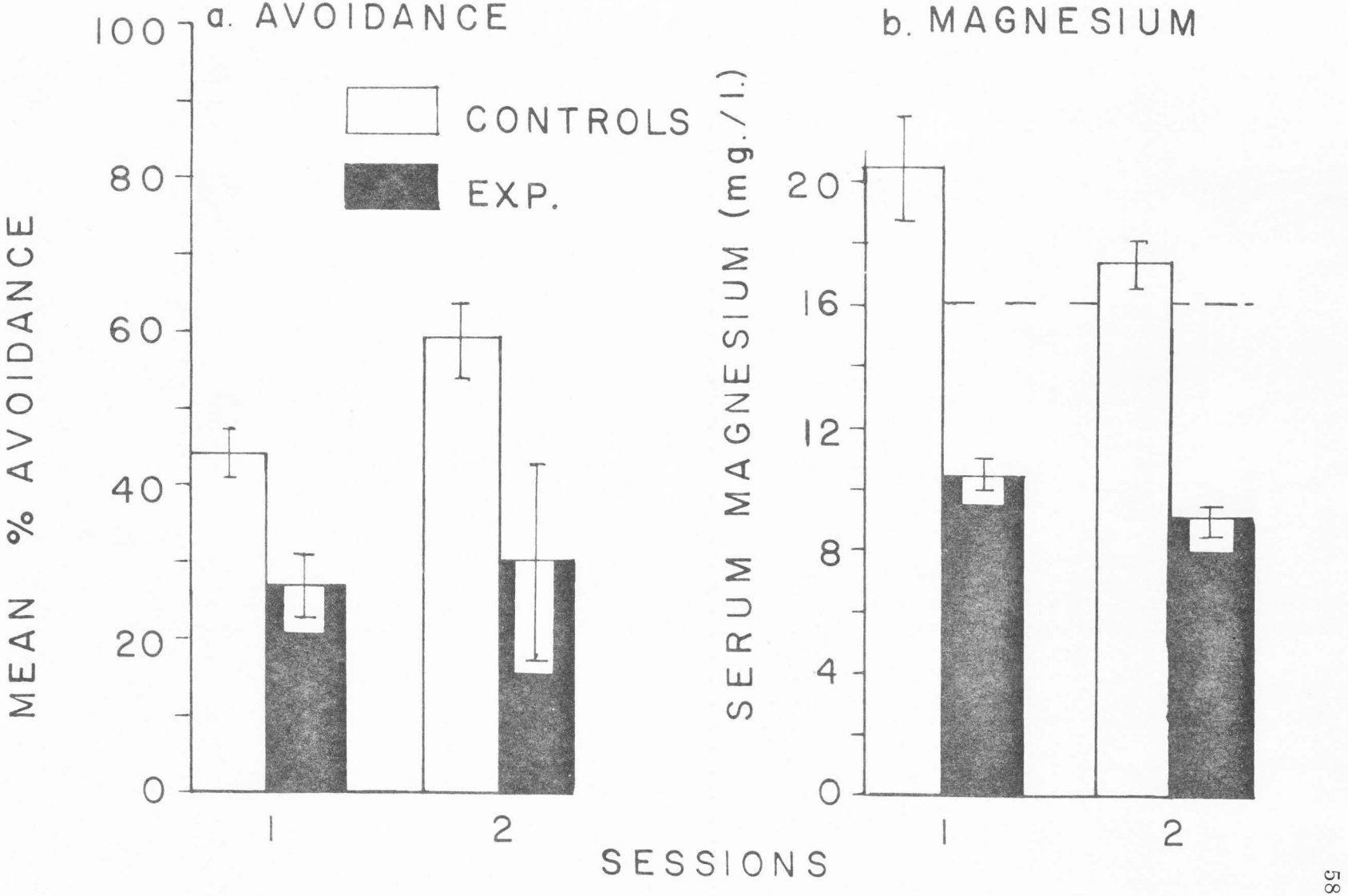
For Experiment III, six experimental animals were fed a magnesium deficient diet for six days before being given avoidance training. Six were maintained on the normal diet. Both experimentals and controls were then given four daily sessions of avoidance training. Blood samples were taken from all subjects after each session to determine serum magnesium (see Appendix B).

