EFFECT OF ANTERIOR OR VENTROMEDIAL HYPOTHALAMIC STIMULATION ON IMMUNOGLOBULIN G.

DISSERTATION

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By

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Although research has linked central nervous system activity with changes in immunoresponsivity, research on the possible role of the central nervous system in altering a specific class of antibody is lacking. This study was an investigation of the possible relationship between anterior or medial hypothalamic functions on Immunoglobulin G. concentrations in rat serum.

Thirty-six male albino rats were randomly assigned to three groups of equal size. Animals within the anterior hypothalamic group received bilateral electrode implants in the anterior hypothalamus while animals in the medial hypothalamic group received electrode implants within the ventromedial area of the hypothalamus. A control group received bilateral electrode implants within the lateral hypothalamus. Electrical brain stimulation was administered to animals in both experimental groups. Control animals spent a comparable time in an operant chamber but did not receive electrical brain stimulation. Following brain stimulation of animals within the experimental groups, Immunoglobulin G. concentrations were determined for all groups 3, 6, 12, and 24 hours

post-stimulation sessions. Brain stimulation did not significantly alter Immunoglobulin G. concentrations. A statistical analysis was performed to assess the effect of obtaining multiple serum samples from each subject. The results of this analysis indicated that obtaining multiple samples from subjects significantly altered the concentration of Immunoglobulin G. These findings point to the need for future research in order to determine the conditions under which neural activity may modify the concentration of immunoglobulins.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

EFFECT OF ANTERIOR OR VENTROMEDIAL HYPOTHALAMIC STIMULATION ON IMMUNOGLOBULIN G.

An organism's capacity to combat the effects of bacteria, toxins, viruses, and other foreign agents is dependent upon the functional competence of the immune system. The immunological response basically consists of the removal of antigen and the production of serum antibodies (Barrett, 1978; Carr, 1973). The functional integrity of the immune system provides the organism with protection from infections and disease. At the same time, the immune system may be the source of great discomfort and in some cases death for that organism. Although basic research continues to elucidate the physiological and biochemical mechanims responsible for immune functioning, the results of this research provides investigators with different perspectives with respect to the interactions of the immune system with other physiological systems. The following brief presentation of some of the basic aspects of immune functioning will provide a background against which research relevant to the present study may be discussed.

Immune System

The cells of the immune system are formed, mature and are dispersed from the bone marrow and are then reclassified as either cells of the phagocytic or lymphoid systems dependent upon the functions they acquire outside the bone marrow.

Phagocytic cells are represented as either microphages or macrophages, both of which comprise the reticuloendothelial system. Foreign material or antigens which come in contact with a phagocytic cell induces the cell to engulf the particle whereupon enzymes within the phagocyte digest the foreign material to its antigenic determinents. These determinents may be passed to lymphocytes for antibody formation. The lymphoid system consists of the central and peripheral lymphoid organs. The bone marrow and thymus serve as the central lymphoid organs, while in fowl a third organ, the bursa of Fabricius, serves the function of developing immunoglobulin synthesizing cells. The peripheral lymphoid organs include the lymph nodes, spleen, tonsils, Peyer's patches, and appendix. Lymphocytes consist of two major types, those with a short (five to seven days) life span, and those with a life span which may extend for years. The former are B lymphocytes while the latter are T lymphocytes.

Lymphocytes that leave the bone marrow and mature in the thymus are called T lymphocytes. Maturation includes the reduction in size (seven um) of the lymphocyte as well as the acquisition of a specific antigen. T cell functions include participation in delayed hypersensitivities, such as contact dermatitis, in the graft rejection response, in tumor rejection, in the production of lymphokines which effect phagocytosis as well as producing effects on B cell immunoglobulin synthesis.

Lymphoblasts which are to become B lymphocytes leave the bone marrow and mature in the bursa of Fabricius or the hypothesized mammalian bursal equivalent. Maturation involves the acquisition of the beta antigen and the ability upon contact with antigen to synthesize immunoglobulins. Specifically, upon contact with an antigen B cells are transformed into plasma cells able to synthesize and secrete IgA, IgD, IgE, IgM, or IgG classes of immunoglobulins or antibodies. Differences among these immunoglobulins include molecular weight and the particular types of immune reactions in which the immunoglobulins participate. All immunoglobulins studied thus far are proteins which are composed of basically two types of chains or sequences of amino acids. The two types of chains are called heavy chains and light chains. Each chain is composed of a variable region and a constant region with constant regions being composed of constant sequences of amino acids and variable regions composed of variable sequences of amino acids. Both the variable light chains and the variable heavy chains contribute to form the antigen binding site (Barrett, 1978).

Immunoglobulin A is most prevalent in cerebrospinal fluid, aqueous humor, and other internal secretions. Immunoglobulin A is also found in external secretions such as in clostrum and early milk, nasal and respiratory mucus, and saliva. Secretory IgA has a molecular weight of 405,000 daltons, a half life of about six days, and little is known

of its specific role in immunity. Phylogentically, all vertebrates studied so far produce secretory IgA.

Immunoglobulin D has a molecular weight of 180,000 daltons and IgD was first identified from human serum as the result of the discovery of a myeloma protein that was antigentically dissimilar from other immunoglobulins (Barrett, 1978). IgD exists in relatively low concentrations in human serum and has a half life of three days. Understanding the role of IgD in immunity has been hampered by several factors. IgD is very heat and acid labile thus making it difficult to isolate the molecule for further study. Also, IgD tends to aggregate rapidly which can alter its biologic activity as well as its structure (Barrett, 1978). Phylogentically rhesus, patas, and macque monkeys along with the chimpanzee and baboon have serum proteins which will react with antihuman IgD. The role of IgD in immunity is uncertain. Antibody activity against diptheria toxoid, bovine insulin, and penicillin has been related to IgD.

Immunoglobulin E has a half life of about three days, a molecular weight of 190,000 daltons and most noted for its involvement in the anaphylactic reaction. The anaphylactic reaction results when IgE antibodies which are bound to Mast cells throughout the body come into contact with a particular antigenic substance. Following the binding of antigen to antibody, the Mast cell releases vasoactive substances called lymphokines. Most notable of the lymphokines

is histamine which acts upon tissues to increase their permeability which results in edema. Mast cells are found commonly in or around blood vessels, connective tissues, uterus, and lung. Their degranulation produces bronchoconstriction, and an increase in the clotting time of the blood. The sudden onset of bronchoconstriction and edema may result in death within a few minutes following contact with an antigen. It is cytotropic IgE which produces lethal anaphylaxis and not the circulating form of the antibody. Other IgE dependent allergies include asthma and penicillin sensitivity. Phylogentically IgE has been identified in all mammalian species thus far studied.

