BEHAVIORAL CONDITIONING OF A PRIMARY HUMORAL IMMUNE RESPONSE IN SELECTIVELY BRED LINES OF RATS*

by James Cameron Keith Porter

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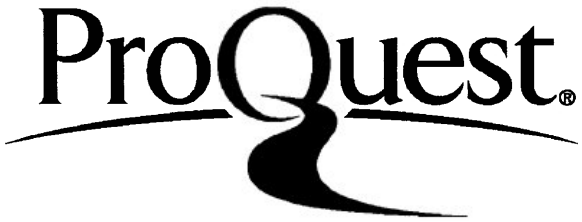

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Abstract

Behaviorally conditioned immunosuppression of a primary humoral immune response was investigated in both the sexes of five selectively bred lines of rats. In the experimental group, an illness-induced taste aversion was produced by pairing sucrose (CS) and cyclophosphamide (US), an immunosuppressant that induces gastrointestinal distress. A nonconditioned group received water as the CS and cyclophosphamide (CY) as the US while a placebo group received sucrose as the CS and isotonic saline as the US. Three days following the CS-US pairing, the animals received an injection of sheep erythrocytes (SRBC). A hemagglutinating antibody titer was used to assess the humoral antibody response (CR) six days later. Overall, there was evidence of behaviorally conditioned immunosuppression. Taste aversion was present 24 hours following conditioning in the MR/Har/Lu ($p<.01$), MNR/Har/Lu ($p<.05$), SHS ($p<.01$) and RLA/Lu ($p<.01$) lines with persistant extinction in MNR/Har/Lu animals and the appearance of taste aversion in the RHA/Lu ($p<.05$) animals 48 hours following conditioning. Results are discussed on behavioral and biochemical levels with a conclusion emphasizing the importance of research in behavioral immunology and calling for greater attention to the field.

Exciting new vistas of research are unfolding with the recent integration of behavioral and biomedical sciences. For instance, one area of research recently opened for exploration is psychoneuroimmunology. Psychoneuro- immunology is the study of the interrelationship between the central nervous system, behavior and immune system (Ader, 1980). One avenue of investigation purporting to demonstrate this relationship is behaviorally conditioned immunosuppression.

In 1975, Ader and Cohen were able to produce behaviorally conditioned immunosuppression of a primary humoral immune response in male rats. Following their initial work, attention has been focused on investigating the parameters of behaviorally conditioned immunosuppression. This study examines two hitherto unexplored parameters; sex and genetic line differences. To facilitate a greater appreciation and understanding of the mechanics of such a study, a brief review of the immune system and the field of psychoneuroimmunology follows.

The Immune System

The immune system is responsible for defending the body from any foreign macromolecules intruding upon an organism's internal milieu. A part of the reticuloendothelial system (RES), the immune system is subdivided anatomically into the central organs and peripheral organs. The central organs include the bone marrow and the thymus, while the lymph nodes, spleen and lymphatics comprise the peripheral organs.

Central Organs

Originating within the bone marrow, lymphoid stem cells give rise to T-cell and B-cell precursors (see Section 1 of Figure 1, page 16). The T-cell precursors migrate to the thymus where they mature into T-lymphocytes (see Section 2 of Figure 1, page 16). The B-cell precursors evolve directly into B-lymphocytes without thymic processing. In addition to its role in the maturation and development of T-lymphocytes, the thymus serves as a regulator of the immune system via the secretion of thymic hormones.

Peripheral Organs

The lymph node is composed of a fibrous network of reticular cells filtering the lymph which is conveyed by the lymphatics. The network of cells--medullary and paracortical areas of the node--also contain T-lymphocytes and macrophages. B-lymphocytes are located in follicles surrounding the paracortical area and contained within the medullary area (see Section 4 of Figure 1, page 16). The spleen is essentially a circulatory system analogue of the lymph node; it is composed of a fibrous network of T-cell areas and follicles containing B-cells and filters the blood (see Section 5 of Figure 1, page 16).

Immune Response

Exposure to an antigen marks the genesis of an immune response. In

describing an immune response, the immune system is divided into three limbs; the afferent, central and efferent (Amkraut & Solomon, 1975). The actual response begins within the afferent limb in which there is a direct or macrophagic presentation of the antigen to the appropriate immunocompetent cells. This may also include degradation of antigen by neutrophils through phagocytosis (see Section 6 of Figure 1, page 16). Central limb functioning, the focal point of the immune response, is manifested subsequent to the presentation of an antigen to the immunocompetent cells. Within the central limb, an immune response will follow the course of one or both of the two existing component systems of the immunologic apparatus. The two component systems include humoral and cell-mediated immunity. Cell-mediated immunity involves the thymus dependent-- T-lymphocytes--while humoral immunity involves thymus independent-- B-lymphocytes. Depending on the type of antigen the evoked immune response will be either humoral, cell mediated or involve a combination of B- and T-cell activation.

Cell-Mediated Immunity. Cell-mediated immunity is primarily restricted to delayed hypersensitivity and tissue graft rejection. When a T-lymphocyte is activated, which is achieved when an antigen comes in contact with surface receptors on the lymphocytes, lymphokines, non-antibody substances are produced. Inflammatory graft rejections result from the interaction between the antigen and lymphokines.

Humoral Immunity. Humoral immunity is restricted to antibody reactions. In a humoral response, the novelty of the antigen determines which of two

general patterns of antibody production will be evidenced. The pattern of antibody production associated with a novel antigen--one that an organism has never encountered--is referred to as the primary response. If the organism has been exposed to the antigen in the past, the pattern of antibody production evidenced is known as the secondary response. The key to antigen stimulation of a B-lymphocyte immune response rests in the receptor molecules, termed surface immunoglobulins, which essentially "identifies" an antigen. There are five classes or structural types of immunoglobulins--IgG, IgM, IgA, IgD, IgE--potentially serving as receptor molecules. A surface immunoglobulin reacts with a specific antigen and marks the transformation of a B-lymphocyte into an immunoblast which in turn transforms into a plasma cell, a type of white blood cell which produces antibodies. The antibody produced is essentially an anti-immunoglobulin with an antigen-binding specificity identical to the surface immunoglobulin receptor.

As previously alluded, certain antigens are capable of evoking an immune reponse involving a combination of B- and T-cell activation. This synergetic functioning of B- and T-cells ("helper" T-cells) is evidenced when sheep erythrocytes are used as an antigen. The macrophage pools the antigen; then, B- and T-cells attach to different determinants of the pooled antigen. At this stage, information passes from the T-helper cell to the B-cell. The information acquired from the T-helper cell initiates the differentiation of the B-cell into a plasma cell which leads to subsequent antibody secretion (see Section 7 of Figure 1, page 16).

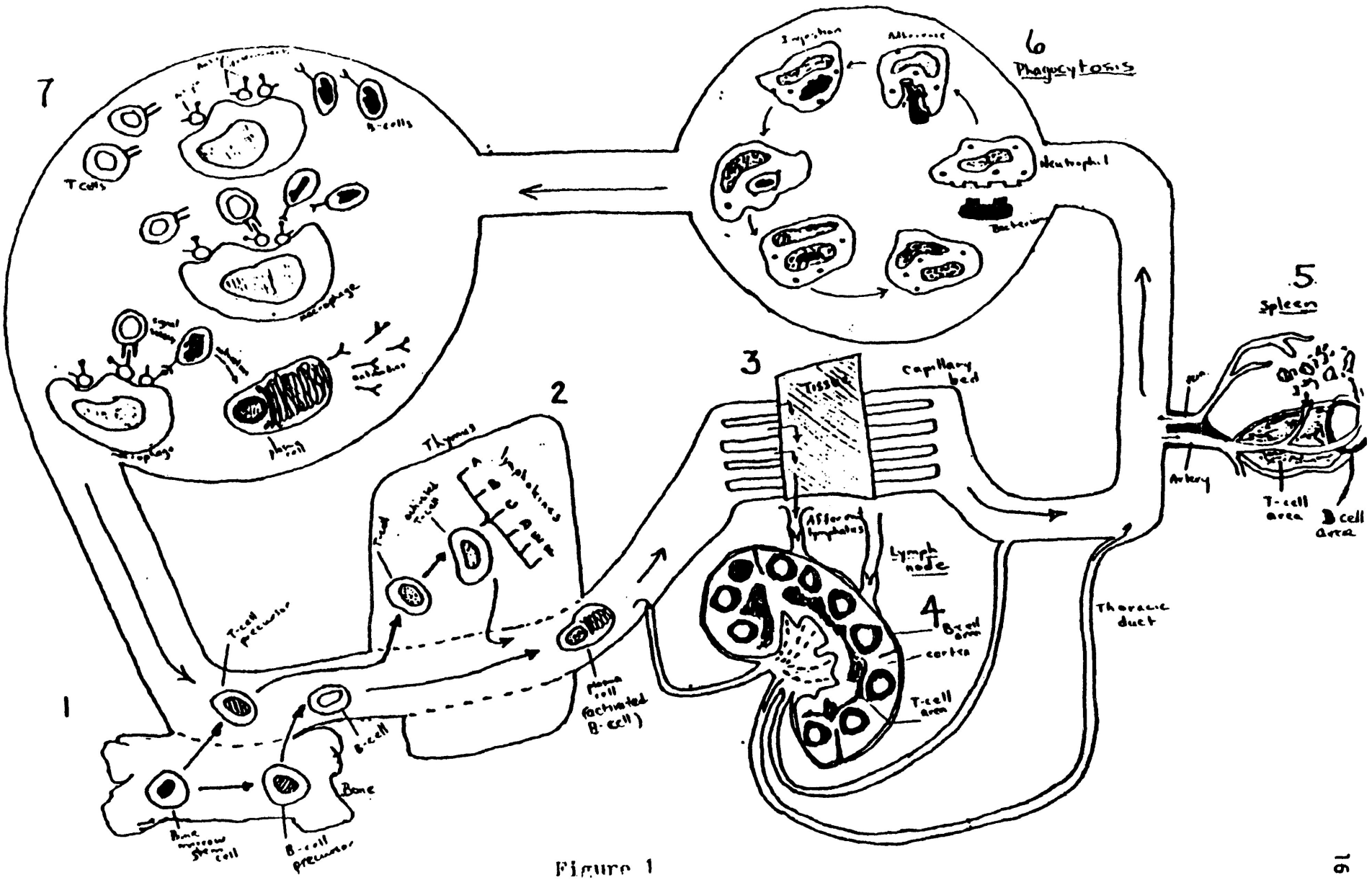


Figure 1

Schematic Representation of Immune System, Immune Response and Lymphocyte Circulation (Thaler, Klausner & Cohen, 1977)

Psychoneuroimmunology

Traditionally, the immune system has been perceived as functioning independently of the central nervous system and hence independently of CNS mediated psychosocial phenomena (Fauman, 1982). In more recent years however, investigations of the relationship between the CNS and immune system suggest that the CNS and CNS mediated psychosocial phenomena are capable of exerting an influence on immune function (Ader, 1981). For instance, studies of the relationship between the CNS and immune system in animals have included the study of "stress" and susceptibility to neoplastic disorders (Riley, 1975), viral disease (Hamilton, 1974; Johnsson, Lavender, Hultin & Rasmussen, Jr., 1963; Rasmussen, Jr., Marsh & Brill, 1957) and the effect of "stress" on lymphocyte function (Gisler, Bussard, Mazie & Hess, 1971; Joason & McKenzie, 1976), antibody production (Beden & Brain, 1982; Hill, Greer & Felsenfeld, 1967) and morphology of immune organs (Jensen, Rasmussen, Jr., 1963; Marsh & Rasmussen, Jr., 1960; Monjan & Collector, 1977). Human studies have focused on life changes/stresses and susceptibility to illness (Rabkin & Struening, 1976; Rahe, 1972) and the effect of bereavement on the immune system (Bartrop, Lazarus, Luckhurst, Kiloh & Penny, 1977). In addition to these studies, there are a number of general reviews examining the relationship between the CNS and immune system (Hurst, Jenkins & Rose, 1976; Rogers, Dubey & Reich, 1979; Solomon & Amkraut, 1981; Solomon & Moos, 1964) and the field of psychoneuroimmunology (Ader, 1980, 1981, 1983; Udelman & Udelman, 1983).

While these studies have implicated the CNS in the modulation of immune function, conditioning of an immune response has been advanced as an area of inquiry yielding evidence of a more direct nature (Ader, 1981). Behaviorally conditioned immunosuppression is demonstrated by a paradigm in which an attenuated immune response is produced as a consequence of a conditioned taste aversion that utilizes an immunosuppressant as an unconditioned stimulus. This conditioned suppression of an immune response has been termed behaviorally conditioned immunosuppression (Ader and Cohen, 1975). The recent impetus for research in conditioned immunologic responses was sparked by Ader and Cohen in 1975. In their study, Ader and Cohen employed a Pavlovian conditioning paradigm in which saccharin was paired with an immunosuppressive agent, cyclophosphamide, to condition illness-induced taste aversion. The saccharin, which possesses a distinctive flavour, served as a conditioned stimulus (CS). Cyclophosphamide, which produces gastrointestinal distress, served as an unconditioned stimulus (US). As a result of this pairing, the animals developed an aversion to the saccharin solution. In addition, cyclophosphamide is an immunosuppressant and thus immune suppression became paired with saccharin. In Ader and Cohen's study, animals received an injection of sheep erythrocytes (SRBC) three days after the initial CS-US pairing. Six days after the SRBC injection, a decreased relative to control groups for which the conditioned and unconditioned stimuli were never paired - hemagglutinating antibody titer (conditioned response) was observed in animals that received the saccharin (conditioned stimulus)-CY

(unconditioned stimulus) pairing on the day of conditioning.