Results

Figure 10a shows acquisition for both sessions of the pilot study. Using a T-test (Steele and Tori, 1961), a significant difference ($P < .01$) was found between the experimental and control animals in percent avoidance responses. Figure 10b shows the concentration of magnesium in the serum for the experimental and control animals corresponding to the same days as that of Figure 10a.

Figure 10a. Pilot study to Experiment III. The mean and standard error of the acquisition of the percent avoidance responses for the two sessions. Controls, N = 3; experimentals, N = 3.

10b. Pilot study to Experiment III. Mean and standard error of the serum magnesium concentration in milligrams/liter for the corresponding two sessions of Figure 10a. The dotted line at 16 milligrams/liter represents normal magnesium concentration. Controls, N = 3; experimentals, N = 3.

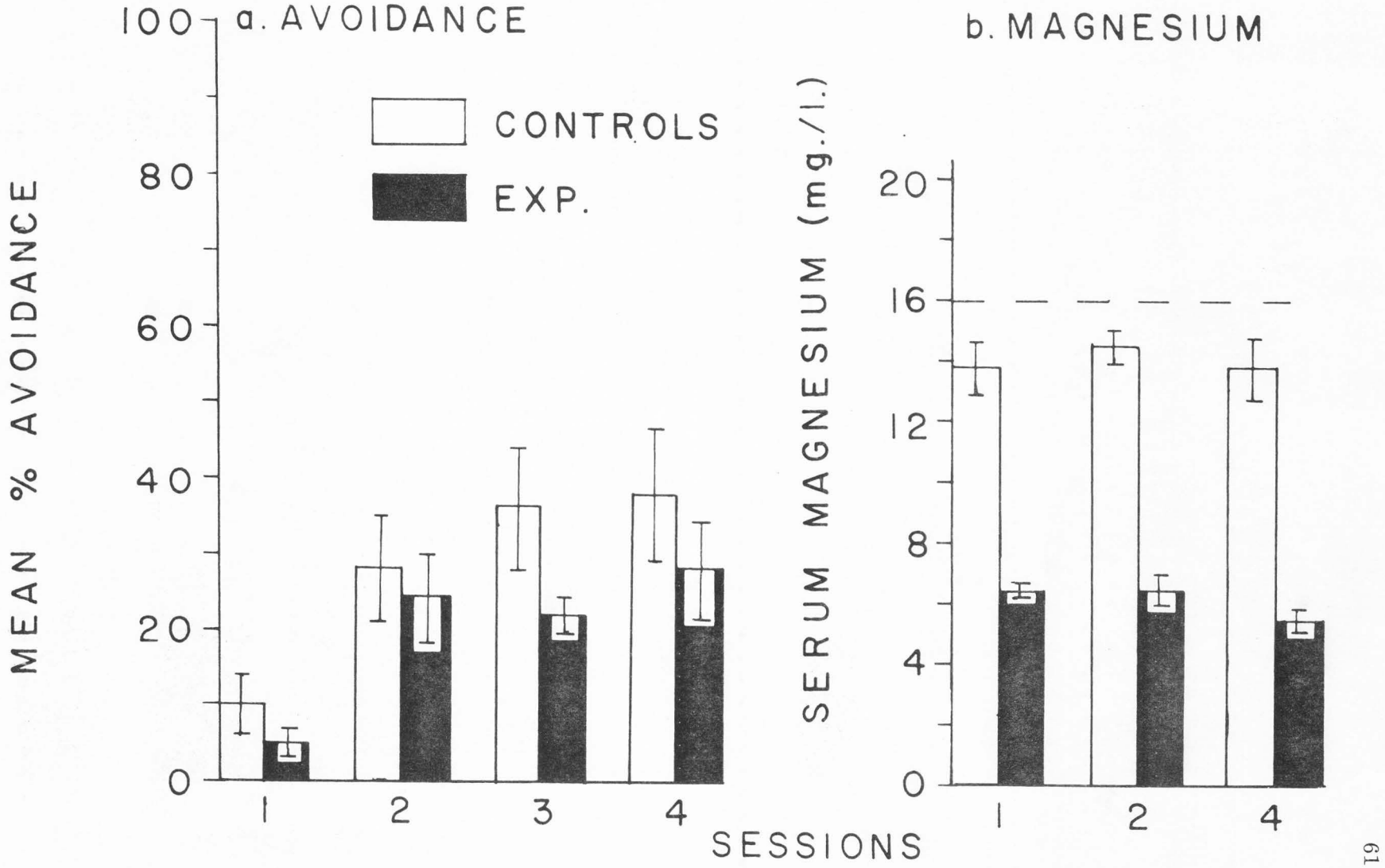

a. AVOIDANCE
b. MAGNESIUM
CONTROLS
EXP.
MEAN % AVOIDANCE
SERUM MAGNESIUM (mg./l.)
100
80
60
40
20
0
20
16
12
8
4
0
1
2
1
2
SESSIONS

A statistical difference ($P < .01$) was found between the two groups in terms of magnesium level. Figures 10a and 10b illustrate, for this pilot study that a deficiency in magnesium affected the acquisition of avoidance of the experimental rats by significantly lowering avoidance rates.