Immunoglobulin M is the largest of the immunoglobulins with a molecular weight of 950,000 daltons and has a half life of approximately 10 days. IgM participates in the primary immune response and in autoimmune diseases such as arthritis. In the primary immune response IgM levels rise following injection of antigen for up to 20 days. During this period of time, the amount of antibody gradually increases over a period of a few days to a few weeks, plateaus, and begins to drop. The magnitude of this response depends upon such factors as amount of antigen, species studied, and route of administration. IgM's participation in autoimmune diseases is unclear at this time, but IgM has been implicated in Hashimotos disease, rheumatoid arthritis, and myesthenia gravis. Furthermore, IgM is also the antibody found on the surface

of erythrocytes and this antibody forms the bases of various blood grouping tests.

Immunoglobulin G. has a molecular weight of 150,000 daltons and is considered the most primitive of the immunoglobulins as it is found in lower vertebrates. IgG is also most prevalent in the serum of immunized animals and can traverse the placenta, is involved in the secondary antibody response, and has a half life of about 30 days. The secondary immune response differs from the primary immune response in that a sharp drop in circulating antibody occurs followed by an abbreviated latent period, a heightened antibody titer and an extended duration of detectable antibody in the blood stream. IgG dependent allergies include a condition known as serum sickness which results when an individual receives a dose of antitoxin against tetanus or other toxins for therapeutic purposes. Pencillin is currently a common agent causing serum sickness. Serum sickness appears to result from the mutual presence of antigen and antibody in the bloodstream over an extended period of time (seven to 10 days) and although the mechanisms for serum sickness and anaphylaxis are similar, in IgG mediated serium sickness, the sensitizing dose and shocking dose of antigen are one in the same. The most notable hemolytic disease which is IgG mediated is called erythroblastosis fetalis which is also known as Rh incompatibility disease. This condition occurs when an Rh negative mother carries an Rh-positive child and through isoimmunization produces

antibodies to the Rh antigen. Since IgG is capable of passing through the placenta, this antibody binds to the fetal red blood cells causing cell lysis and in many cases death follows. Other immunological reactions involving IgG as the primary molecule include Farmers Lung, Mushroom Workers Lung, and other conditions known as immune complex pneumotitis (Barrett, 1978; Gordon, 1972; Carr, 1973).

Traditionally research in immunology concentrated on identifying the specific components of the immune system, the activity of these components in the face of antigenic challenge, and the interactions of the components of the system (Barrett, 1978; Gordon, 1972). The identification of separate markers for T and B cells, the discovery of the ABO blood grouping system, the identification of the chemical structure of immunoglobulins as well as the mapping of the primary and secondary immune response serve as examples of earlier approaches in the study of immunoresponsivity (Barrett, 1978; Gordon, 1972; Frobisher, Hindsill, Crabtree, & Goodhart, 1974). These traditional approaches provided and continue to provide information regarding the basic mechanisms of the immune system. However in order to understand the interaction of immune mechanisms with other physiological systems other approaches may be beneficial (Ader & Cohen, 1975).

Interdisciplinary Research

More recent investigations directed toward the study of immunology have emphasized an interdisciplinary approach.

Influences on the magnitude of the immune response to various antigens have been investigated with respect to a) environmental variables, b) endocrine secretions, and c) neural structures possibly involved in altering immunoresponsivity (Besedovsky & Sorkin, 1977; Rogers, Dubey, & Reich, 1979; Stein, Schiavi, & Camerino, 1976; Corson, 1966; Lambert, Harrell, & Achterberg, 1981; Stern, Mickey, & Gorski, 1969).

For example, rats exposed to environmental stimuli such as loud noise were more susceptible to anaphylactic shock as well as displaying enhanced reactivity to viral infections, and mice housed in groups were shown to have either lower antibody titers or altered responsivity to mammary tumors (Jenson & Rasmussen, 1963; Vessey, 1964; LaBarba, 1970). Jenson and Rasmussen (1963) determined that exposure of mice to high intensity sound for several hours a day for three days resulted in a diminished quantity of leuckocytes during stress induction and a transient period of leukocytosis following termination of the stressor. Intranasal injections of vesicular stomatititus virus showed a similar biphasic response in that mice infected just before sound onset were more susceptable while mice injected following sound offset were more resistant to death than were controls (Jenson & Rasmussen, 1963). LaBarba (1970) also demonstrated that handling lessened the susceptibility of mice to leukemic cells while Rashkis (1952) showed that mice subjected to forced swimming survived longer following challenge with

tumor cells than did not-stressed controls. Electric shock administered to either rats or mice has been associated with increased susceptibility to herpes simplex, poliomyelitis, coxsackie B., and polyoma viruses (Rasmussen, Marsh, & Brill, 1957; Rasmussen, 1965; Johnsson, Lavender, & Rasmussen, 1963; Rasmussen, 1969). However, the ability of the organism to escape or avoid electric shock appears to influence the susceptibility to poliomyelitis since Marsh, Lavender, Chang, and Rasmussen (1963) demonstrated that monkeys exposed to an avoidance paradigm were less susceptible to this virus while Friedman, Ader, & Grota (1973) demonstrated a protective effect of noxious stimulation in mice challenged with rodent malaria.

Physiological mechanisms mediating the effects of environmental events on the activity of the immune system may include changes in the endocrine system because alterations in endocrine responses parallel certain changes in immunoresponsivity (Besedovsky & Sorkin, 1977; Heller, Meir, Zuker, & Mast, 1957). For example, gonadectomy of experimental animals results in an increase in the size of the thymus and gonadectomy also appears to conteract the thymic involution induced by stress and fasting (Ahlqvist, 1976). Thyroidectomized rats displayed a decrease in the ability to produce antibody to an injection of sheep red blood cells as compared to an intact control group (Fabris, 1973). In addition, thymectomized mice show adrenal hypertrophy and slowed sexual development (Rogers, Dubey, & Reich, 1979).

Adrenalectomy has been demonstrated to increase phagocytosis, increase interferon titers as well as affect susceptability to anaphylaxis (Reichard, Edelman, & Gordon, 1956; Dews & Code, 1951). Hypophysectomy or administration of ACTH has been shown to either increase or decrease phagocytosis (Reichard et al., 1956; Keefe, Helman, & Smith, 1967).