Rogers, Reich, Strom and Carpenter (1976) conducted a replication of Ader and Cohen's study. Their results, while essentially replicating the findings of Ader and Cohen, deviated from Ader and Cohen's in two respects: (i) Rogers et al only found behaviorally conditioned immunosuppression with two CS-US pairings, while Ader and Cohen found conditioned immunosuppression with a single CS-US pairing (ii) conditioned animals injected with CY following treatment with antigen (US animals) possessed higher mean titers in the Rogers et al study (approximately 3.0) than in the Ader et al study (less than 1.0). No explanation is provided for the first deviation and the second difference was attributed to the more sensitive method of determining the hemagglutinating antibody titer by Rogers et al. Since their original study, Ader and Cohen have examined the effect of various manipulations of the original experimental paradigm (1981). For instance, one study examined the time sampling--variations in the time elapsed between immunogenic stimulation and hemagglutinating antibody titers. Hemagglutinating antibody levels were measured 4, 6, 8, and 10 days after the injection of antigen. It was discovered that optimal serum levels of antibody were present six days after immunogenic stimulation. In addition, Ader and Cohen concluded that the qualitative results of behaviorally conditioned immunosuppression produce a transient delay in the appearance of antibody in the serum as opposed to an inhibition of antibody production. Additional variations of the original paradigm include investigations using preference vs. forced choice fluid consumption to control for effects of relative fluid deprivation on the day of antigen administration; dose of cyclophosphamide and concentration of antigen; and residual effects of cyclophosphamide (Ader

& Cohen, 1981).

In addition to modification of the original experimental paradigm, the generalizability of behaviorally conditioned immunosuppression has also been investigated (Ader & Cohen, 1981). For instance, methotrextate replaced cyclophosphamide as the unconditioned immunosuppressive stimulus. It was concluded that methotrextate has particular advantages making it appropriate for future studies. However, Ader & Cohen cautioned that their results were somewhat equivocal and studies were performed in the absence of data indicating optimal or appropriate experimental conditions for using methotrextate. Additional investigations extending the generalizability of behaviorally conditioned immunosuppression include studies by Cohen, Ader, Green and Bovbjerg (1979) and Wayner, Flannery and Singer (1978). Wayner et al (1978) examined behaviorally conditioned immunosuppression of a T-cell independent response as opposed to Ader and Cohen's (1975) use of a T-cell dependent response. Wayner et al examined both T-cell dependent (SRBC) and T-cell independent (Brucella abortus) antigens. Wayner et al found that the use of cyclophosphamide-saccharin induced taste aversion conditioning produced statistically significant behaviorally conditioned suppression of the immune response to a T-cell dependent (SRBC) antigen but not the immune response to a T-cell independent (Brucella abortus) antigen. Conversely, Cohen, Ader, Green and Bovbjerg (1979) successfully produced a behaviorally conditioned suppression of an immune response to a T-cell independent (TNP-LPS) antigen. Cohen et al (1979) suggested that their successful suppression of a T-cell independent response in contrast to Wayner et al's failure to produce suppression of the T-cell independent response may have

been the result of species differences. Cohen et al used male mice for their study and Wayner et al used male albino rats. Suppression of a cellular immune response, as opposed to humoral immune response, has also been behaviorally conditioned (Bovbjerg, Ader and Cohen, 1982). Splenic leukocytes obtained from female donors were injected into the footpads of experimental animals. An increased weight of popliteal lymph nodes would typically occur subsequent to this injection. Conditioned animals had significantly lower lymph node weights than control animals, thereby demonstrating relative behaviorally conditioned immunosuppression. The generalizability of behaviorally conditioned immunosuppression was expanded by the Bovbjerg et al (1982) study and then expanded further by Ader and Cohen (1982). Ader and Cohen (1982) examined the impact of behaviorally conditioned immunosuppression on the development of an autoimmune disease (systemic lupus erythematosus) in female mice. Behaviorally conditioned animals received saccharin solution paired with CY injections. For distinguishing experimental group differences, the rate of development of proteinuria and the rate of death for the initial 50 percent of the experimental population was used. The rate, as opposed to the incidence of mortality and development of proteinuria were used because all animals were expected to eventually develop proteinuria and die; thus making treatment effects difficult to discern. Ader and Cohen (1982) found that, relative to control groups, conditioned animals had a lower mortality rate and lower development of proteinuria thereby displaying properties reflecting behaviorally conditioned immunosuppression.

In an attempt to uncover potential mechanisms and mediators involved in

conditioned immunosuppression, Ader, Cohen and Grota (1979) investigated the possibility of adrenal involvement in behaviorally conditioned immunosuppression. On the basis of their results, they concluded that behaviorally conditioned immunosuppression is not mediated by the adrenal system. Finally, Ader, Cohen and Bovbjerg (1982) confirmed previous reports of behaviorally conditioned immunosuppression of a humoral immune response and found that conditioning was evident with as long as 25 days between conditioning and antigenic stimulation.

None of the previously cited studies on behaviorally conditioned immunosuppression have examined sex or genetic line as variables. Indeed, it is not uncommon to find the sex of animals unspecified (Ader & Cohen, 1981; Ader, Cohen & Bovbjerg, 1982; Ader, Cohen & Grota, 1979). A survey of past studies revealed the exclusive use of male animals in behavioral conditioning of a humoral immune response (Ader & Cohen, 1975; Cohen, Ader, Green & Bovbjerg, 1979; Rogers et al, 1976; Wayner, Flannery & Singer, 1978); all cellular immune response studies (Bovbjerg, Ader & Cohen, 1982; Bovbjerg, Cohen & Ader, 1980; Klosterhalfen & Klosterhalfen, 1983) and the sole autoimmune study (Ader & Cohen, 1982) used females exclusively; and all T-cell independent studies used male animals (Cohen, Ader, Green & Bovbjerg, 1979; Wayner, Flannery & Singer, 1978). Further, genetic line differences have not been investigated at all.

The five genetic lines of rats used in this study include two genetic lines selectively bred for bidirectional avoidance behavior (RHA/Lu and RLA/Lu animals), two genetic lines selectively bred for bidirectional

open-field reactivity behavior (MNR/Har/Lu and MR/Har/Lu) and a control line (SHS: Satinder's heterogenous stock) produced by a 4-way cross among the four selectively bred lines. These genetic lines vary in a number of ways. Broadhurst (1975) presents a comprehensive survey of investigations comparing the MR and MNR genetic lines in four broad topic areas; behavioral, psychophysiological, psychoendocrinological and psychopharmacological. It includes studies that have shown that the MR animals possess significantly lower amounts of thyroid hormones in comparison to the MNR animals (Feuer & Broadhurst, 1962); the MR animals have a lower general metabolism than the MNR animals (Watson, 1960); greater GABA production in MNR animals (Rick, Huggins & Kerkut, 1967). In addition, more recent studies have indicated that the MNR animals have higher concentrations of norepinephrine in descending and transverse colon (Blizard, Altman & Freedman, 1982); the MR animals, under resting conditions, exhibit lower basal plasma norepinephrine levels and have a higher systolic and diastolic blood pressure and heart rate (Blizard, Liang & Emmel, 1980). The Maudsley nonreactive animals have higher heart, spleen and hypothalamic levels of norepinephrine (Slater, Blizard & Pohorecky, 1977). Studies comparing the Roman lines have shown that the RHA animals have a significantly lower basal level of plasma corticosterone whereas under "stressful" conditions -- novel environment -- the RLA animals manifest higher defecation scores, prolactin secretion, ACTH secretion and corticosterone secretion (Gentsch, Lichsteiner, Driscoll and Feer, 1982). These hormonal differences were also evidenced following an ip injection of saline (Gentsch et al, 1982). Comparative studies that include both Roman lines, both Maudsley lines and the SHS line have found that the SHS animals'

avoidance learning is inferior to that of the MNR and RHA animals and comparable to MR and RLA animals; SHS animals' open-field defecation rates are comparable to the MR animals' and higher than the MNR, RHA and RLA animals; SHS animals' activity level in the open-field is higher than the RHA, MR and RLA animals and comparable to the MNR animals (Satinder, 1980).

Neurohormones have been shown to play a role in both immune function (Ader, 1981; Soloman and Amkraut, 1981) and taste aversion (Chambers, 1976; Chambers and Sengstake, 1976; Hennessy, Smotherman and Levine, 1976). As the preceding review indicated, there is neurohormonal and learning variability in the genetic lines selected for this study. This variability provides a focal point for investigation of any genetic line differences in taste aversion and/or behaviorally conditioned immunosuppression that might occur.

This study has departed from the original paradigm used by Ader and Cohen in a number of ways. Firstly, a 5% sucrose solution was used instead of a saccharin chloride solution. Ader and Cohen (1981) report that they have successfully used sucrose to produce behaviorally conditioned immunosuppression. No details were provided so, presumably, there were no significant differences in the degree of success. Ader and Cohen (1975) used subgroups within the conditioned group. All subgroups received the Day 0 Saacharin-CY pairing, but varied in Day 3 and Day 6 drinking solution - injection pairings. This study used a conditioned group (sucrose-CY) with no subgroups, a nonconditioned group (water-CY) and a placebo group which paired sucrose with an isotonic saline injection (sucrose-isotonic

solution). The conditioned group received the sucrose-CY pairing to behaviorally condition immunosuppression. The water-CY pairing was selected to provide as a control for the effects of CY on immune function per se. The sucrose-isotonic solution pairing provided a control for the effects of sucrose solution consumption per se and the effects of an injection per se on the immune response. This study also included a procedure for the measurement of fluid preference for two days following each injection. This provided a measure of taste aversion. This two bottle method of determining fluid preference provides a more sensitive measure than using a single bottle of the CS fluid (Dragoin, McCleary & McCleary, 1971). One further departure from Ader and Cohen's original study was the larger dose of cyclophosphamide used in this study. A pilot study revealed that the larger dose was required in order to increase the probability of the acquisition of taste aversion in both male and female animals.

In an adherence to the goal of exploring the parameters of behaviorally conditioned immunosuppression, this study examined potential differences in behaviorally conditioned immunosuppression of a primary humoral (T-cell dependent) immune response in male and female rats from five genetic lines.

Method

Animals

Sixty experimentally naive rats approximately 100 days of age equally represented by five genetic lines (MNR/Har/Lu, MR/Har/Lu, SHS, RHA/Lu, RLA/Lu) and both sexes (n=2 for sex x experimental condition x genetic line cell) plus an additional three males from the SHS line comprised the sixty-three animals used in this study. The Maudsley nonreactive and Maudsley reactive lines have been selectively bred for low and high open-field emotional reactivity respectively (Broadhurst, 1960). The Roman high avoidance and Roman low avoidance have been selectively bred for high and low rates of two-way active avoidance, respectively (Bignami, 1965; Satinder, 1971, 1972a). The SHS line (Satinders Heterogenous Stock) is a genetic line developed through a four-way cross among the Maudsley and Roman lines. (Satinder, 1980). Additional details regarding the genetic history and behavioral characteristics of these lines have been previously discussed in Satinder (1972b, 1981).

Animals were individually housed on a 12:12 hour light/dark cycle and a laboratory temperature of $22 \pm 1^{\circ}$ C. Animals were used in groups of three same-sexed litter mates with one litter mate assigned to one of the three experimental conditions. The use of same-sexed litter mates meant that animals were matched to minimize individual differences in immune function due to individual genetic differences. Serum immunoglobulin concentrations

have been shown to vary among individuals (Grundbacher, 1974). These individual differences have been shown by twin studies to be partially genetic in origin (Kalff & Hijmans, 1969; Rowe, Boyle & Buchanan, 1968). Assignment to conditions was made by matching for body weights and adjunctively for average fluid and food intakes. Nonconditioned animals were housed in a different laboratory room than were the conditioned and placebo animals to control for the role of olfaction in taste aversion (Ader, 1977). Ader (1977) has reported that olfaction can influence acquisition of a taste aversion by decreasing the consumption of fluid by control animals or by decreasing the magnitude of the avoidance response. As a result, Ader (1981) recommends that different groups of animals be housed in separate rooms.

Materials

Sucrose Solution. A 5% (w/v) sucrose solution which served as a conditioned stimulus, was prepared by dissolving 25 mg of sugar in 500 ml volume of distilled water. Fresh preparations of the sucrose solution were made on a regular basis throughout the experiment.

Cyclophosphamide. Cyclophosphamide (CY), which served as the unconditioned stimulus, was obtained from Aldrich chemicals in the form of a powdered crystaline hydrate and stored at 4°C to increase its shelf life. In a pilot study, it was discovered that a dose of 50 mg/kg in a volume of 1.5 ml/kg was insufficient to produce taste aversion in a few of the female animals, whereas a dose of 75 mg/kg was found to be sufficient to produce

taste aversion in female animals and was therefore used throughout the experiment for all animals. As per the recommendations of Calabresi and Parks (1970), the cyclophosphamide solution was prepared no more than 30 minutes in advance of administration.

Buffered Physiological Saline. A phosphate-buffered 0.85% M physiological saline solution (Kwapinski, 1965) was used for injections in the placebo condition, to wash sheep's blood used to prepare sheep red blood cell suspensions and for dilutions used in the hemagglutinating antibody assay. The pH of the solution was routinely tested with hydrion pH paper to ensure that it was in the order of 7.2. Fresh preparations of the solution were made on a regular basis throughout the experiment.

SRBC Suspension (Antigen). A 1% thrice-washed suspension of SRBC's was prepared from sheep's blood (Nowotny, 1969) and preserved in a modified Alseiver's solution. The sheep blood was initially secured from a local Abattoir and subsequently from National Biological Laboratories. The blood was stored at approximately 4°C and suspensions were prepared no more than 20 minutes in advance of administration.

Additional Materials/Equipment. Additional materials/apparatus that were used throughout the experiment included Plastipak 1cc tuberculin hypodermic syringes, serological pipettes, micro cavity microscope slides, 18 gauge needles, 26 gauge needles, a Gilson P200 and a P1000 air displacement pipette, Carl Zeiss compound microscope and a Sorvall S-24 centrifuge.

Procedure

Baseline. The baseline phase of the experiment was two days in duration. On the first day, Day B0, animals were weighed and placed in individual cages that were placed on a cage rack. The animals were then supplied, ad libitum, with food that had been weighed and water in calibrated bottles. On Day B1, 24 hours later, the animal and its food were weighed and water consumption was recorded. On Day B2, 48 hours after the animal was first placed in its cage, the animal and its food were weighed and the fluid consumption was recorded. On Day B2, after all data was recorded, the fluid bottles were removed from the cage. Therefore, animals no longer had an ad libitum supply of water. Removing the fluid bottles from the cage marked the end of the baseline phase and the beginning of the preconditioning phase of the experiment.