In Experiment III, contrary to the pilot study (Figure 10a), no significant difference between the controls and experimentals was found in the acquisition of the avoidance task. Referring to Figure 11b, a significant difference was found ($P < .01$) between the amount of magnesium in the serum between the two groups. Figure 11b does show that the controls were slightly below the expected normal magnesium level of 16 milligrams/liter.

Figure 11a. Experiment III. The mean and standard error of the acquisition of the percent avoidance responses for the four sessions. Controls, N = 6; experimentals, N = 6.

11b. Experiment III. The mean and standard error of the serum magnesium concentration in milligrams/liter for the corresponding four sessions in Figure 11a. The dotted line at 16 milligrams/liter represents normal magnesium concentration. Controls, N = 6; experimentals, N = 6.


a. AVOIDANCE
b. MAGNESIUM
CONTROLS
EXP.
MEAN % AVOIDANCE
SERUM MAGNESIUM (mg./l.)
100
80
60
40
20
0
20
16
12
8
4
0
1
2
3
4
1
2
4
SESSIONS

DISCUSSION

Results from Experiments I and II clearly illustrate that a magnesium deficiency affects the discriminative bar-press avoidance behavior of rats by decreasing, over a period of time, the number of avoidance responses emitted per session. The conclusion reached, at this time, is only applicable provided that the rats at the time of deficiency are avoiding shock at a substantial rate per session. Unfortunately, data as to the concentration of magnesium for the rats in Experiment I were not available; however, observations of the physical signs of deficiency were made. The vasodilation symptom did occur in these rats after approximately five to seven days of deficiency. Experiment II confirmed and expanded the previous observations as to the serum magnesium concentrations. It was revealed that it took only one day to produce a deficiency of serum magnesium (Figures 4, 5, 6 and 7). It was observed that physical manifestations of the deficiency (i.e., vasodilation) in some cases took as little as two days to develop. These results confirm Aikawa's (1963) statement that the onset of deficiency is very rapid, being detectable within a few days. It should be noted that the rats used for these experiments were young and had just been weaned. Weights of these rats on arrival to the laboratory ranged between 50 and 100 grams; an adult rat weighs from 250 to 300 grams. The age of the rats used in these studies probably accounts for the short period of time necessary to develop a deficiency.

As mentioned above (Guthrie, 1971; Krehl, 1967; Wacker and Parisi, 1967), decreases in magnesium produce absorption changes of other nutrients such as an increase in calcium. No attempts at regulating other nutrients was employed in these experiments. Suffice it to say that conclusions from these experiments are the results of a magnesium deficiency plus any other nutrient changes that accompany this deficiency.

It may also be concluded that a magnesium deficiency did not seem to affect rats that were escaping as opposed to avoiding shock. It was shown in Experiment I (Figure 3) that rats escaping shock at a relatively high rate per session did not exhibit any decrease in escape behavior when made deficient. It was also seen in Experiment II (Figure 8a) that when rats decreased in their avoidance responses, they continued to respond, but responded after shock was presented. In effect the avoidance behavior was replaced by escape behavior.

These findings suggested two conclusions: 1) that motor functions involved in the bar-press response were not severely imparied; and 2) that shock still functioned as an aversive stimulus. The qualification in conclusion 1, that motor functions were not severely impaired, has been added because it was noted that as the deficiency continued, the rats' total time in shock per session did increase (Figure 9). Whether or not increasing the deficiency beyond ten days would in fact severely impair motor functions has not been determined. But if, as reported by Aikawa (1963), death does occur after a prolonged deficiency, one would be inclined to agree that motor functions would eventually be impaired. For this particular research though, results indicated that the avoidance

response was much more severely affected than the escape response.