Injections of hormonal substances have also been used to study the relationships between hormonal mechanisms and immunoresponsivity. For instance, large doses of estrogens have been demonstrated to induce thymic involution in rats and mice while administration of testosterone somewhat decreases thymic size (Ahlqvist, 1976). Moderate doses of thyroxine have been shown to induce significant differentiation of large lymphocytes and of plasma cells in medullary sections of the lymph nodes in guinea pigs (Aoki, Wakisaka, & Nigota, 1976). Thyroxine treatment has also been demonstrated to increase T cell formation while restoring the anaphylatic reaction in guinea pigs (Aoki et al., 1976; Flipp & Mess, 1969). Administration of glucocorticoids have also been implicated in the inhibition of lymphokine synthesis along with being correlated with breast cancer in humans (Wahl, Altman, & Rosenriech, 1975; Desphapande, Hayward, & Bubrook, 1965; Solomon, 1969).

In addition to removal of endocrine organs and injections of hormones, pharmacologic approaches have also been used while investigating the relationships between hormonal levels and antibody synthesis (Pierpaoli & Maestroni, 1978a). Haloperidol

5-hydroxytryptophan, and phentolamine were administered singly and in combination along with sheep red blood cells to experimental animals. 5-hydroxytryptophan has been demonstrated to inhibit the release of thyroid stimulating hormone from the pituitary while phentolamine (α blocker) has been shown to inhibit the release of adrenocorticotropic hormone. Haloperiodol is a powerful neuroleptic and it has been demonstrated to decrease leutinizing hormone and follicle stimulating hormone in female mice (Levy & Munson, 1976). Pierpaoli and Maestroni (1978a) found that only a combination of 5-HTP, phentolamine, and haloperiodol were successful in inhibiting the antibody production of splenic cells in response to antigenic stimulation. Pierpaoli and Maestroni (1978b) demonstrated that the combination of 5-HTP, haloperidol, phentolamine, and dopamine retarded the graft rejection response in mice. Mice which are deficient in somatotropic hormone display decreased T cell responsivity and replacement therapy with somatotropic hormone restores the normal T cell response (Fabris, Pierpaoli, & Sorkin, 1971).

The third factor that has recently been under investigation are the relationships between central nervous system structures and the immune system. The discovery of receptors on T and B cells which bind insulin, dopamine, and acetylcholine (Rogers, Dubey, & Reich, 1979) suggests the importance of research in this area. Most of the studies on the involvement of the central nervous system on the functioning of the

immune system have concentrated on the role of hypothalamic mechanisms involved in immunity.

Anatomically, the hypothalamus consists of a group of nuclei within the diencephalic portion of the brain lying on either side of the third ventricle. The hypothalamus is connected to the pituitary gland by a neurovascular stalk through which in some cases releasing factors from the hypothalamus trigger the release of many anterior pituitary hormones (STH, ACTH, TSH) or the substances which act directly on target tissues are manufactured within the hypothalamus and are stored for release in the posterior pituitary gland (e.g., Vasopressin, Oxytocin). The hypothalamus can be divided into medial and lateral sections which are separated in part by the columns of the fornix. Medial hypothalamic areas contain neurosecretory cells involved with pituitary functioning while lateral hypothalamic areas primarily consist of a multisynaptic system which interconnects the limbic forebrain with the mesencephalon. Through the lateral hypothalamus passes the medial forebrain bundle which interconnects the hypothalamus to the forebrain and hindbrain. Posterior hypothalamic structures are interconnected with the thalamic and limbic areas through the mammillary bodies via the fornix. Hypothalamic neurons receive input from multiple neural pathways as well as from the bloodstream and cerebrospinal fluid. Research on the role of hypothalamic mechanisms on immunoresponsivity have primarily utilized lesioning or stimulation techniques (Lambert,

et al., 1981; Besedovsky & Sorkin, 1977; Korneva & Khai, 1964; Ado & Goldstein, 1973; Schiavi, Adams, & Stein, 1966; Shekoyan, Khasman, & Uchital, 1975; Thrasher, Bernadis, & Cohen, 1971; Macris, Schiavi, Camerino, & Stein, 1970; Fessell & Forsyth, 1963; Kavetsky, Turkevich, Akimova, Khayetsky, & Matuechuck, 1969; Stein, Schiavi, & Camerino, 1976; Flipp & Mess, 1969; Tyrey & Nalbandov, 1972; Andervont, 1944; Segal, Izak, & Feldman, 1971).

Among the hypothalamic areas demonstrated to effect immunoresponsivity include the anterior and medial hypothalamus. The integral relationships between these hypothalamic structures and the endocrine and autonomic systems have been well established (Martin, 1980; Turner & Bagnara, 1976), and as previously discussed there exists a body of data suggesting that there is an interdependence between the immune and endocrine systems, thus anterior and medial hypothalamic structures appear to be likely candidates for playing a role in some aspects of immunological responsivity.

For example, Lambert, Harrell, and Achterberg (1981) investigated the effects of medial hypothalamic stimulation on the reticuloendothelial system. Following brain stimulation, animals were injected with a colloidal suspension of carbon particles through a jugular cannulae. All animals were injected with the colloidal suspension at either 3, 6, 12, or 24 hours after brain stimulation in a counterbalanced sequence. The rate at which the carbon-suspension cleared from the bloodstream served as a measure of the activity of

the reticuloendothelial system. The results of this investigation suggested that medial hypothalamic stimulation decreased the rate of which phagocytes removed foreign particles from the bloodstream. The precise mechanism through which these decremental effects on reticuloendothelial system activity were produced are unknown at this time.

Besedovsky and Sorkin (1977) suggested that medial hypothalmic areas may be involved with respect to the synthesis of IgM class antibodies. These authors immunized rats with one of two antigenic substances, sheep red blood cells or trinitrophenylated-hemocyanin. The kinetics of the immune response and hypothalamic activity were measured at various intervals after antigenic challenge. Rats immunized with sheep red blood cells showed no placque forming cells on Day 1 as well as no significant change in hypothalamic firing rates as compared to controls. However by Day 5 which corresponds with the time in which splenic cells are most active in synthesizing IgM class antibodies there was more than a two fold increase in medial hypothalamic firing rates as compared to controls. Hypothalamic responses to trinitrophenylated-hemocyanin were studied in rats over a period of five days. The highest firing rates were observed on Day 2 of the experiment which preceded the peak of placque forming cells in the spleen. The results of the studies indicate that two separate antigenic substances elicited not only the conventional immune response but also apparently influenced the

rate of neuronal activity in the hypothalamus. Szentivanyi and Filipp (1958) found that tuberal lesions within the area of the medial hypothalamus offered protection to guinea pigs from lethal anaphylaxis; thus medial hypothalamic structures appear to influence some aspects of immunoresponsivity.