Preconditioning. Prior to assignment to experimental groups and actual testing, a water intake regimen was established. This constituted the preconditioning phase. Animals were restricted to two, one hour morning and one hour afternoon, drinking periods. Each animal's daily water consumption was restricted to these two, sixty minute drinking periods throughout the remainder of the experiment. Body weights were recorded daily throughout the entire preconditioning phase. On the seventh day of the preconditioning phase, animals were assigned to experimental groups by matching same-sexed litter mates for average body weight, average daily food, and water consumption.

Conditioning

Conditioned Group. The initial day of conditioning was designated as Day 0. On Day 0, two drinking bottles containing a 5% sucrose solution and one empty bottle were affixed to the front of the cage at the beginning of the morning drinking period. Fluid levels were then recorded. At the end of one hour, the fluid levels were recorded and the drinking bottles were removed. An intraperitoneal (ip) injection of CY was administered thirty minutes later. In the interim period between the end of the drinking period and the ip injection, the animals, their food and their food wastage were recorded. The procedure for weighing the animals, their food and food wastage was performed at the same time each day throughout the remainder of the experiment. On Day 3, the morning drinking period was the same as outlined for Day 0 with animals receiving an ip injection of SRBC in place of the CY. The SRBC suspension was injected to initiate a primary humoral immune response which would provide a measure of the conditioning of the immune response by means of a hemagglutinating antibody titer. Further, the SRBC injection was administered 30 minutes following the drinking period -- CS exposure -- to simulate the Day 0 CS-US pairing. It has been repeatedly demonstrated (Hutton, Woods & Makous, 1970; Seigel, 1975) that the injection itself becomes an integral part of the CS. The SRBC injection thus serves the dual role of supplying both the antigen and the "injection complex". In addition, procedures followed for the morning drinking period on Day 6 were as outlined for Day 3 and Day 0 but with no injection following the drinking period.

On Days 1, 2, 4 and 5, the drinking bottles affixed to the front of the cage for the morning drinking period included one empty bottle, one bottle containing a 5% sucrose solution and one bottle containing distilled water. By providing both a bottle containing water and one containing a sucrose solution, the animals were provided with a choice between fluids. This choice is important, as it provides a measure of an animal's fluid preference -- a measure of taste aversion. As per the procedures outlined for Days 0, 3 and 6, the fluid levels for each bottle were recorded both at the beginning and end of the drinking period and all bottles were removed with the termination of the 60 minute drinking period.

On Days 7 and 8, the three bottles affixed to the cage for the morning drinking period included one empty bottle and two bottles containing distilled water. No sucrose solution was provided during these drinking periods in order to minimize the extinction of the taste aversion by limiting the number of times the animal was exposed to the sucrose solution. Again, fluid levels were recorded at both the beginning and end of the drinking period and all bottles were removed at the end of the drinking period. The bottles are, of course, removed in order to continue the restriction of fluid consumption to the two daily drinking periods.

In addition to the morning drinking period, there was also a daily afternoon drinking period. The afternoon drinking period also lasted 60 minutes and involved affixing three bottles to the front of the cage. The contents of the bottles were the same for each day of the experiment; two

contained distilled water while the third was empty. The afternoon drinking period was included in this study to reduce fluid deprivation resulting from the restricted fluid intake and to minimize relative fluid deprivation on Day 0 and Day 3.

In addition to the procedures outlined above, it should be noted that the bottles were rotated for both drinking periods on each day of the experiment. This was done to prevent animals from developing a preference for a drinking position per se as opposed to a fluid.

On Day 9, blood was collected from animals for the subsequent determination of hemagglutinating antibody titers.

Nonconditioned Group. The procedures followed for the nonconditioned group of animals was the same as those outlined above for the conditioned group of animals. The only difference that existed in the procedure was that the animals in the nonconditioned group received distilled water instead of the 5% sucrose solution the conditioned animals received on Days 0, 3 and 6. The distilled water was used instead of the sucrose solution so that an examination of the nonconditioned group of animals would show the effects of CY on the immune system per se. In addition, the nonconditioned animals were housed in a different room than that in which the conditioned and placebo animals were housed.

Placebo Group. The procedures employed for animals in the placebo group

were essentially identical to those outlined above for the conditioned group. The only difference between the two groups was that animals in the placebo group received an ip injection of physiological saline on Day 0 instead of the CY which the conditioned animals received. The physiological saline solution was used instead of the CY in this group so that the effects of exposure to a novel stimulus -- sucrose -- per se could be observed. In addition, the physiological saline reflects the effects of an injection per se. (See Table 1 for experimental protocol).

Blood Collection

Procedure

Blood collection occurred on Day 9 after the morning drinking period. The animals, their food and food wastage were weighed.

After the animals were weighed, an ip injection of euthanyl was administered. The dosage of euthanyl--one half the lethal dose--was .001 mg/ml. Once the drug had taken effect, blood was obtained by cardiac puncture using an 18 gauge needle attached to a tuberculin syringe. Blood was drawn into the syringe, the syringe was detached from the needle and the blood was then released into a test tube labelled with the animal's experimental number. This procedure was continued until approximately 4 ml of blood was extracted from the animal. On a number of occasions it was necessary to surgically open the heart and then draw blood into a syringe

for transfer to a labelled test tube. Once blood was in a test tube, the blood was placed into a refrigerator (4°C) where it was allowed to clot overnight.

Hemagglutinating Antibody Titer

Procedure

On Day 10, the blood was removed from the refrigerator and centrifuged at 1000Xg for 10 minutes. One mililiter of the resulting blood serum was transferred to a clean test tube and placed in a water bath at 57°C for 30 minutes to inactivate any complement (Ader & Cohen, 1975). For each titer, 11 test tubes were arranged in a test tube rack and labelled for a serial twofold dilution--ratios of 1:2, 1:4 through to 1:1024 and a control. To each test tube was added .5 ml of a buffered physiological saline solution using an air-displacement pipette. Using an air-displacement pipette, .5 ml of blood serum was added to the first test tube. The solution was mixed, .5 ml of the solution was transferred to the next test tube, mixed and the process continued for each test tube. The .5 ml serum-saline mixture drawn from the test tube labelled 1:1024 was discarded. Once all the appropriate dilutions were prepared, .5 ml of a 1% thrice washed suspension of sheep red blood cells was added to each test tube; including the test tube labelled "control". Following this, all test tubes were placed in a water bath at 37° C for a minimum of 1 hour, after which test tubes were centrifuged at 200Xg for 2 minutes. Once the solutions had been centrifuged, a drop of the solution was placed on a clean microscope slide and examined under a

magnification of 100X to ascertain the presence of agglutination. An examination of all test tubes was made and the reciprocal of the highest dilution producing definite hemagglutination, the end point of the titer, was recorded and expressed as a power of the base two (Kwapiniski, 1965). The criteria for determining the end point of the titer were adopted from Moore, Humphreys and Lovett-Moseley (1972, pp. 67-71). The procedures for blood collection and assaying the hemagglutinating antibody titer were repeated for each individual animal.

Table 1

Experimental Protocol

	Day 0		Day 1 & 2	Day 3		Day 4 & 5	Day 6-8	Day 9
	Morning		Morning	Morning		Morning	Morning	
	Fluid	Inj	Fluid	Fluid	Inj	Fluid	Fluid	
Conditioned	Suc	CY	Suc vs H_2O	Suc	SRBC	Suc vs H_2O	H_2O	Sacrificed
Nonconditioned	H_2O	CY	Suc vs H_2O	H_2O	SRBC	Suc vs H_2O	H_2O	Sacrificed
Placebo	Suc	Sal	Suc vs H_2O	Suc	SRBC	Suc vs H_2O	H_2O	Sacrificed

Note (1) distilled water was the sole fluid provided for the afternoon drinking period
(2) Suc - sucrose solution, Sal - physiological saline solution, CY - cyclophosphamide.

Results

For statistical analysis, data was acquired from the five phases of the experiment; baseline, preconditioning (fluid intake regimen), dr/saline (post-drug/saline injection), SRBC (post-SRBC injection) and post conditioning (return to preconditioning status).

Immunosuppression

Evaluation by 2 (sex) x 3 (expt'al condition) x 5 (genetic line) anova of hemagglutinating antibody titers revealed a significant main effect for the sex [$F(1,33) = 14.11$, $p<.01$] and experimental condition [$F(2,33) = 36.70$, $p<.01$] variables. Further examination of experimental condition differences by use of a Newman-Keuls pairwise comparison test (see Table 2) shows that all groups differed significantly ($p<.01$) from each other.

Mean titers for male and female animals and experimental groups are presented in Table 3. In addition, Figure 2 shows mean titers of experimental groups for each genetic line.

Table 2

Newman-Keuls Test for
Experimental Condition Differences

Experimental Condition	Conditioned	Nonconditioned	Placebo
Conditioned	———	Diff=1.47(Cr=.69**)	Diff=2.90(Cr=.78**)
Nonconditioned	———	———	Diff=1.43(Cr=.69**)
Placebo	———	———	———

Note (1) Diff: Difference between means
(2) Cr: Newman-Keuls critical value
(3)**: indicates significance at $p<.01$

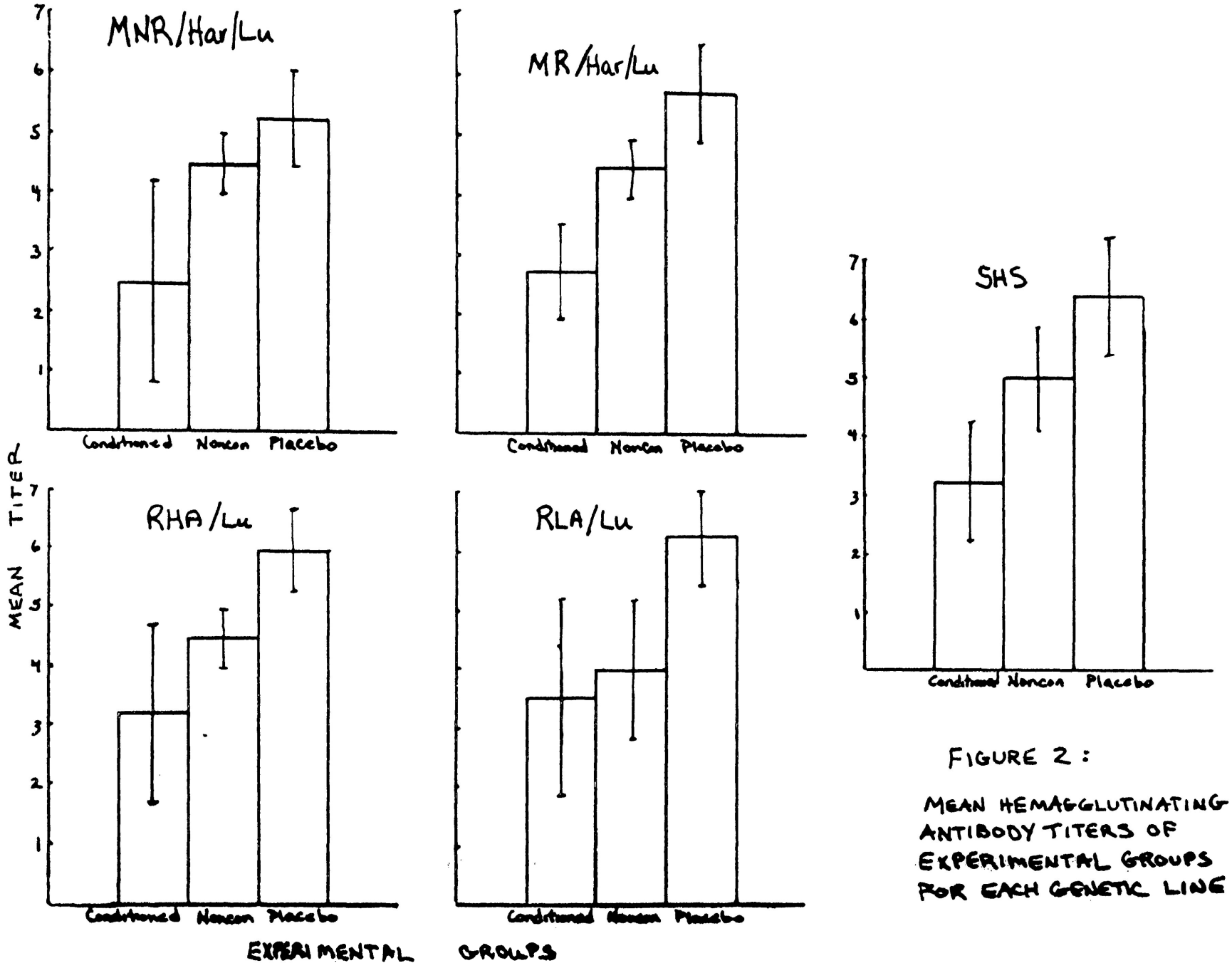


FIGURE 2:

MEAN HEMAGGLUTINATING ANTIBODY TITERS OF EXPERIMENTAL GROUPS FOR EACH GENETIC LINE

Table 3

Mean Titers for Male and Female Animals and for Each Experimental Group

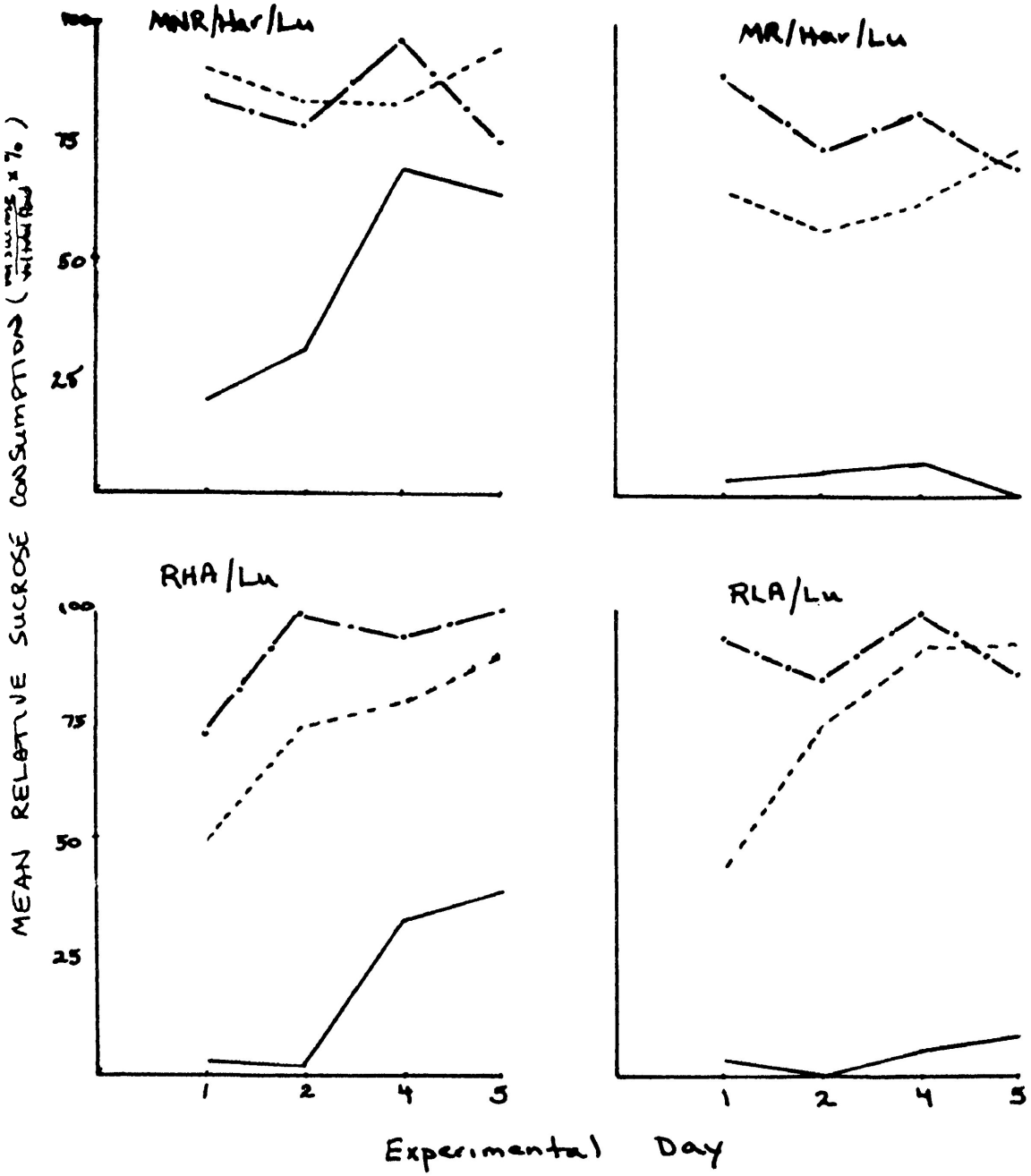
Experimental Variables				
Sex		Experimental Groups		
Males	Females	Conditioned	Nonconditioned	Placebo
4.03±1.5 (n=33)	5.03±1.8 (n=30)	3.05 ± 1.4 (n=21)	4.52 ± .9 (n=21)	5.95 ± 1.0 (n=21)

Note (1) n= number of animals

Taste Aversion

Consumptions of sucrose solution and distilled water during morning drinking periods for Days 1,2,4 and 5 were expressed as ratios of sucrose to total fluid consumed and plotted for experimental conditions for each genetic line (see Figure 3). These ratios (fluid preferences) were also analyzed with a variety of factorial Anova's.

Table 4 summarizes a series of 2 (sex) x 3 (expt'al condition) x 5 (genetic line) Anova's for each day a fluid choice was provided. The statistically significant experimental condition and genetic line differences indicated in Table 4 were further analyzed by Newman-Keuls pairwise comparison tests and are presented in Tables 5 and 6 respectively. Interaction effects were also analyzed.



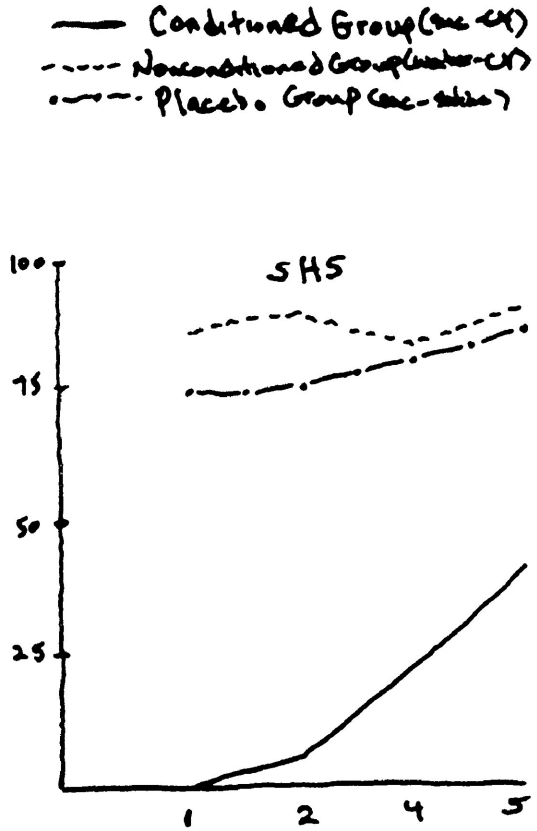


FIGURE 3: GRAPHIC REPRESENTATION OF TASTE AVERSION IN EXPERIMENTAL GROUPS FROM EACH GENETIC LINE

Table 4

Summary of 2(sex)x3(expt'al condition)x5(genetic line) Anova's Examining Fluid Preferences

	Experimental Day			
Variable	Day1	Day2	Day4	Day5
Sex d.f.(1,33)	F=3.6	F=3.5	F=4.1^{+}	F=12.0**
Con d.f.(2,33)	F=82.1**	F=73.4**	F=114.8**	F=104.5**
Gl d.f.(4,33)	F=2.1	F=1.3	F=7.5**	F=9.1**
SexCon d.f.(2,33)	F=.1	F=.8	F=3.4*	F=16.6**
SexGl d.f.(4,33)	F=1.3	F=.02	F=3.0*	F=2.6
ConGl d.f.(8,33)	F=1.8	F=1.1	F=4.3**	F=4.2**
SexConGl d.f.(8,33)	F=1.6	F=1.5	F=1.8	F=2.4*

Note (1)Con- Experimental condition, Gl- genetic line
(2)$p < .05^{*}$, $p < .01^{**}$, $p = .052^{+}$

The taste aversion is composed of two components: (i) a novel stimulus (sucrose solution) followed by (ii) an aversive consequence resulting in a subsequent reduction in relative sucrose solution consumption. Therefore, for true taste aversion to occur, the effects of both these components should be evidenced. In other words, there should be a significant difference in subsequent relative sucrose solution between

sucrose-cyclophosphamide and water-cyclophosphamide groups (reflecting the novel stimulus aspect) and between sucrose-cyclophosphamide and sucrose-saline groups (reflecting the aversive consequence aspect). On the

Table 5

Newman-Keuls Pairwise Comparison Tests for Experimental Condition Differences in Fluid Preferences

	Day1		
	Con	Noncon	Plac
Con	—	.63(.17**)	.78(.19**)
Noncon	—	—	.15(.13*)
Plac	—	—	—

	Day2		
	Con	Noncon	Plac
Con	—	.68(.18**)	.74(.20**)
Noncon	—	—	.06(ns)
Plac	—	—	—

	Day4		
	Con	Noncon	Plac
Con	—	.53(.12**)	.63(.14**)
Noncon	—	—	.10(.09*)
Plac	—	—	—

	Day5		
	Con	Plac	Noncon
Con	—	.53(.12**)	.58(.13**)
Plac	—	—	.05(ns)
Noncon	—	—	—

Note (1) Con- conditioned group, Noncon- nonconditioned group, Plac- placebo group

(2) significant at $p < .05^{*}$, $p < .01^{**}$ and nonsignificant(ns)

basis of this criteria, the significant experimental condition differences ($p<.01$) evident on Day 1 (see Tables 4 and 5) indicate overall taste aversion. Further, animals in the placebo group showed a statistically significant preference ($p<.05$) for the sucrose solution relative to animals in the nonconditioned group (see Tables 5 and 7). By Day 2, there was still evidence of overall taste aversion ($p<.01$) but the significant difference between the nonconditioned and placebo groups evidenced on Day 1 was no longer present (see Table 5).

Table 7.

Experimental Group's Mean Fluid Preferences

	Day1	Day2	Day4	Day5
Conditioned	.05 ± .15	.08 ± .20	.27 ± .30	.31 ± .36
Nonconditioned	.68 ± .30	.76 ± .21	.80 ± .17	.89 ± .10
Placebo	.83 ± .22	.82 ± .26	.90 ± .13	.84 ± .19

Note (1) Values represent a ratio of volume of sucrose consumption to total fluid consumption

On Day 4, overall, taste aversion was present ($p<.01$; see Tables 4 and 5). In addition, a significant sex difference was begining to develop ($p=.052$) with male animals showing less preference for the sucrose solution than the female animals (see Tables 4 and 8). There was also a significant

Table 8

Mean Male and Female Fluid Preferences

	Day1	Day2	Day4	Day5
Male	.47 ± .41	.51 ± .41	.62 ± .37	.62 ± .38
Female	.57 ± .40	.61 ± .39	.70 ± .33	.74 ± .30

Note (1) Values represent a ratio of volume of sucrose consumption to total fluid consumption

($p<.01$) genetic line difference (see Table 4). Table 6 reveals that these genetic line differences are due to the significantly lower preference shown by MR/Har/Lu animals relative to RHA/Lu ($p<.05$), RLA/Lu ($p<.05$) and

Table 6

Newman-Keuls Pairwise Comparison Tests for Genetic Line Differences in Day4 and Day5 Fluid Preferences

Day4					
	MR/Har/Lu	SHS	RLA/Lu	RHA/Lu	MNR/Har/Lu
MR/Har/Lu	——	.11(ns)	.15(.14*)	.18(.15*)	.32(.20**)
SHS	——	——	.04(ns)	.07(ns)	.21(.20**)
RLA/Lu	——	——	——	.03(ns)	.17(.16*)
RHA/Lu	——	——	——	——	.14(ns)
MNR/Har/Lu	——	——	——	——	——
Day5					
	MR/Har/Lu	RLA/Lu	SHS	RHA/Lu	MNR/Har/Lu
MR/Har/Lu	——	.14(.11*)	.24(.17**)	.27(.18**)	.29(.19**)
RLA/Lu	——	——	.10(ns)	.13(ns)	.15(ns)
SHS	——	——	——	.03(ns)	.05(ns)
RHA/Lu	——	——	——	——	.02(ns)
MNR/Har/Lu	——	——	——	——	——

Note (1)significant at $p<.05^{*}$, $p<.01^{**}$ and $p>.05^{ns}$

MNR/Har/Lu ($p<.01$) animals; in addition to greater preference shown by

MNR/Har/Lu animals relative to the SHS and RLA/Lu animals. As Table 4 indicates, there were significant sex-genetic line ($p<.05$), sex-experimental condition ($p<.05$) and genetic line-experimental condition ($p<.01$) interactions. Further analysis of the genetic line-experimental condition interaction (see Table 9) indicated that genetic line differences were significant ($p<.01$) within each experimental group and that experimental

Table 9

Genetic Line-Experimental Group Simple Interaction Effects For Fluid Preferences on Day 4

Source	df	SS	MS	F
Genetic line for Conditioned group	4	3.33	.83	23.79**
Genetic line for Nonconditoned group	4	.57	.14	4.08**
Genetic line for Placebo group	4	.34	.09	2.43**
Experimental group for MNR/Har/Lu	2	1.54	.77	22.00**
Experimental group for MR/Har/Lu	2	12.04	6.02	172.00**
Experimental group for SHS	2	7.66	3.88	110.86**
Experimental group for RHA/Lu	2	7.83	3.97	113.29**
Experimental group for RLA/Lu	2	21.54	10.77	307.71**
Pooled Error Term	41	1.43	.035	

Note (1) $p<.01$**

group differences were present in each genetic line ($p<.01$). Additional analysis of the sex-experimental condition interaction (see Table 10) showed that experimental condition differences -- taste aversion (see Table 5) -- were present for both males ($p<.01$) and females ($p<.01$). However, the sex

Table 10

Experimental Group-Sex Simple Interaction Effects For Fluid Preferences on Day 4

Source	df	SS	MS	F
Experimental group for Males	2	3.39	1.70	70.83**
Experimental group for Females	2	1.68	.84	35.00**
Sex for Conditioned group	1	.23	.23	9.75**
Sex for Nonconditioned group	1	.05	.05	2.13ns
Sex for Placebo group	1	.04	.04	1.71ns
Pooled Error Term	35	.84	.024	

Note (1) $p<.01$**, $p>.05$ns

difference was restricted to the conditioned group ($p<.01$; see Table 10). Mean fluid preferences for males and females within experimental groups are presented in Table 11. As indicated by Table 12, the sex difference in fluid preferences were limited to the SHS ($p<.05$) and RHA/Lu ($p<.01$) animals and genetic line differences were limited to female animals ($p<.01$).