In an attempt to explain this phenomenon, an understanding of the physiological functions of magnesium plus an understanding of the schedule effects of the avoidance paradigm must be analyzed.

Magnesium deficiency affects the nervous and muscular systems (Guthrie, 1971; Krehl, 1967; Wacker and Parisi, 1967). As the deficiency proceeds, the animal becomes more susceptible to any noise or other stimulation which startles it, and which may precipitate tremors and convulsive seizures. It was found in our experiments that these reactions did take place. Observations of rat A-1's behavior after being deficient for five days showed that during the CS presentation, A-1 vocalized, quivered and sometimes froze until the onset of shock; other times, he moved back and forth in front of the lever trembling until shock was presented. It was also observed in Experiment II that the average avoidance latency before deficiency was between five and eight seconds after the onset of the CS. This meant that when the rat did emit an avoidance response, it was usually toward the end of the ISI. Very seldom was the response made immediately after the onset of the CS.

Examining the physical reactions of the deficient rats plus the analysis of the avoidance latency time, it is suspected that the magnesium deficiency produced the phenomenon called conflicting response tendency as described by Hoffman (1966). In effect the onset of the CS could have produced a delay in an avoidance response when the rats were deficient. A short delay of just seconds could create a condition in which the beginning movements of the avoidance response are

punished by shock; resulting in a decrease in avoidance responses with a corresponding increase in escape responses.

The rehabilitation results from Experiment II are less conclusive. Figures 4 and 5 showed that a rehabilitation program did in fact re-establish avoidance responding in two of the four rats. Figure 6 shows conflicting data. After one day of a magnesium supplemented diet, avoidance behavior increased to a level that even surpassed A-3's BL level, but a continual decrease was noted thereafter. Figure 7 shows that A-5 never did recover its original avoidance behavior after rehabilitation.

An explanation of these two rats' rehabilitation treatment might be explained as a schedule effect rather than a deficiency effect. Coons, Anderson and Myers (1960) reported that some of their animals which had learned the avoidance well, eventually lost the avoidance response with no change in the parameters of the experiment. Such might be the case with rats A-3 and A-5 (Figures 6 and 7). In fact as seen in Fibures 8b and 9, both A-3 and A-5 did exhibit a rehabilitation effect with escape latency and total time in shock as dependent variables. Figure 8b is interesting in that A-3 and A-5 exhibited escape latencies of approximately 0.4 seconds. During deficiency this latency increased. After rehabilitation both rats escaped shock rather than avoided shock at approximately the same escape latency as during baseline. Obviously, there was a rehabilitation effect. The rehabilitation effect seen in Figures 8b and 9 for rats A-3 and A-5 suggests that the effects of magnesium deficiency are not irreversible. Rats A-1 and A-2 (Figures 4 and 5) support this

conclusion as qualified. As mentioned previously, deficiencies of magnesium lasting longer than eight days might produce results differing from those obtained in this experiment.

The results from Experiment III were ambiguous. The pilot phase (Figures 10a and 10b) used six female rats that were larger than the other rats used throughout the experiments. This study showed a statistical difference between the controls and experimentals in the acquisition of avoidance behavior. The experimentals displayed less avoidance responses during both sessions. Experiment III (Figures 11a and 11b) used twelve male rats that were between 50 and 100 grams in weight. No statistical difference in acquisition of avoidance behavior between the two groups was found in this experiment.

Several possibilities exist to explain the contradictory findings. One concerns the sex and the age of the rats in the two studies. The pilot study used females which were older than the male rats used in Experiment III (Figure 11b). Even though a statistical difference in magnesium was found between the controls and experimentals, it is seen that the controls were below the expected normal magnesium level of 16 milligrams/liter (represented by the horizontal dotted line). The female control rats of the pilot study exhibited a magnesium level above the expected normal level. The assumption is that the subnormal level of magnesium within the control male rats of Experiment III could have affected their avoidance behavior. It is further borne out by comparing the avoidance behavior of the control rats in the pilot study (Figure 10a) to the control rats in Experiment III. As can be seen, the control rats in the pilot study, session 1,

avoided approximately 44 percent of the trials. The controls for Experiment III, session 1, avoided approximately 10 percent of the trials.

In further defense of the assumption that magnesium deficiency affects the acquisition of avoidance, it is seen that the trend of Experiment III (Figure 11a) is that for each of the four sessions the controls did exhibit more avoidances than did the experimentals.