Anterior hypothalamic structures have been implicated in other aspects of immunoresponsivity, most notably in the anaphylaxis or acute hypersensitivity reaction. Luparello, Stein, and Park (1964) found that anterior but not, posterior hypothalamic lesions inhibited development of anaphylaxis in the rat. Macris et al., (1970) placed bilateral electrolytic lesions in the anterior, medial, and posterior hypothalamus in guinea pigs. Significant protection against the anaphylactic reaction was found for the animals with lesions in the anterior hypothalamus while posterior and medial lesions produced no significant effect on the anaphylactic reaction. Tyrey and Nalbandov (1972) demonstrated that bilateral lesions of the anterior hypothalamus in albino rats reduced the production of antibody to ovalbumin and that hypophysectomy blocked the effect of anterior lesions. Anterior hypothalamic lesions have also provided albino rats some protection to increase in tumor growth as compared to non-lesioned controls (Kavetsky et al., 1969).

Other hypothalamic areas have been implicated in reducing the immunocompetence of experimental animals. Lesions of posterior hypothalamic structures decreased the formation of

complement fixing antibodies up to 40 days following challenge with experimental allergic polyneuritis in rabbits as well as producing lower antibody titers and fewer antibody secreting cells in the spleens of albino rats (Konovalov, Korneva, & Khai, 1971; Kishkovaskaya, Zufarov, Saakov, & Polyak, 1974). Lateral hypothalamic areas have also been shown to be involved in some aspects of antibody production. Fessell and Forsyth (1963) demonstrated that electrical stimulation of lateral hypothalamic areas resulted in the increase of gamma globulin levels in rats for periods up to 10 days after brain stimulation. Investigators utilizing lesioning or stimulation have determined antibody responsivity using wheal reactions, hemolysin titers, precipitation titers, or complement fixation tests. Such methods are highly sensitive but parametric studies attempting to identify the particular antibody or antibodies involved (e.g., IgG, IgM, IgE, etc.) and the time course of the particular antibody response is lacking. The studies discussed above used lesioning and or stimulation of the brain. While the studies discussed above demonstrated that lesioning or stimulation of hypothalamic structures may influence the immunocompetence of the species studied, these investigations have not identified the specific antibodies involved or the time course of the particular antibodies involved.

In the studies previously discussed, hypothalamic areas which appear to be likely candidates for participating in

the immune response, include the lateral, anterior, and ventromedial nuclei of the hypothalamus. Lambert and Harrell (1982) investigated the effects of lateral hypothalamic stimulation on IgG concentrations in rat serum. Electrodes were implanted within the lateral hypothalamus of male albino rats. Animals in one group received electrical brain stimulation while a control group spent a comparable time in an operant chamber, but did not receive brain stimulation. IgG concentrations were determined for both groups 3, 6, 12, and 24 hours post-brain stimulation. These measurement intervals were counterbalanced across the groups to control for the possibility of order effects. There was no significant difference between the experimental and control groups on IgG concentrations. The data from this investigation is presented in Appendices D and E.

The purpose of this investigation was to determine whether or not electrical stimulation of the anterior or medial hypothalamus produces changes in the concentration of IgG and the time course for any observed effects. Specifically, it was hypothesized that electrical brain stimulation of anterior or medial hypothalamic areas would change the serum concentration of Immunoglobulin G.

Method

Subjects

Subjects were 36 experimentally naive male, albino Sprague-Dawley rats (ARS Sprague-Dawley, Madison, Wisconsin) weighing 300-250 grams at the beginning of the experiment.

All animals were individually housed and allowed food and water ad-libitum throughout the experiment.

Apparatus

During intercranial stimulation, subjects were placed in a Gerbrands operant chamber with a hole in the ceiling to permit passage of a shielded cable. The dimensions of the chamber were 38.58 cm x 21.59 cm x 21.59 cm. The floor consisted of stainless steel bars, and the walls and ceilings were constructed of plexiglass (Ralph Gerbrands, Arlington, Maryland).

Brain stimulation was delivered by two Grass S48 brain stimulators (Grass Instruments, Quincy, Massachusetts). A bifurcated shielded cable 120 cm in length with a stainless steel spring covering and two Plastic Products #303 electrode plugs were used to deliver brain stimulation (Plastic Products, Roanoke, Virginia).

A Kopf stereotaxic apparatus (Kopf Instruments, Tujunga, California) was used in the surgical implantation of electrodes. The eletrodes were MS/303 stainless steel, 32 mm in length and 0.15 mm in diameter. Self-tapping stainless steel mounting screws 0.16 mm in length along with crainioplastic liquid and powder were utilized in the permanent fixing of the implanted electrodes.

All electrodes, mounting screws, and cement were supplied by Plastic Products, Inc., Roanoke, Virginia.

Procedure

Animals were randomly assigned to a ventromedial, anterior, or sham (dummy electrode) group with each group containing

12 animals. Following assignment to groups, animals were subjected to the surgical procedure.

Surgery

Animals were anesthetized with sodium pentobarbital at a dose of 50 mg/kg. Following anesthesia, each animal was placed in a stereotaxic apparatus and the skull was exposed by an incision approximately one inch in length. Following the incision, the skull was trephined and electrodes were implanted bilaterally in either the ventromedial or anterior hypothalamus. The stereotaxic coordinates for the implantations were 6.7 mm anterior of the interaural line, 0.75 mm lateral of the midsaggital sinus, and 8.5 mm ventral from the dural surface of the brain for animals in the ventromedial group while animals in the anterior group received implants with the coordinates 1.0 mm posterior of bregma, 0.7 mm lateral of the midsaggital sinus, and 7.0 mm ventral from the dural surface of the brain. The dummy electrode group received bilateral implants within the lateral hypothalamus with coordinates of 6.5 mm anterior to the interaural line, 1.0 mm lateral of the midsaggital sinus and 8.0 mm ventral from the dural surface of the brain (Sherwood & Timeras, 1970; Tyrey & Nalbandov, 1972).