Table 11

Male and Female Fluid Preferences Within Experimental Groups for Day4

	Conditioned	Nonconditioned	Placebo
Male n=11 per cell	.18 ± .22	.77 ± .19	.92 ± .12
Female n=10 per cell	.37 ± .34	.84 ± .12	.88 ± .15

Note (1) Values represent a ratio of volume of sucrose consumption to total fluid consumption

Table 12

Sex-Genetic Line Simple Interaction Effects For Fluid Preferences on Day4

Source	df	SS	MS	F
Sex for MNR/Har/Lu	1	.043	.043	1.65ns
Sex for MR/Har/Lu	1	.036	.036	1.39ns
Sex for SHS	1	.118	.118	4.54*
Sex for RHA/Lu	1	.235	.235	9.04**
Sex for RLA/Lu	1	.001	.001	.04ns
Genetic line for Males	4	.21	.052	1.92ns
Genetic line for Females	4	.85	.212	8.08**
Pooled Error Term	37	.951	.026	

Note (1) $p<.01^{**}$, $p>.05^{ns}$, $p<.05^{*}$

On Day 5, overall, taste aversion existed ($p<.01$) but the difference between placebo and nonconditioned groups found on Day 4 were no longer present on Day 5 (see Tables 4 and 5). There was also a significant sex ($p<.01$) and genetic line ($p<.01$) difference with MR/Har/Lu animals--relative to the other genetic lines--showing a significantly greater taste aversion to the sucrose solution (see Table 6). In addition, a sex-experimental condition interaction existed in which both sexes displayed evidence of taste aversion, but with a difference in the magnitude of the effect (Table 13 and 14). Likewise, there was a significant experimental condition-genetic line interaction in which genetic lines differed in conditioned

Table 13

Sex-Experimental Group Simple Interaction Effects for Fluid Preferences on Day 5

Source	df	SS	MS	F
Sex for Conditioned Group	1	.842	.842	21.59**
Sex for Nonconditioned Group	1	.032	.032	.82ns
Sex for Placebo Group	1	.058	.058	1.49ns
Experimental Group for Males	2	3.930	1.965	50.38**
Experimental Group for Females	2	1.070	.535	13.72**
Pooled Error Term	35	1.356	.039	

Note (1) $p<.01^{**}$, $p>.05^{ns}$

Table 14

Mean Male and Female Fluid Preferences Within Experimental Groups for Day5

	Conditioned	Nonconditioned	Placebo
Male n=11 per cell	.12 ± .19	.88 ± .11	.86 ± .12
Female n=10 per cell	.52 ± .37	.89 ± .09	.81 ± .24

Note (1) Values represent a ratio of volume of sucrose consumption to total fluid consumption

(p<.01) and placebo (p<.01) but not nonconditioned (p<.05) groups (see Table 15). There was also a sex difference in the magnitude of the experimental condition-genetic line interaction (see Table 16).

Table 15

Experimental Group-Genetic Line Simple Interaction Effects for Fluid Preferences on Day 5

Source	df	SS	MS	F
Experimental Group for MNR/Har/Lu	2	1.83	.91	27.58**
Experimental Group for MR/Har/Lu	2	13.85	6.93	210.00**
Experimental Group for SHS	2	2.21	1.11	33.64**
Experimental Group for RHA/Lu	2	26.15	13.08	396.36**
Experimental Group for RLA/Lu	2	17.23	8.63	261.52**
Genetic Line for Conditioned Group	4	4.24	1.06	32.12**
Genetic Line for Nonconditioned Group	4	.30	.07	2.24ns
Genetic Line for Placebo Group	4	.93	.23	7.09**
Pooled Error Term	41	1.37	.033	

Note (1) $p < .01^{**}$, $p > .05^{ns}$

Table 16

Sex-Experimental Group-Genetic Line Higher Order Interaction Effect for Fluid Preferences on Day5

Source	df	SS	MS	F
Females: Genetic Line-Experimental Group	8	8.646	1.081	41.54**
Males: Genetic Line-Experimental Group	8	1.500	.190	7.31**
Pooled Error Term	41	1.074	.026	

Note (1) $p < .01^{**}$

Conditioned Response

A Pearson moment - product correlational analysis was performed between titers and fluid preference consumptions for each day a fluid preference was provided (see Table 17). A positive correlation indicates that animals with a higher sucrose solution consumption also had higher titers (high antibody concentrations). In other words, a positive correlation would indicate that taste aversion (low sucrose solution consumption) would be associated with immunosuppression (low antibody) within the confines of the paradigm used in this study.

Table 17

Pearson Correlation Coefficients for Correlation Between Titer and Fluid Preferences for Each Experimental Day

Experimental Day			
Day1	Day2	Day4	Day5
.62**	.64**	.55**	.49**

Notes (1) Fluid preferences represent a ratio of sucrose consumption to water consumption

(2) A one tailed analysis was used since a significant negative correlation would not be expected to occur

(3) Significant at $p < .01$**

Food Consumption

Figure 4 shows each genetic line's mean food consumption for experimental conditions by experimental phases. Table 18 summarizes a series of 2 (sex) x 3 (expt'al condition) x 5 (genetic line) Anova's examining food consumption for the five experimental phases.

FIGURE 4: Mean Food Consumption of Experimental Groups For Each Genetic Line According to Experimental Phases

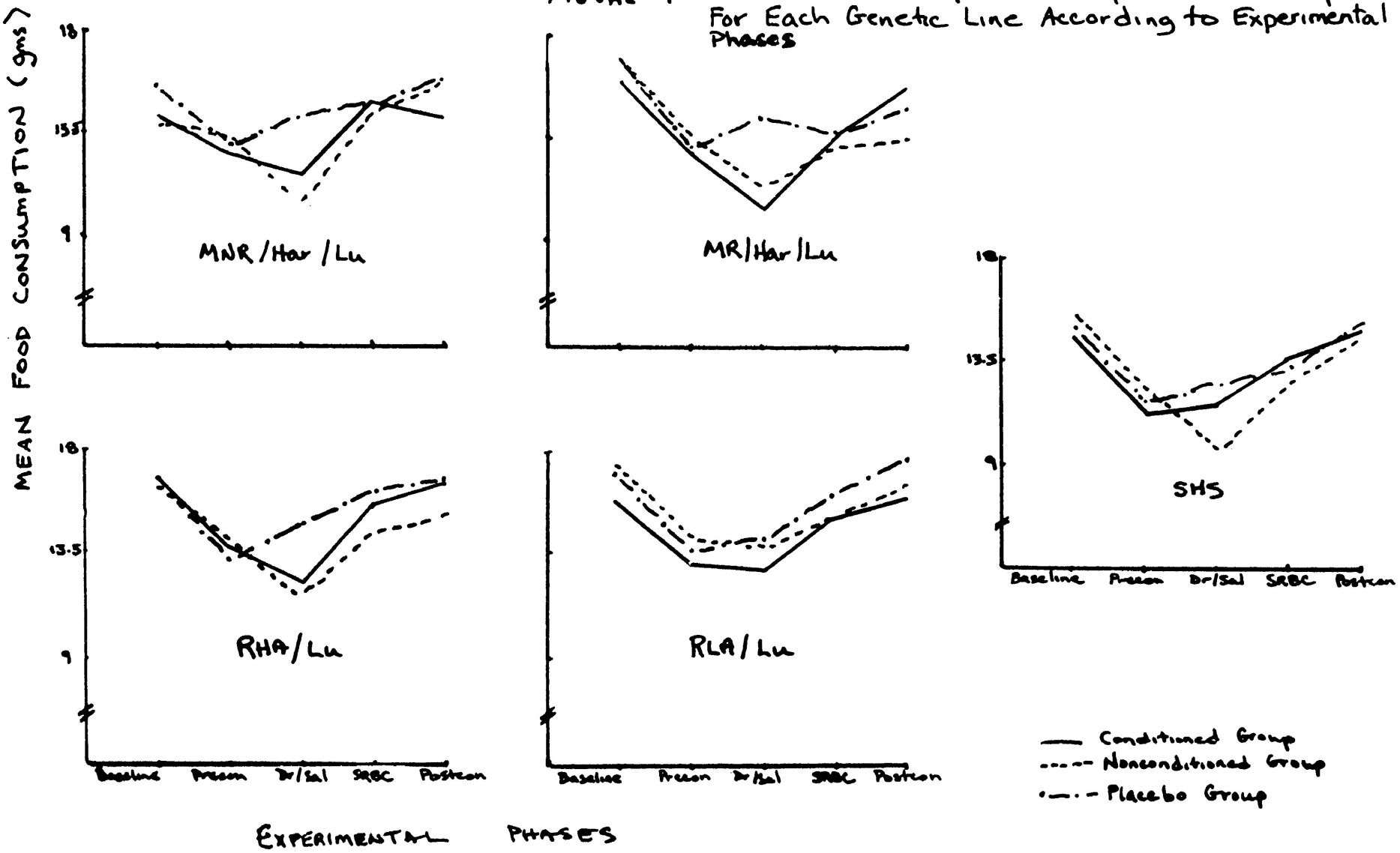


Table 18

Summary of 2(sex)x3(expt'al condition)x5(genetic line) Anovas Examining Food Consumption

Experimental Variables	Experimental Phases				
	Baseline	Precond	Dr/Saline	SRBC	Postcond
Sex	F= 51.3**	F= 94.1**	F= 68.1**	F=179.9**	F=159.6**
Genetic Line	ns	F= 3.9**	F= 4.5**	F= 13.5**	F= 5.4**
Exp'tal Condition	ns	ns	F= 19.0**	F= 3.3*	ns
Sex-Exp'tal Cond	ns	ns	F= 5.8**	F= 9.7**	ns
Sex-Genetic Line	ns	ns	ns	F= 2.6*	ns

Note (1) $p<.01^{**}$, $p<.05^{*}$, $p>.05^{ns}$

The statistically significant main effect for the genetic line and experimental condition variables were further analyzed with Newman-Keuls pairwise comparison tests (see Table 19). In addition, statistically significant interaction effects were analyzed and are presented in Table 20. Overall, there was a significant genetic line difference in food consumption for all but the baseline phase (see Table 18). However, as Table 18 indicates, during the preconditioning phase none of the genetic lines differed significantly. Further, the only significant difference between genetic lines in the dr/saline phase was between the SHS and RLA/Har/Lu ($p<.01$) lines; while the only significant difference in the postconditioning phase was between the MR/Har/Lu and RHA/Lu lines and SHS and RHA line. The

major genetic line differences in food consumption were primarily evidenced in the SRBC phase where the SHS and MR/Har/Lu lines did not differ significantly but did differ from each of the other genetic lines. The experimental condition differences for the dr/saline

Table 19

Newman-Keuls Pairwise Comparison Tests of Genetic Lines' Food Consumption for Experimental Phases

	SHS	MR/Har/Lu	MNR/Har/Lu	RHA/Lu	RLA/Lu
SHS	---	1.38(1.39)ns	1.48(1.67)ns	1.70(1.83)ns	1.76(1.95)ns
MR/Har/Lu		------	.10(1.39)ns	.32(1.67)ns	.38(1.83)ns
MNR/Har/Lu			------	.22(1.39)ns	.28(1.67)ns
RHA/Lu				------	.06(1.39)ns
RLA/Lu					------

Dr/Saline Phase

	SHS	MR/Har/Lu	MNR/Har/Lu	RHA/Lu	RLA/Lu
SHS	---	.07(1.20)ns	1.12(1.45)ns	1.37(1.59)ns	2.06(1.69)*
MR/Har/Lu		------	.42(1.20)ns	.67(1.45)ns	1.36(1.59)ns
MNR/Har/Lu			------	.25(1.20)ns	.94(1.45)ns
RHA/Lu				------	.69(1.20)ns
RLA/Lu					------

SRBC Phase

	SHS	MR/Har/Lu	MNR/Har/Lu	RHA/Lu	RLA/Lu
SHS	---	.45(.97)ns	2.01(1.47)**	2.23(1.58)**	2.29(1.66)**
MR/Har/Lu		------	1.56(1.29)**	1.78(1.47)**	1.84(1.58)**
MNR/Har/Lu			------	.22(.97)ns	.28(1.17)ns
RHA/Lu				------	.06(1.29)ns
RLA/Lu					------

Postconditioning Phase

	MR/Har/Lu	SHS	MNR/Har/Lu	RHA/Lu	RLA/Lu
MR/Har/Lu	----	.37(1.20)ns	.97(1.45)ns	1.50(1.59)ns	2.17(2.05)
SHS		------	.60(1.20)ns	1.13(1.45)ns	1.80(1.59)*
MNR/Har/Lu			-----	.53(1.20)ns	1.20(1.45)ns
RHA/Lu				------	.67(1.2)ns
RLA/Lu					-----

Note (1) $p < .01^{**}, p < .05^{*}, p > .05^{ns}$

Table 20

Newman-Keuls Pairwise Comparison Tests of Experimental Groups' Food Consumption for Dr/Saline Phase

	Nonconditioned	Conditioned	Placebo
Nonconditioned	------	.49(.93)ns	2.23(1.40)**
Conditioned		------	2.72(1.23)**
Placebo			------

Table 21

Newman-Keuls Pairwise Comparison Tests of Experimental Groups' Food Consumption for SRBC Phase

	Nonconditioned	Conditioned	Placebo
Nonconditioned	----------	.70(.75)ns	.92(.90)*
Conditioned		--------	.22(.75)ns
Placebo			-----

Note (1) $p < .01^{**}$, $p < .05^{*}$, $p > .05^{ns}$

phase appear to be restricted to the males (see Table 22) while the differences during the SRBC phase (see Table 23) were present for both males ($p<.05$) and females ($p<.01$). The genetic line differences found in the SRBC phase (see Table 24) were present in both males ($p<.01$) and females ($p<.05$).