The results of Experiment III are by no means conclusive, but it is felt that magnesium deficiency does affect the acquisition of a shuttle avoidance response. More information, though, is needed before a definite conclusion can be asserted.

CONCLUSIONS

Conclusions are as follows:

1. A magnesium deficiency decreases over a period of time the ongoing bar-press discriminative avoidance behavior of rats.
2. A magnesium deficiency of a relatively short duration (i.e., eight to ten days) does not seem to seriously affect the bar-press discriminative escape behavior of rats.
3. Providing a magnesium supplemented diet to magnesium deficient rats appears to reverse the behavioral symptoms caused by the deficiency.
4. The effects of a magnesium deficiency on the acquisition of a disciminative shuttle avoidance response have not conclusively been shown. The results of this research do indicate that an effect is possible, but further research is needed.

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APPENDIXES

Appendix A

Diet composition		Mineral mixture	
Ingredients	g/kg	Salt	g/kg
Casein	200	$CuSO_4 \cdot 5H_2O$	0.290
Dextrose	590	$FeSO_4 \cdot 7H_2O$	1.000
[1]Corn oil	100	KCl	200.000
Fiber	50	$Na_2HPO_4 \cdot H_2O$	360.000
[2]Vitamin mix	20	*$MgCO_3$	37.600
Mineral mix	40	$CaCO_3$	395.000
		KI	0.790
		$MnSO_4 \cdot H_2O$	3.780
		$ZnSO_4$	0.288
		$CoCl_2 \cdot 6H_2O$	0.0226
		$Na_2MoO_4 \cdot 2H_2O$	0.0417

[1]Mazola Corn Oil, a product of Best Foods Corp., division of CPC International Inc., Englewood Cliffs, N. J.

[2]Vitamin Diet Fortification Mixture, Nutritional Biochemical Corporation, Cleveland, Ohio.

*$MgCO_3$ is left out of this mixture for the magnesium deficient diet.

Appendix B

Serum Magnesium Analysis

The procedure for determining the serum magnesium content of each rat was as follows:

1. Approximately .5 to 1.0 milligrams of blood was extracted from the retro-ocular vein of each rat.
2. The blood was allowed to clot for one hour.
3. The blood was then centrifuged at approximately 2,500 rpm for twenty minutes.
4. The serum was collected and diluted 50:1 with distilled water. In practice 2 milligrams of distilled water was added to 50 ul of serum.
5. Magnesium standards of 10 ppm, 7.5 ppm, 5.0 ppm, 2.5 ppm and 0.0 ppm with distilled water were made.
6. Triplicate samples of serum were analyzed with comparisons to the standards by an atomic absorption spectrophotometer set at 285.7 mM wavelength.

Appendix C

Glossary

ABA design - the procedure of establishing a baseline (A), introducing a treatment condition (B), returning to baseline condition (A).

Aversive stimulus - 0.6 - 1.0 milliamperes of scrambled electrical shock.

Avoidance conditioning - Training of a rat to either bar-press or move to another chamber (shuttle) in order to avoid being shocked.

Baseline (BL) - The initial behavior prior to any treatment conditions.

Bar-press response - A specific measurable response in which the animal depresses a protruding bar.

Controls - Rats which were fed the control diet throughout the experiments.

Control diet - The diet which contained the required amount of magnesium for normal maintenance (see Appendix A).

Conditioned stimuli (CS) - The red light or lights and tone which preceded the onset of shock in the discriminative avoidance paradigm.

Experimentals - Rats which were fed the magnesium deficient diet.

Interstimulus interval (ISI) - The interval of time between an avoidance or escape response and the onset of the CS in the discriminative avoidance paradigm.

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Latency - The time from the onset of the CS to either an avoidance response or an escape response. Avoidance latency is the time from the onset of the CS to an avoidance response. Escape latency is the time from the onset of the CS to an escape response.

Magnesium deficient diet - The diet which was identical to the control diet except for the omission of $MgCO_3$ (see Appendix A).

Nutritional psychology - The study of the interaction between nutrition and behavior.

Rehabilitation - The feeding of the control diet to rats previously fed the magnesium deficient diet.

Response - A specified and measurable behavior. Avoidance response is that behavior which when emitted, avoids the shock. Escape response is that behavior which when emitted, terminates shock.

Shuttle response - A specified and measurable response in which the animal crosses from one chamber to another.

Total time in shock - The amount of time in seconds that the animal receives shock per session.

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