Measurement Procedures

Following the return of each animal to his preoperative weight (seven to 10 days), subjects from each group were removed from their home cages and IgG concentrations were determined through the use of radial immunodiffusion as

outlined by Mancini, Carbonara, and Heremans (1965). A 0.5 ml blood sample was collected by cutting the tail vein with a sterile scalpel blade. The blood sample was allowed to clot, and following clot formation, the sample was centrifuged at 3500 rpm for 15 minutes in order to remove cells obtained during serum collection from the clotted sample. Next, 16.6 ul of serum from each sample was pipetted into a 4 mm well cut within a 1% agarose-phosphate buffered (ph 7.2) sterile saline medium contained on a 3 x 1 inch corning glass microscope slide (Fisher Scientific Products, Dallas, Texas). The agarose medium contained a 1:20 dilution of rat anti-IgG (Sigma Chemical Company, St. Louis, Missouri) and the serum-agarose anti-IgG complex was allowed to incubate at 25^{o}C for 24 hours. At the end of the 24 hour period, ring diameters were measured using a Bel Art caliper (Fisher Scientific Products, Dallas, Texas). Concentrations of IgG were determined for the samples by comparing the sample ring diameters with ring diameters produced by reacting known concentrations of rat IgG (Sigma Chemical Company, St. Louis, Missouri) with the identical agarose anti-IgG medium used to determine the sample ring diameters. The IgG concentrations were determined from the ring diameters through the use of a linear regression equation. The logarithm of the known concentrations of IgG were correlated with the ring diameters obtained during the determination of a standard curve. The resulting values of the slope and intercept of the line were used to determine

the IgG concentrations of sample ring diameters. The equation $y = mx + b$ was used to determine the log concentration of IgG. The antilog of y produced the predicted IgG concentration of sample. The average linear correlations between ring diameters and known concentrations of IgG were determined to be 0.98 for all standards. The above measurement served as a baseline measure of IgG concentrations for each subject.

Brain Stimulation

Following the determination of baseline concentrations of IgG, a period of four days elapsed before the next phase of the experiment. At the end of this period, each animal was placed in an operant chamber where intracranial brain stimulation was delivered by a Grass S48 brain stimulator through a bifurcated cable. Animals in the ventromedial and anterior hypothalamic groups received one train of rectangular wave form pulses every 10 seconds up to a maximum of six volts with the duration of each train lasting one second. The intensity of brain stimulation was adjusted for each animal by beginning the first session at one volt and increasing the intensity by one volt increments every two minutes until either a maximum of six volts was reached or until an aversive reaction to brain stimulation was observed. If an aversive reaction was observed, the intensity of brain stimulation was lowered in one volt units until the reaction(s) were no longer observed and that value of intensity was held constant for each animal throughout the experiment. An aversive reaction was defined

as flinching, jumping, and/or vocalization at stimulus onset. Animals in the dummy electrode group had the bifurcated cable attached but brain stimulation was not delivered. Each train of brain stimulation delivered 100 stimulus pulses per second with the duration of each stimulus pulse lasting 0.2 msec. Following one hour of brain stimulation, IgG concentrations for each animal was determined using the Mancini technique at 3, 6, 12, and 24 hours postbrain stimulation in a counter-balanced sequence. A four day period was interposed between each brain stimulation IgG determination to allow for the possibility of carry over effects. Following the conclusion of the experiment, histological analysis of the brain was performed for animals in the anterior and medial hypothalamic groups. The brain was removed from the skull following perfusion was 0.9% physiological saline and 10% formalin. The brain tissue was embedded in celudodin and sliced with a clinical microtome at 30 um. The tissue was next stained with Thionin and mounted on 3 x 1 inch Corning glass slides for microscopic examinations.

Results

Results of the effects of brain stimulation on IgG concentraionts are presented in Figure 1. The points along with ordinate represent adjusted mean IgG concentrations while the points along the abcissa represent the post stimulation measurement intervals. The unadjusted means and standard deviations are presented in Appendix A. Animals receiving anterior

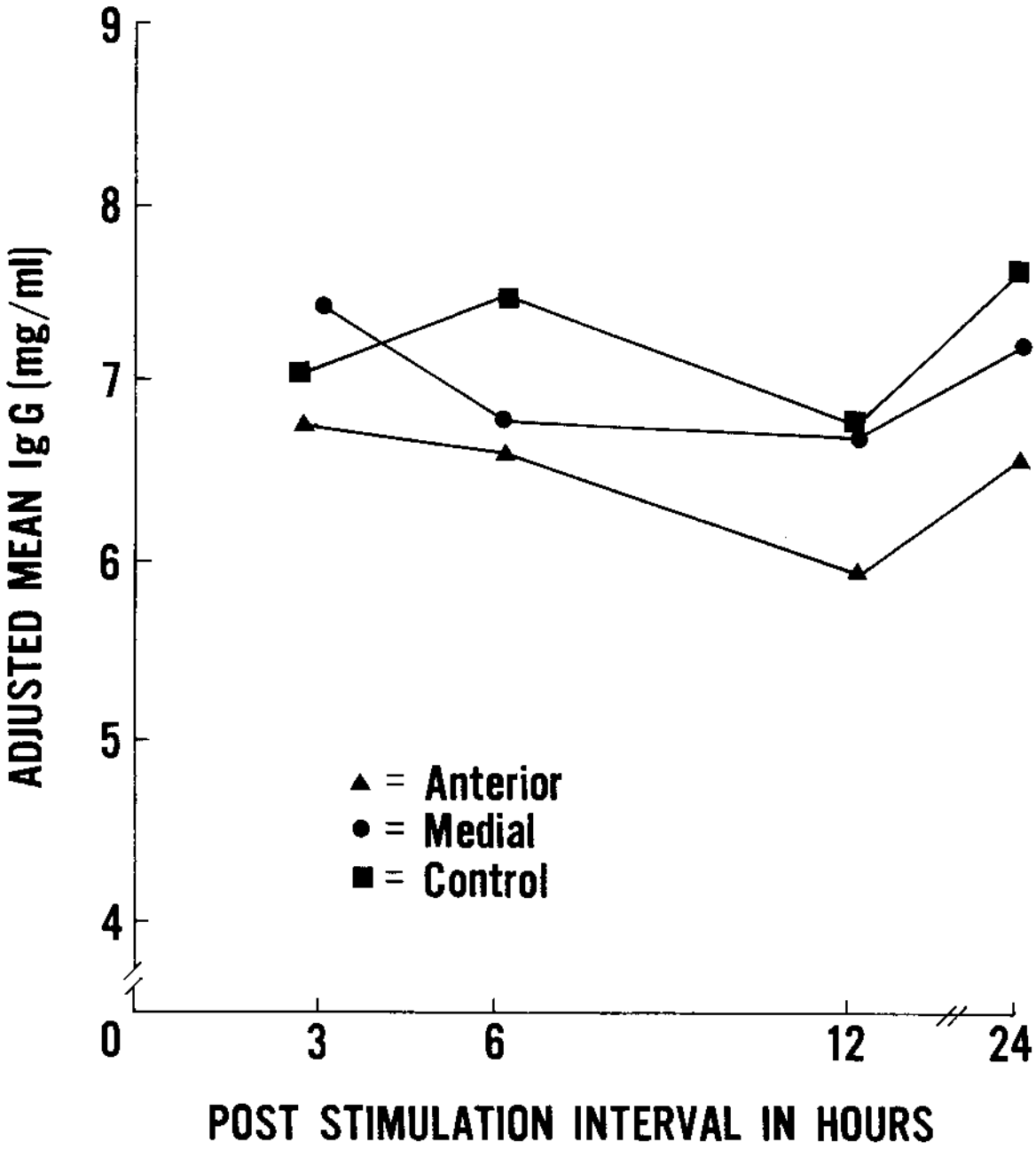

Figure 1. Adjusted mean immunoglobulin G. concentrations as a function of time since hypothalamic stimulation.

hypothalamic stimulation were lower in IgG concentrations over all post stimulation measurement periods as compared to either medial hypothalamically stimulated animals or control animals. Baseline IgG concentrations for all groups are represented in Figure 2. Medial and nonstimulated control groups show comparable serum IgG concentrations while the anterior hypothalamic group displays lower baseline IgG values as compared to either control or medial groups.