Table 22

Sex-Experimental Group Interaction Effects for Dr/Saline Food Consumption

Variable	df	SS	MS	F
Sex for Conditioned Group	1	21.00	21.00	7.29**
Sex for Nonconditioned Group	1	30.23	30.23	10.50**
Sex for Placebo Group	1	125.81	125.81	43.68**
Experimental Group for Males	2	101.23	50.62	17.58**
Experimental Group for Females	2	10.38	5.19	1.80ns
Pooled Error Term	35	100.90	2.88	

Note (1) $p < .01^{**}$, $p < .05^{*}$, $p > .05^{ns}$

Table 23

Sex-Experimental Group Interaction Effects for SRBC Phase Food Consumption

Variable	df	SS	MS	F
Sex for Conditioned Group	1	57.65	57.65	25.97**
Sex for Nonconditioned Group	1	108.78	108.78	49.00**
Sex for Placebo Group	1	150.34	150.34	67.72**
Experimental Group for Males	2	18.06	9.03	4.07*
Experimental Group for Females	2	23.96	11.98	5.40**
Pooled Error Term	35	77.64	2.22	

Note (1) $p < .01^{**}$, $p < .05^{*}$, $p > .05^{ns}$

Table 24

Sex-Genetic Line Interaction Effects for SRBC Phase Food Consumption

Variable	df	SS	MS	F
Sex for MNR/Har/Lu	1	90.20	90.20	51.84**
Sex for MN/Har/Lu	1	24.88	24.88	14.30**
Sex for SHS	1	37.39	37.39	21.49**
Sex for RHA/Lu	1	45.80	45.80	26.32**
Sex for RLA/Lu	1	86.25	86.25	49.57**
Genetic Line for Males	4	67.92	16.98	9.76**
Genetic Line for Females	4	19.28	11.08	2.77*
Pooled Error Term	37	64.49	1.74	

Note (1) $p < .01^{**}$, $p < .05^{*}$, $p > .05^{ns}$

Total Fluid Consumption Figure 5 shows the mean fluid consumption for each phase of the experiment according to experimental conditions and for each of the genetic lines. A series of 2 (sex) x 3 (expt'al condition) x 5 (genetic line) Anova's are summarized in Table 25. The genetic line differences were further analyzed by Newman-Keuls pairwise comparison tests to indicate

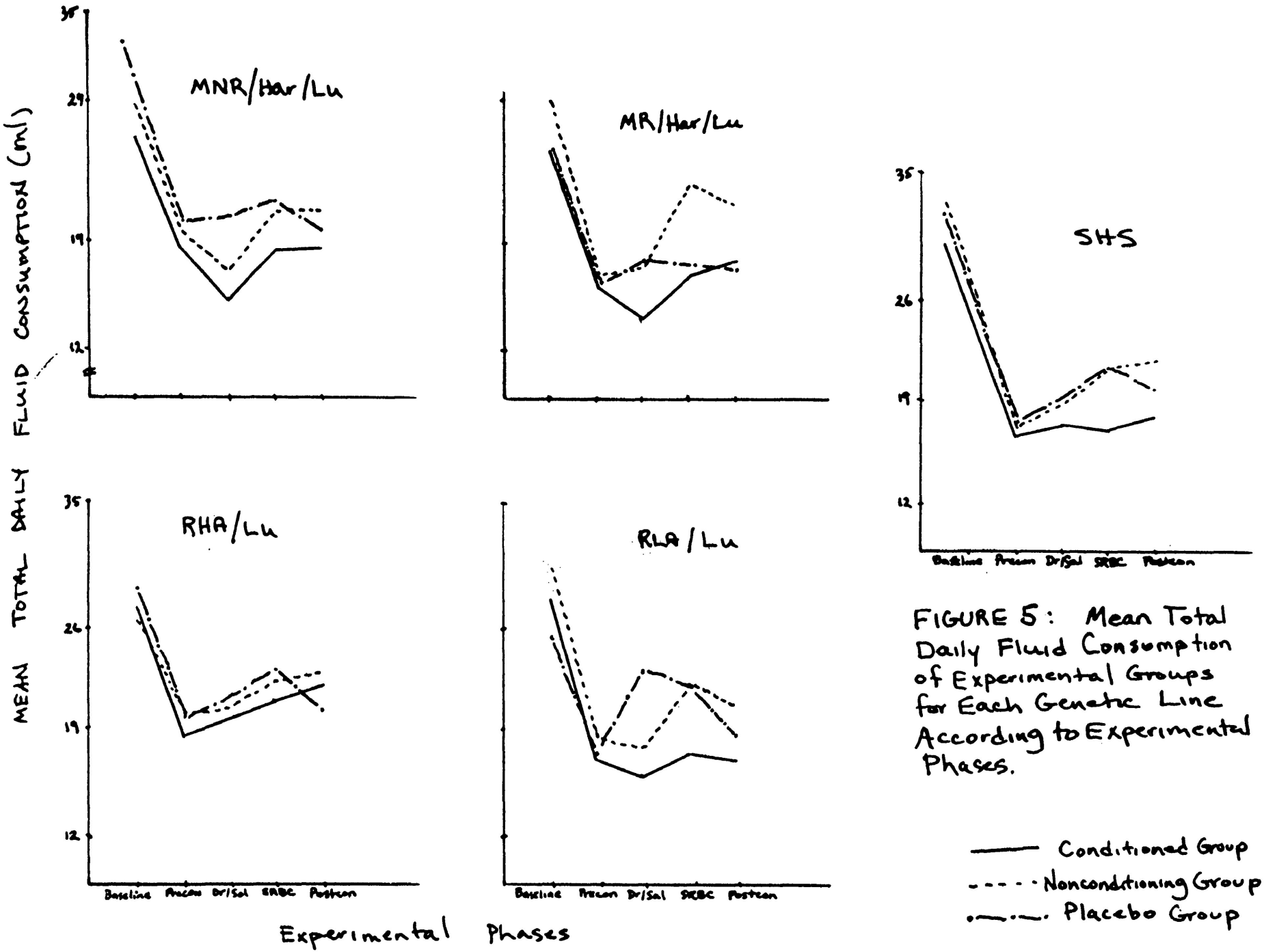


FIGURE 5: Mean Total Daily Fluid Consumption of Experimental Groups for Each Genetic Line According to Experimental Phases.

Table 25

Summary of 2(Sex)x3(Exp 'tal Condition)x5(Genetic Line) Anova
Examining Total Fluid Consumption for Experimental Phases

		Experimental Phases				
Variable	df	Baseline	Preconditioning	Dr/Saline	SRBC	Postconditioning
Sex	1	F=10.68**	F=52.22**	F=35.29**	F=36.57**	F=52.71**
Genetic Line	4	ns	F= 4.63**	F= 2.99*	F= 2.68*	ns
Expt'al Condition	2	ns	ns	F= 9.63**	F=11.05**	F= 7.32**
Sex-Genetic Line	4	ns	ns	ns	F= 2.78*	ns
Total=	33					

Note (1) $p < .01^{**}$, $p < .05^{*}$, $p > .05^{ns}$

precisely which lines differed significantly from one another (see Table 26). In addition, experimental condition differences (see Table 27) were further analyzed with Newman-Keuls pairwise comparisons test. Table 28 shows results of analyses investigating the sex-genetic line difference seen in Table 25.

Table 26

Newman-Keuls Pairwise Comparison Tests Examining Genetic Line Differences in Total Fluid Consumption

Preconditioning Phase					
	MR/Har/Lu	SHS	RLA/Lu	RHA/Lu	MNR/Har/Lu
MR/Har/Lu	-----	.54(ns)	.84(ns)	3.09(2.84)*	3.34(3.03)*
SHS		---	.30(ns)	2.55(ns)	2.80(ns)
RLA/Lu			-----	2.25(2.15)*	2.50(ns)
RHA/Lu				-------	.25(ns)
MNR/Har/Lu					-------
Dr/Saline Phase					
	MR/Har/Lu	SHS	RLA/Lu	RHA/Lu	MNR/Har/Lu
MR/Har/Lu	------	1.25(ns)	1.85(ns)	2.25(ns)	4.08(3.42)*
SHS		---	.60(ns)	1.00(ns)	2.83(ns)
RLA/Lu			----	.40(ns)	2.23(ns)
RHA/Lu				----	1.83(ns)
MNR/Har/Lu					-----
SRBC Phase					
	MR/Har/Lu	SHS	MNR/Har/Lu	RLA/Lu	RHA/Lu
MR/Har/Lu	-----	.88(ns)	1.58(ns)	1.66(ns)	3.66(3.46)*
SHS		----	.70(ns)	.78(ns)	2.78(ns)
MNR/Har/Lu			------	.08(ns)	2.08(ns)
RLA/Lu				----	2.00(ns)
RHA/Lu					-----

Note (1) $p < .01^{**}$, $p < .05^{*}$, $p > .05^{ns}$

Table 27

Newman-Keuls Pairwise Comparison Tests Examining
Experimental Group Differences in Total Fluid Consumption

	Experimental Group	Nonconditioned Group	Placebo Group
Dr/Saline Phase			
Experimental Group	----------	1.81(ns)	4.10(2.83)**
Nonconditioned Group		----------	2.29(1.87)*
Placebo Group			--------
SRBC Phase			
Experimental Group	----------	3.19(2.52)**	4.29(2.87)**
Nonconditioned Group		----------	1.05(ns)
Placebo Group			----------
Postconditioning Phase			
Experimental Group	----------	.57(ns)	3.09(2.61)**
Nonconditioned Group		----------	2.52(2.29)**
Placebo Group			---------

Note (1) $p < .01^{**}$, $p < .05^{*}$, $p > .05^{ns}$

Table 28

Genetic-Line-Sex Simple Interaction for
Total Mean Fluid Consumption for SRBC Phase

Variable	df	SS	MS	F
Genetic Line for Males	4	164.10	41.02	3.65*
Genetic Line for Females	4	36.03	9.01	.80ns
Sex for MNR/Har/Lu	1	3.11	3.11	.28ns
Sex for MR/Har/Lu	1	24.86	24.86	2.21ns
Sex for SHS	1	67.71	67.71	6.03*
Sex for RHA/Lu	1	224.29	224.29	19.97**
Sex for RLA/Lu	1	131.15	131.15	11.68**
Pooled Error Term	37	415.40	11.23	

Note (1) $p < .01^{**}$, $p < .05^{*}$, $p > .05^{ns}$

Morning Fluid Consumption A 2 (sex) x 3 (expt'al condition) x 5 (genetic line) Anova was used to analyse morning fluid consumption for each phase of the experiment. Results are presented in Table 29. There was a significant sex difference ($p<.01$) for each phase of the experiment (see Table 29). Sex differences found in the conditioning and postconditioning phases were further analyzed and the results indicate that sex differences were limited to specific genetic lines (see Table 30).

Table 29

Summary of Significance from 2(Sex)x3(Exp'tal Con) x5(Genetic Line) Anova for Mean Morning Fluid Consumption

	Experimental Phase			
	Conditioning	Dr/Saline	SRBC	Postconditioning
Sex(1,33)	$F=45.83^{**}$	$F=10.63^{**}$	$F=16.93^{**}$	$F=36.67^{**}$
Experimental Con(2,33)	ns	$F=90.81^{**}$	$F=12.14^{**}$	ns
Genetic Line (4,33)	ns	ns	$F=2.84^{*}$	ns
Sex-Genetic Line(4,33)	$F=2.98^{*}$	ns	ns	$F=2.84^{*}$
Con-Genetic Line(8,33)	ns	$F=2.39^{*}$	ns	ns

Note (1) $p<.01^{**}$, $p<.05^{*}$, $p>.05^{ns}$

Table 30

Analysis of Sex-Genetic Line Simple Interaction for Mean Morning Fluid Consumption

Preconditioning Phase				
Variable	df	SS	MS	F
Sex for MNR/Har/Lu	1	7.68	7.68	1.34ns
Sex for MR/Har/Lu	1	34.68	34.68	6.06*
Sex for SHS	1	14.70	14.70	2.57ns
Sex for RHA/Lu	1	96.45	96.45	16.86**
Sex for RLA/Lu	1	158.25	158.25	27.67**
Pooled Error Term	37	211.45	5.72	

Postconditioning Phase				
Variable	df	SS	MS	F
Sex for MNR/Har/Lu	1	7.78	7.78	1.71ns
Sex for MR/Har/Lu	1	53.68	53.68	11.82**
Sex for SHS	1	1.64	1.64	.36ns
Sex for RHA/Lu	1	74.20	74.20	16.34**
Sex for RLA/Lu	1	45.40	45.40	10.00**
Pooled Error Term	37	167.89	4.54	

Note (1) $p < .01^{**}$, $p < .05^{*}$, $p > .05^{ns}$

Experimental condition differences found in the dr/saline and SRBC phases were analyzed with Newman-Keuls pairwise comparison tests (see Table 31). Table 32 shows the results of a Newman-Keuls pairwise comparison test performed to examine the significant genetic line differences found in the SRBC phase. Further, the experimental condition-genetic line interaction of

Table 31

Newman-Keuls Pairwise Comparison Tests Examining Experimental Group Differences in Mean Morning Fluid Consumption

Dr/Saline Phase			
	Experimental Group	Nonconditioned Group	Placebo Group
Experimental Group	----------	4.02(1.76)**	4.87(2.00)**
Nonconditioned Group		----------	8.89(1.76)**
Placebo Group			---------

SRBC Phase			
	Experimental Group	Nonconditioned Group	Placebo Group
Experimental Group	----------	3.24(2.38)**	4.22(2.71)**
Nonconditioned Group		----------	3.25(2.38)**
Placebo Group			---------

Note(1) $p < .01^{**}$, $p < .05^{*}$, $p > .05^{ns}$

Table 32

Newman-Keuls Pairwise Comparison Test
Examining Genetic Line Differences in
Mean Morning Fluid Consumption

	MR/Har/Lu	SHS	RLA/Lu	MNR/Har/Lu	RHA/Lu
MR/Har/Lu	------	.24ns	1.59ns	2.30ns	3.00(2.68)*
SHS		----	1.34ns	2.06ns	2.76(2.52)*
RLA/Lu			-------	.71ns	1.41ns
MNR/Har/Lu				------	.70ns
RHA/Lu					----------

Note(1) $p < .01^{**}$, $p < .05^{*}$, $p > .05^{ns}$

the dr/saline phase was examined in greater detail (see Table 33).

Table 33

Analysis of Mean Morning Fluid Consumption
Experimental Condition-Genetic Line
Interaction for Dr/Saline Phase

Variable	df	SS	MS	F
Experimental Condition for MNR/Har/Lu	2	160.12	80.06	13.73**
Experimental Condition for MR/Har/Lu	2	75.52	36.26	6.22**
Experimental Condition for SHS	2	168.63	84.32	14.46**
Experimental Condition for RHA/Lu	2	203.25	101.63	17.43**
Experimental Condition for RLA/Lu	2	317.86	158.93	27.26**
Genetic Line for Experimental Group	4	21.41	5.35	.92ns
Genetic Line for Nonconditioned Group	4	6.74	1.69	.23ns
Genetic Line for Placebo Group	4	111.33	27.83	4.77**
Pooled Error Term	41	238.94	5.83	

Note (1) $p < .01^{**}$, $p < .05^{*}$, $p > .05^{ns}$

Afternoon Fluid Consumption There were statistically significant ($p<.01$) overall sex differences in the amount of fluid consumption during the afternoon drinking period for all phases of the experiment (see Table 34). In addition, there were significant ($p<.01$) genetic line differences for the

conditioning phase and postconditioning phase. These genetic line differences were evaluated by Newman-Keuls pairwise comparison tests (see Table 35).

Examination of Table 34 revealed significant experimental condition differences in amount of fluid consumed during afternoon drinking perid for the dr/saline ($p<.01$), SRBC ($p<.01$) and postconditioning ($p<.01$) phases. These experimental condition differences were further analyzed with Newman-Keuls pairwise comparison tests (see Table 36).

Table 34

Summary of 2(sex)x3(exp'tal condition)x5(genetic line) Anova Showing Statistically Significant Differences in Mean Afternoon Fluid Consumption

	Preconditioning	Dr/Saline	SRBC	Postconditioning
Sex	$F=17.13^{**}$	$F=16.78^{**}$	$F=24.62^{**}$	$F=17.97^{**}$
Experimental Con	$F=1.13^{ns}$	$F=6.48^{**}$	$F=29.70^{**}$	$F=17.79^{**}$
Genetic Line	$F=5.90^{**}$	$F=1.191^{ns}$	$F=1.30^{ns}$	$F=6.79^{**}$

Note (1) $p<.01^{**}$, $p<.05^{*}$, $p>.05^{ns}$

Table 35

Newman-Keuls Pairwise Comparison Tests Examining Genetic Line Differences in Mean Afternoon Fluid Consumption

		RLA/Lu	MR/Har/Lu	SHS	MNR/Har/Lu	RHA/Lu
RLA/Lu	Con	----	.15ns	1.08ns	1.61(1.38*)	2.13(1.78**)
	Post	----	1.20ns	1.70ns	2.42(2.01*)	3.84(2.59**)
MR/Har/Lu	Con		------	.93ns	1.42(1.26*)	1.98(1.69**)
	Post		------	.56ns	1.22ns	2.64(2.47**)
SHS	Con			-----	.53ns	1.05ns
	Post			-----	.65ns	2.08(1.83*)
MNR/Har/Lu	Con				-----	.52ns
	Post				-----	1.42ns
RHA/Lu	Con					--------
	Post					--------

Note (1) $p<.01^{**}$, $p<.05^{*}$, $p>.05^{ns}$
(2) Con- Conditioning phase, Post- Postconditioning phase

Table 36

Newman-Keuls Pairwise Comparison Tests Examining Experimental Condition Differences in Mean Afternoon Fluid Consumption

	Dr/Saline Phase		
	Placebo Group	Experimental Group	Nonconditioned Group
Placebo Group	---	1.52(1.46**)	1.58(1.32*)
Experimental Group		---	.06(ns)
Nonconditioned Group			---
	SRBC Phase		
	Placebo Group	Nonconditioned Group	Experimental Group
Placebo Group	---	3.58(1.45**)	3.70(1.65**)
Nonconditioned Group		---	.12(ns)
Experimental Group			---
	Postconditioning Phase		
	Experimental Group	Placebo Group	Nonconditioned Group
Experimental Group	---	.12(ns)	3.09(1.78**)
Placebo Group		---	2.97(1.56*)
Nonconditioned Group			---

Note (1) $p < .01^{**}, p < .05^{*}, p > .05^{ns}$

Additional Variables

CS (Sucrose/Water) Consumption. On Day 0, for the morning drinking period, conditioned and placebo animals were supplied with a sucrose

solution, while nonconditioned animals received water. Using a 2 (sex) x 3 (expt'al condition) x 5 (genetic line) Anova, it was found that the only significant difference was a significant sex difference in the amount of CS fluid consumed on Day 0 [$F(1,33) = 12.0$, $p<.01$]; males consumed more fluid than females.

Injections. A series of 2 (sex) x 3 (expt'al condition) x 5 (genetic line) Anova's were performed to determine the differences in the volumes of injections. The results of these analyses revealed a significant difference in the volume of SRBC for experimental conditions [$F(2,33) = 3.9$, $p=.03$], genetic line [$F(4,33) = 11.5$, $p<.01$] and sex [$F(1,33) = 320.2$, $p<.01$]. There was a significant difference between genetic lines and males and females in the volume of anaesthetic [genetic line: $F(4,33) = 19.0$, $p<.01$; sex: $F(1,33) = 41.6$, $p<.01$] and for the volume of cyclophosphamide/physiological saline [genetic line: $F(4,33) = 9.2$, $p<.01$; sex: $F(1,33) = 312.4$, $p<.01$]. In addition, there was a sex-genetic line interaction in the volume of SRBC [$F(4,33) = 3.2$, $p<.03$].

Body Weight Figure 6 shows mean body weights with respect to experimental phases for each genetic line and all experimental conditions. A 2 (sex) x 3 (expt'al condition) x 5 (genetic line) Anova revealed significant sex differences in body weight ($p<.01$) for all experimental phases (see Table 37). Further, there were significant genetic line differences ($p<.01$) for all experimental phases (see Table 37). Table 38 shows the results of Newman-Keuls pairwise comparisons tests for the genetic line differences in body weight.

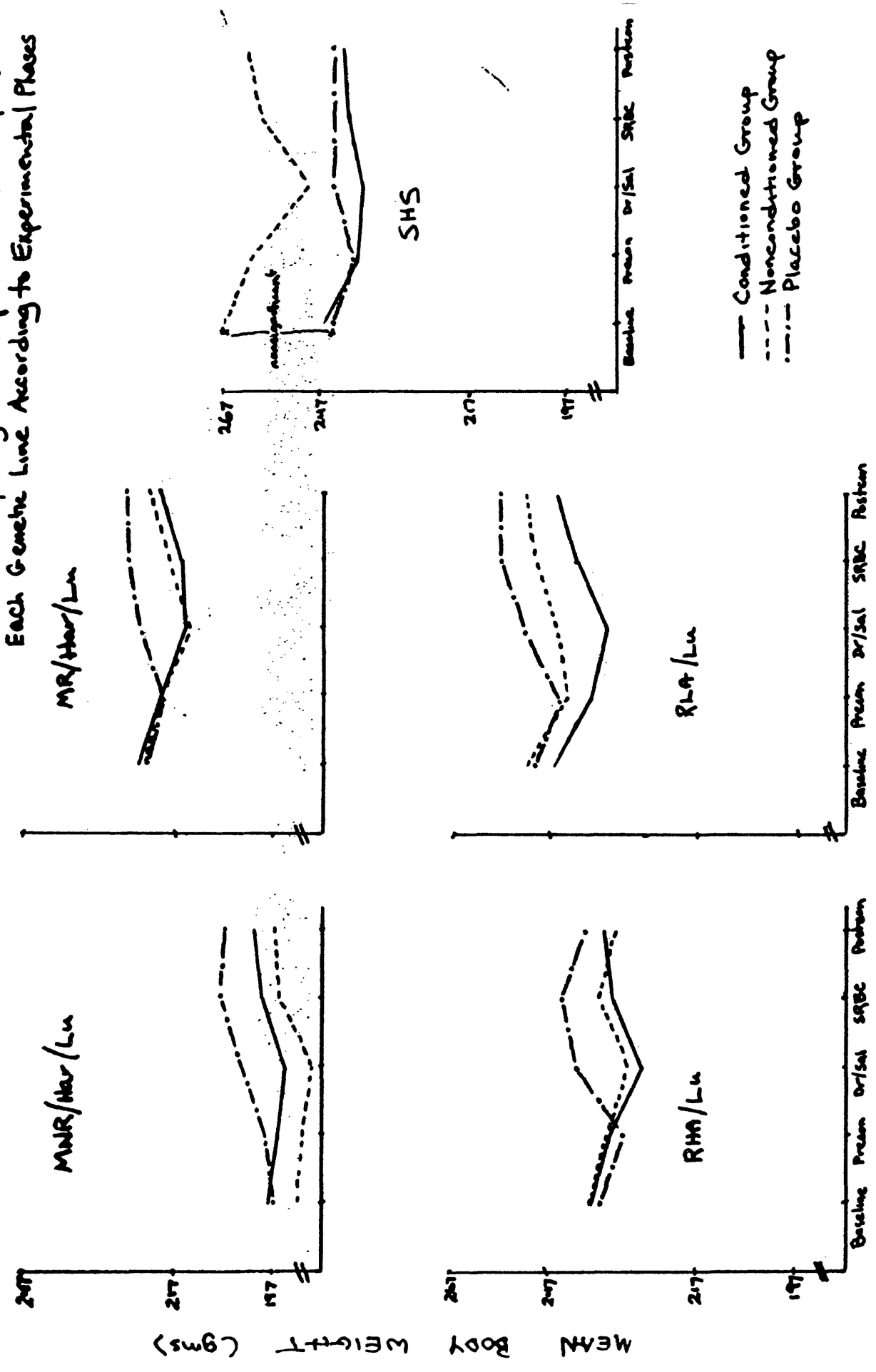


FIGURE 6 : Mean Body Weights of Experimental Groups for Each Genetic Line According to Experimental Phases

Table 37

Summary of Anovas Examining Body Weight Differences for Respective Experimental Phases

Variable(df)	Baseline	Preconditioning	Dr/Sal	SRBC	Postconditioning
Sex(1,33)	F=298.58**	F=313.55**	F=298.27**	F=331.44**	F=370.65**
Con(2,33)	F= .36ns	F= .37ns	F= .47ns	F= 1.21ns	F= .55ns
Gl(4,33)	F= 11.61**	F= 9.62**	F= 9.83**	F= 10.49**	F= 11.21**
SexGl(4,33)	F= 2.23ns	F= 2.52ns	F= 2.53ns	F= 2.81*	F= 2.73*

Note (1) $p<.01^{**}$, $p<.05^{*}$, $p>.05^{ns}$
(2) variables: con- experimental condition
gl- genetic line
sexgl- sex genetic line interaction
(3) df- degrees of freedom

Table 38

Newman-Keuls Analyses Investigating Genetic Line Differences in Body Weight for Each Experimental Phase

Baseline		MNR/Har/Lu	MR/Har/Lu	RHA/Lu	RLA/Lu	SHS
	MNR/Har/Lu	-----	27.97(23.35**)	42.59(26.58**)	54.06(28.50**)	56.44(29.93**)
	MR/Har/Lu	-----	-----	14.62(ns)	26.09(21.11**)	28.47(23.22*)
	RHA/Lu	-----	-----	-----	11.47(ns)	13.85(ns)
	RLA/Lu	-----	-----	-----	-----	2.38(ns)
	SHS	-----	-----	-----	-----	-----
Preconditioning		MNR/Har/Lu	MR/Har/Lu	RHA/Lu	RLA/Lu	SHS
	MNR/Har/Lu	-----	24.73(22.75**)	38.78(25.89**)	47.25(27.77**)	51.29(29.16**)
	MR/Har/Lu	-----	-----	14.05(ns)	22.52(20.57*)	26.56(22.63*)
	RHA/Lu	-----	-----	-----	8.47(ns)	12.51(ns)
	RLA/Lu	-----	-----	-----	-----	4.04(ns)
	SHS	-----	-----	-----	-----	-----
Dr/Saline		MNR/Har/Lu	MR/Har/Lu	RHA/Lu	SHS	RLA/Lu
	MNR/Har/Lu	-----	21.86(16.84*)	37.61(25.47**)	47.34(27.31**)	48.47(28.68**)
	MR/Har/Lu	-----	-----	15.75(ns)	25.48(25.47**)	26.61(22.25*)
	RHA/Lu	-----	-----	-----	9.73(ns)	10.86(ns)
	SHS	-----	-----	-----	-----	1.13(ns)
	RLA/Lu	-----	-----	-----	-----	-----
SRBC		MNR/Har/Lu	MR/Har/Lu	RHA/Lu	SHS	RLA/Lu
	MNR/Har/Lu	-----	19.50(16.33*)	37.46(24.70**)	46.62(26.48**)	48.21(27.81**)
	MR/Har/Lu	-----	-----	17.96(16.33*)	27.12(24.70**)	28.71(26.48**)
	RHA/Lu	-----	-----	-----	9.16(ns)	10.75(ns)
	SHS	-----	-----	-----	-----	1.59(ns)
	RLA/Lu	-----	-----	-----	-----	-----
Postconditioning		MNR/Har/Lu	MR/Har/Lu	RHA/Lu	SHS	RLA/Lu
	MNR/Har/Lu	-----	21.56(20.94**)	34.54(23.84**)	47.69(25.57**)	49.46(26.85**)
	MR/Har/Lu	-----	-----	12.98(ns)	26.13(23.84**)	27.90(25.57**)
	RHA/Lu	-----	-----	-----	13.15(ns)	14.92(ns)
	SHS	-----	-----	-----	-----	1.77(ns)
	RLA/Lu	-----	-----	-----	-----	-----

Note (1) $p < .01^{**}$, $p < .05^{*}$, $p > .05^{ns}$

Discussion

General Discussion

This study confirms previous reports of behaviorally conditioned immunosuppression of a primary humoral immune response (Ader & Cohen, 1975; Ader et al, 1979; Ader et al, 1982; Rogers et al, 1976; Wayner et al, 1978). The overall relative percentage of behaviorally conditioned immunosuppression was 24.81%. This figure is comparable to the 21.7% and 25.6% reported by Ader, Cohen & Grota (1979) and the 22.4% observed by Ader & Cohen (1975). Ader, Cohen & Bovbjerg (1982) report more robust results when--as with this study--the CS precedes antigenic stimulation. Presumably, their more robust results are comparable to those reported here. Further, Ader & Cohen (1975) reported nonsignificant correlations that ranged from -.34 to .16 between hemagglutination titer and volume of CS consumed among conditioned animals. In the present study, it was found that the correlation among conditioned animals between hemagglutination titer and volume of CS on day of conditioning was -.37 (n=21).