Statistical analysis of the data in Figure 1 was performed utilizing a 3 x 4 (groups x measurement interval) analysis of covariance (ANCOVA) using baseline IgG concentrations as the covariate. The among groups main effect showed a nonsignificant effect of hypothalamic stimulation in IgG concentrations ($F = 2.5$, $df = 2,32$, $p < .10$). The results of this analysis indicate that there was not a statistically significant effect of brain stimulation on IgG concentrations over the post stimulation intervals investigated in the present study. There was also no significant main effect of post stimulation in IgG intervals on IgG concentrations ($F = 1.28$, $df = 3,98$, $p < .30$). The F ratio for the interaction between brain stimulation and the post stimulation measurement intervals was statistically nonsignificant ($F = 0.49$, $df = 6,98$, $p < .90$). The summary table of this ANCOVA is presented in Appendix B. The statistical power of the ANCOVA used to determine the effects of brain stimulation on IgG concentrations was calculated to be .99. A check on the assumptions

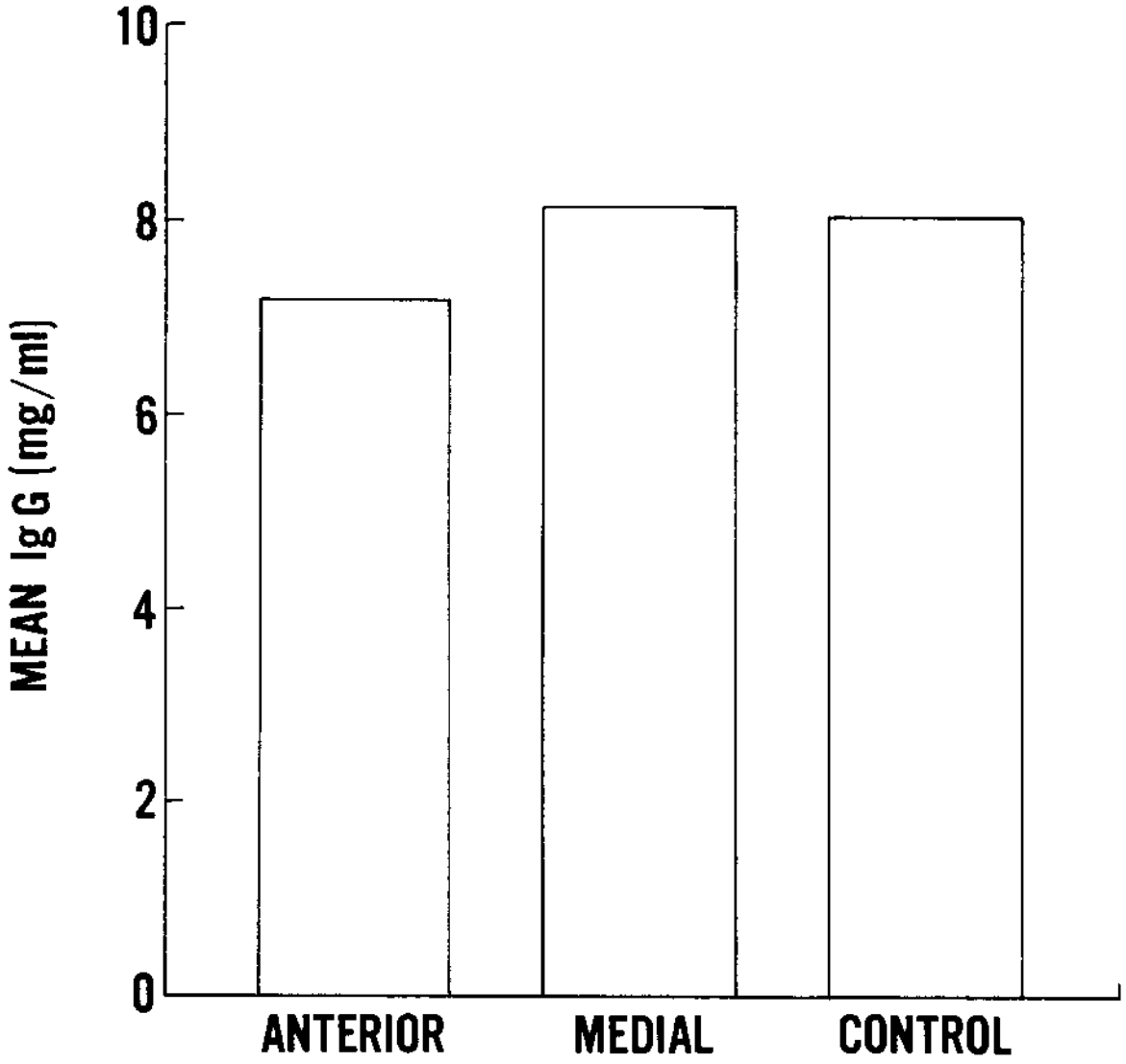

Figure 2. Mean baseline Immunoglobulin G. concentrations for anterior hypothalamic, medial hypothalamic, and control groups.

of equality and symmetry of variance-covariance matrices performed on these data indicate that the assumption of equalty was violated ($X = 36.67$, $df = 20$, $p < .05$) while the assumption of compound symmetry was not violated ($X^2 = 1.56$, $df = 8$, $p < .10$).

The possibility exists that even though a statistically nonsignificant difference was found for the data in Figure 1, there may be a cummulative effect of repeated measurements on the concentration of IgG. Figure 3 displays the cumulative effect of obtaining serum samples from all groups on IgG concentrations. The adjusted mean IgG concentrations over all post stimulation measurement intervals is presented in Figure 4. In order to determine the cumulative effects of the number of measurements taken on IgG concentrations, a 3 x 4 ANCOVA was performed on the data presented in Figure 3 using groups and number of measurements as factors. The ANCOVA performed on these data is presented in Appendix C. These results indicate that there is a significant main effect of the number of measurements taken on the concentration of IgG ($F = 6.9$, $df = 2,32$, $p < .001$).

The results of histological examination of brain tissue indicated that electrodes were in the posterior portion of the ventromedial nucleus for animals in the medial hypothalamic group. Electrode placements for animals in the anterior hypothalamic group were within the central portion of the anterior hypothalamus. There was no evidence of lesioning at the electrode tip for all animals.

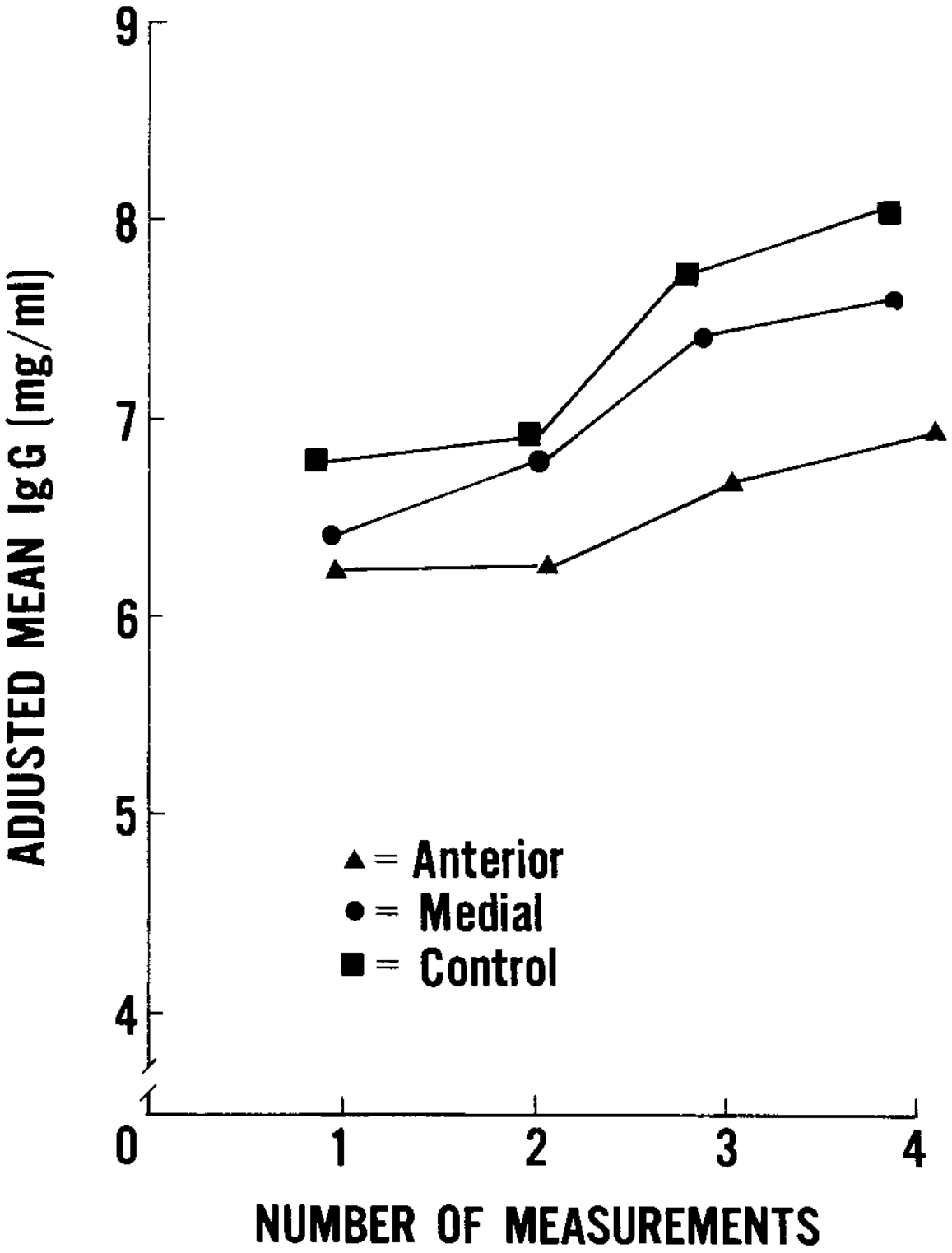

Figure 3. Adjusted mean Immunoglobulin G. concentrations as a function of the number of sessions following hypothalamic stimulation for the anterior hypothalamic, medial hypothalamic, and control groups.