This study reports evidence of overall acquisition of taste aversion with no sex or genetic line differences. However, there were significant sex and genetic line differences in the extinction of the taste aversion with females exhibiting greater extinction than males; with greater extinction among females in the SHS and RHA/Lu genetic lines. Extinction of the taste aversion does not appear to occur to a statistically significant extent until Day 4. Wayner et al (1978) argue that the manifestation of

behaviorally conditioned immunosuppression requires the acquisition of conditioned taste aversion. In other words, if there is no taste aversion evidenced, then there will be no conditioned immunosuppression. The overall success in this study in conditioning a taste aversion may therefore, in part, explain the success in producing conditioned immunosuppression.

It has been demonstrated that under nondeprived conditions, males extinguish taste aversion at a slower rate than females (Chambers & Sengstake, 1976). It appears that the sex difference in extinction of a taste aversion under nondeprived conditions is due to elevated levels of testosterone (Chambers, 1976; Sengstake, Chambers & Thrower, 1978). In this study, there were sex and genetic line differences in extinction of taste aversion but not in its acquisition. Since, in addition there were no sex or genetic line differences in the acquisition of behaviorally conditioned immunosuppression, then one might speculate that it is a component specifically in the acquisition -- independent of any subsequent extinction -- of a taste aversion that is essential to the acquisition of behaviorally conditioned immunosuppression. This would likewise eliminate testosterone -- shown to be both immunosuppressive and stress responsive (Ader, 1983; Deiter & Breitenbach, 1970; Grossman, 1985) -- as playing a role in behaviorally conditioned immunosuppression.

There was a significant sex difference in titers, with males having a lower mean titer than females. In the absence of a signficant sex - experimental condition interaction, the sex difference is assumed to exist in all experimental groups. This would be indicative of the greater

immunoreactivity generally attributed to females (Grossman, 1985) and suggest that males and females did not differ significantly in the incidence of behaviorally conditioned immunosuppression.

There are two avenues of inquiry that may be pursued in attempting to explain the results of this study: behavioral and biochemical. The behavioral approach focuses on the behavioral differences among animals that affect the conditioning of a primary humoral immune response. The biochemical approach focuses on neurohormonal/biochemical and other physiological differences which affect the conditioning of a primary humoral immune response.

Speculative Neurohormonal/Biochemical Explanation

It should be remembered, of course, that the behavioral and biochemical phenomena occur concurrently. Thus, once a behavioral difference has been evidenced then, where possible, the corresponding neurohormonal/biochemical aspects have been examined.

The validity of invoking possible catecholamine influences in the mediation of behaviorally conditioned immunosuppression receives tentative support from research that demonstrates the effects of both beta-and alpha-adrenergic catecholamines on immunocompetence and the relationship between stress and the catecholamines. For instance, Theorell (1971) examined weekly life change unit (LCU) totals and urinary output of catecholamines. The LCU represented point values assigned to life events such as death of spouse,

trouble with boss, etcetera, with larger LCU values assigned to increasingly more dramatic changes. Theorell found that subject's urinary output of epinephrine correlated significantly with weekly LCU totals and similar, but nonsignificant results were found with norepinephrine. Further, these LCU totals also corresponded to increased illness susceptibility and incidence of coronary heart disease (Rahe, 1972). In addition, Bourne (1974) reports that in the presence of B-catecholamines there is a decrease in the number of demonstrable antibody-forming cells (splenic lymphocytes) and the B-catecholamines appear to be capable of inhibiting either the production or secretion of antibody. Therefore, it might be postulated that the the low antibody count (low titer) found in the SHS and MR/Har/Lu animals is the result of B-catecholamine activity triggered by as yet undefined parameters of the experimental paradigm unique to the conditioned experimental group.

There is some indication (Claesson, 1972) that the B-catecholamine epinephrine plays a role in the regulation of the number of thymocytes in the immune system. According to Claesson, thymocytes exhibit a consistent pattern of cellular decay. More precisely, there is a bimodal wave pattern of decay in a 24 hour period of time. In close examination of this pattern of thymic lymphoid cell decay, Claesson investigated the effects of adrenalectomy and subsequent injection of epinephrine on this pattern of cell decay. Claesson reports that adrenalectomy eliminated the bimodality of decay and produced an overall decrease in decay of thymocytes. The injected epinephrine resulted in an increased decay. Therefore, epinephrine may play a natural role in regulating the number of thymocytes found in the immune system. It also appears that stem cells possess a beta-adrenergic receptor

that, when stimulated, results in the synthesis of DNA and hence triggers the development of stem cells into lymphocytes (Byron, 1972). These beta-adrenergic effects are not the only catecholamine effects. The alpha-adrenergic catecholamine norepinephrine has also been found to be associated with immune function and response. Actual measurements of norepinephrine levels in rats following antigenic challenge (SRBC suspension) have been reported by Del Rey, Besedovsky, Sorkin, Da Prada and Bondiolotti (1982). Del Rey et al found that there was a decrease in splenic norepinephrine levels on Day 3 after antigenic challenge with a SRBC suspension. It was further observed that the diminished splenic norepinephrine levels persisted after Day 3 in some animals (high-responders) but not others (low-responders). These high-responders appear to also possess higher numbers of plaque forming cells (antibody-forming cells). Del Rey et al advanced the hypothesis that the norepinephrine level serves as a regulatory signal within the microenvironment of an ongoing immune response. This catecholamine effect has also been studied by Besedovsky, Del Rey, Sorkin, Da Prada, Burri and Honegger (1983) who report that a humoral immune response to SRBC's elicits a decrease in norepinephrine synthesis in the hypothalamus and that soluble products of activated immunological cells induce a decrease in norepinephrine content in the hypothalamus. Besedovsky et al further revealed that there were no changes in norepinephrine turnover rate in the right hemisphere, brain dopamine levels were unaffected and brain levels of epinephrine were not affected in any brain regions. An additional observation included decreased monoamine content in central norepinephrine neurons. It was concluded (Besedovsky et al, 1983) that the immune response

exerted an inhibitory action on central noradrenergic neurons and that the decrease in norepinephrine in the hypothalamus may cause an increase in the firing rates of neurons of the medial hypothalamus. From Besedovsky et al's research, one might speculate that animals manifesting behaviorally conditioned immunosuppression are, in effect, conditioned in some manner to be "ultra low-responders". In other words, as yet unknown parameters of the conditioning paradigm may alter the normal balance of norepinephrine which plays a key role (Del Rey et al, 1982) in an immune response.

There are numerous neurohormones that potentiate modulation of an immune response and that are, in addition, responsive to stress in general and to the numerous stresses associated with a taste aversion paradigm and may, therefore, play a role in conditioned immunosuppression. For instance, one hormone which has been shown to be modulated by stress and is also important in immunocompetence is growth hormone. For instance, Seggie and Brown (1975) found that five seconds of handling and a three minute exposure to a novel environment resulted in a significant decrease in growth hormone (GH) that reaches its nadir 15 minutes following the cessation of the stress and diminished levels extended for further 45 minutes. This was inversely correlated with corticosterone and prolactin which were both elevated and responded more to the three minute stress than the handling. The maximum levels of prolactin and corticosterone were found after about 15 minutes with both responses returning to baseline levels after a further 45 minutes. Further, both had circadian rhythms with less magnitude in the prolactin response. Kokka, Garcia, George and Elliott (1972) also report a decreased GH and elevated ACTH in response to stress. They further found that an ip

injection of saline failed to produce a significant rise in GH levels and thus any elevated GH levels are not solely attributable to the stress of injection. The immunological relevance of GH is shown by MacManus, Whitfield and Youdale (1969) who report that a withdrawal of GH results in a decline in thymocyte multiplication. They suggest that the GH sensitizes the thymocytes to calcium ions thereby promoting mitotic activity and cellular uptake of calcium ions. Further, MacManus et al reveal that GH appears to specifically stimulate the entry of thymocytes into the S phase of the cell cycle. It is the late G1 and early S phases of the cell cycle that immunologlobulin synthesis occurs (Thaler, Klausner & Cohen, 1977). Pierpaoli and Maestroni (1977) further suggest that GH is needed for the differentiation of T- derived cells to immunocompetent cells. Arrenbrecht (1974) reveals the presence of binding sites for GH on thymocytes and suggests that GH is capable of affecting helper T-cell function. Therefore, it is possible that behaviorally conditioned immunosuppression occurred, in part, as a result of impaired helper T-cell function exacerbated by low circulating levels of GH precipitated by undernutrition.

Gonadotropins also appear to be sensitive to the stress inherent in a taste aversion paradigm. The CS used in a taste aversion paradigm results in gastrointestinal distress and hence a reduction in food consumption (see Figure 4). According to Howland and Skinner (1973), gonadotropin secretion in male rats is inhibited during periods of undernutrition or starvation. They further reveal that starvation produces significant reductions in serum levels of FSH and LH within 48 and 24 hours respectively. The same effects were found by Root and Russ (1972) who, in addition, found that there were

higher levels of LH and FSH corresponding to the lower serum levels in male rats. Conversely, Negro-Vilar, Dickerman and Meites (1971) found that starvation resulted in a marked reduction in hypothalamic FSH-RF content and pituitary FSH content and concentration of the male rats used in their study. Female rats with a reduced food intake produced lowered pituitary LH content but no effect on pituitary FSH (see Negro-Vilar, Dickerman & Meites, 1971). Pierpaoli and Maestroni (1977) reveal that the immunologic significance of the gonadotropins lay in their role in the differentiation of the antigen-sensitive cells to antibody-forming cells. Also, Amkraut, Solomon, Kasper and Perdue (1973) found a suppressed GvH (Graft vs. Host) response to a limited feeding schedule. During the dr/saline phase, animals in the placebo group consumed significantly ($p<.01$) more food than animals in the nonconditioned and conditioned groups. However, these experimental group differences evidenced during the dr/saline phase in food consumption appear to be restricted to males. Apparently, as one might expect, the cyclophosphamide inhibits food consumption; and to a greater extent among males than females. This may indicate that males are more sensitive to the gastrointestinal distress than females and this greater sensitivity may further play a role in the sex differences in the extinction of a taste aversion. However, there is no evidence to indicate that this cyclophosphamide induced relative undernutrition definitely contributed to the behaviorally conditioned immunosuppression and thus remains highly speculative at best.

Perhaps the most widely studied hormones are the glucocorticoids and ACTH. Glucocorticoid levels increase, in rodents, in response to stress and this

increase has been used to explain low titers found in male and female mice exposed to crowded housing conditions (Brayton & Brain, 1975). Gisler, Bussard, Mazie and Hess (1971) have observed that, following stress induced increases in ACTH and corticosterone, there is a decrease in the number of circulating lymphocytes. This decrease was attributed to a temporary retention of lymphoid cells in lymphatic tissues. Further, Gisler and Schenkel-Hullinger (1971) have reported an inverse relationship between corticosterone levels and the number of antibody-forming spleen cells. Gisler and Schenkel-Hullinger further suggested that a pituitary factor was necessary for the reconstitution of immune reactivity after its depression by corticosteroids and they believed that the pituitary factor might be somatotropic hormone (STH). In a taste aversion paradigm, both ACTH and glucocorticoid levels become elevated (Hennessy, Smotherman & Levine, 1976). In fact, ACTH appears to play a vital role in the acquisition and extinction of a taste aversion (Smotherman & Levine, 1978). In light of the effects of glucocorticoids on the immune system (Claman, 1972), the relevance of glucocorticoids in taste aversion, ACTH-secreting cell's affinity for immunoglobulins (Pouplard, Bottazzo, Doniach & Roitt, 1976) and the conditionability of corticosterone levels (Ader, 1976); the glucocorticoids would appear to be a possible mediator of conditioned immunosuppression. Ader, Cohen and Grota (1979) examined this possibility and concluded that there was no evidence to indicate adrenocortical mediation of conditioned immunosuppression. However, the study by Ader et al (1979) does not preclude the possibility of synergetic activity between the immunosuppressive drug and glucocorticoids. For example, perhaps, following administration of an immunosuppressive agent the immune system communicates

with the brain to inform the brain of the current status of the immune system. This "communication" would interact with the CNS "communication" that would be triggered by taste aversion. Therefore, while both LiCl (lithium chloride) and CY groups used by Ader, Cohen and Grota (1979) included taste aversion and elevated glucocorticoid levels, only the CY group would have exhibited the hypothesized immune system-brain communication. Communication between the immune system and the brain (hypothalamus) has, in fact, been found. Stein, Schiavi and Camerino (1976) have reviewed a number of studies illustrating the effect of hypothalamic lesions on the immune system. Besedovsky, Sorkin, Felix and Haas (1977) have reported that antigenic challenge (SRBC) produced a significant increase in electrical activity of individual neurons in the ventromedial hypothalamus in female rats.

It appears that there are a number of candidates for the role of the mediator in conditioned immunosuppression. It seems logical, in view of the preceeding discussion, that given the ubiquitious nature of neurohormones that there is no sole mediator of conditioned immunosuppression. It is more likely a complex of neurohormonal communication between the immune system and CNS. Continued study in psychoneuroimmunology should be encouraged and promoted by its introduction into psychology programs at both the undergraduate and graduate level of post secondary institutes. Pragmatically, the value of any research lays in its application. The potential application of psychoimmunologic findings are reflected in a study by Smith and McDaniel (1983) who, in a paradigm similar to behavioral conditioning, produced an apparently psychologically mediated reduction in a

delayed hypersensitivity reaction (cell-mediated immune response) in human subjects.

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Footnotes

1. Food consumption refers to mean food consumptions uncorrected for body weight unless otherwise stated.

2. Since fluid was provided ad libitum during the baseline phase, there was no data for either a morning or afternoon drinking period. Therefore, the baseline phase is not included in analyses of morning or afternoon fluid consumption.