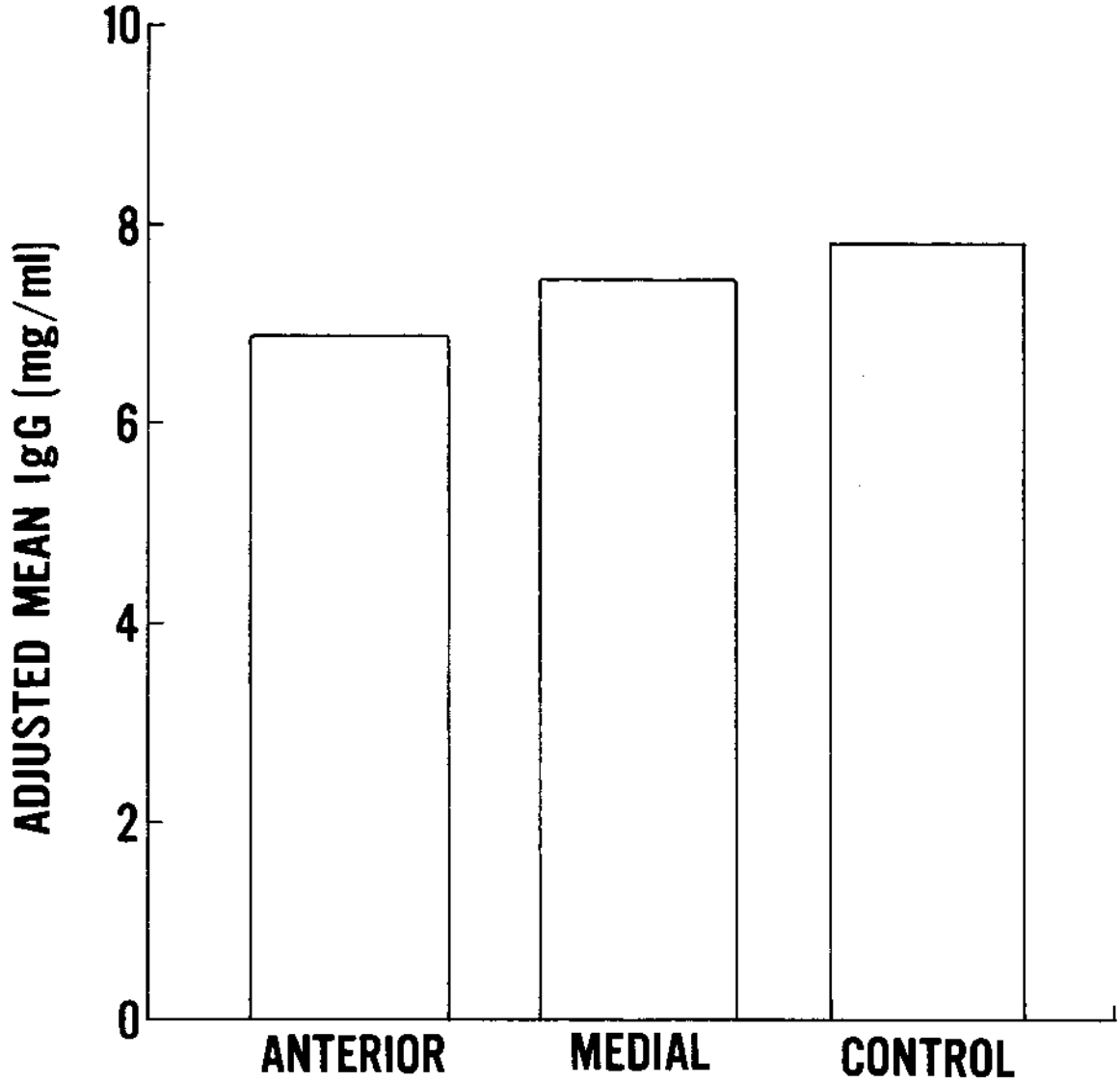

Figure 4. Adjusted mean Immunoglobulin G. concentrations for the anterior hypothalamic, medial hypothalamic, and control groups collapsed over all post-stimulation measurement intervals.

Discussion

The results of this investigation did not support the hypothesis that anterior or medial hypothalamic stimulation affected the concentration of IgG in rat serum under the conditions studied in the present experiment. Although the effects of brain stimulation across the post stimulation intervals was statistically nonsignificant for the anterior or medial hypothalamically stimulated animals, anterior hypothalamically stimulated animals showed a slight decrease in IgG concentrations as compared to either medial hypothalamically stimulated animals or control animals which approached statistical significance. However baseline concentrations of IgG were somewhat lower for anterior hypothalamically stimulated animals as compared to either medial hypothalamically stimulated animals or control animals (see Figure 2). However the analysis of covariance is designed to adjust treatment values for differences on the covariate, thus differences in baseline IgG concentrations would not produce differences in IgG concentrations under treatment conditions.

The present study employed a repeated measures design whereby each animal serves as his/her own control thereby reducing error variance (Winer, 1971). However, the repeated measures paradigm by itself does not control for the cumulative effects of the measurement procedure per se. Figure 3 displays the cumulative effects of obtaining serum samples from all groups on IgG concentrations. The ANCOVA performed

on these data indicate that there is a significant effect of the number of measurements taken on the concentration of IgG. The present investigation employed a counterbalancing procedure whereby order effects are controlled and the effectiveness of this technique is shown by the results presented in Appendix B. Although order did not have any differential effects, higher order interactions of treatment with a specific sequence cannot be ruled out because all possible sequences were not used. The F ratio for the data presented in Figure 3 indicates a cumulative effect of repeated samples does exists in the present study. Although the repeated measures design is a powerful tool for reducing error variance, physiological investigations utilizing this design should be aware of the possibility of order effects and subsequently employ counterbalancing as a control procedure.

A statistically nonsignificant difference on IgG concentrations across the treatment conditions may result from a number of factors. Among such factors include inadequate control groups, inconsistent environmental conditions, inadequate measurement of the dependent variable, inability of a test to detect a real difference, and differences that may exist among the groups before any treatment was administered.

The repeated measures design offers a means by which sources of error variance due to differences between subjects at the beginning of the experiment are controlled through the use of each subject as his/her own control. The analysis of

covariance provides another statistical method of controlling differences among the experimental subjects prior to the administration of the experimental treatment which in the case of the present study was brain stimulation. Furthermore, the use of a dummy electrode control group provided still another technique of experimental control. Because the present investigation employed the above control procedures along with counterbalancing of the experimental subjects, inadequate control or design procedures do not appear to account for the results obtained.

Inconsistent environmental conditions may introduce sources of error which could attenuate the magnitude of the effect of brain stimulation on IgG concentrations. In the present investigation, all experimental subjects were individually housed and maintained by the experimenter throughout the duration of the experiment in a temperature controlled environment. Although drastic changes in environmental conditions could attenuate treatment effects, such conditions were not observed to have occurred during the present study.

Adequate measurement of the dependent variables is necesary in order to accurately assess the effects of a particular treatment. Insensitive measures could mask differences which may be produced by that treatment, thus resulting in a statistically nonsignificant analysis. The present study employed the use of radial immunodiffusion which has been demonstrated to be highly sensitive and specific assay for the presence or

absence of a particular antibody (Barrett, 1970; Frobsher, Hindsill, Crabtree, & Goodhart, 1976; Burrell, 1980; Mancini et al., 1965). Furthermore, the specificity of antigen-antibody reactions has enabled this technique to be used reliability and successfully in immunological assays (Barrett, 1978). As reported previously, the radial immunodiffusion technique developed by Mancini and utilized in the present investigation was found to be highly reliable. Although insensitive or unreliable measurement methods could effect the ability to demonstrate a statistically significant analysis, the present study employed a technique which has been demonstrated not only in this investigation, but in previous research as well as in applied laboratories to be a sensitive and reliable measurement tool for use in immunological assays (Barrett, 1978; Gordon, 1972). The evidence that radial immunodiffusions was a highly reliable technique in the present study was the high correlation (.98) between ring diameters on IgG concentrations.

The inability of a statistical test to detect a real difference if in fact one exists is determined by the magnitude of a Type II error. This might be of particular concern for the present study because of the near significant main effect of groups. The power of a statistical test is the ability of a test to detect a real difference and is given by 1-B or one minus the probability of a Type II error (Winer, 1971). The power of the test used in the present investigation was

determined to be 0.99 (i.e., the probability of a Type II error is 0.01, thus the statistical test used in the present investigation was determined to have adequate power to detect a real difference).

The assumptions of equality and compound symmetry of variance covariance matrices is necessarily tested when using a repeated measures design. The assumption of equality was violated which indicates that the hypothesis that the covariance matrices belong to a population of covariance matrices that are equal is unsupported (Winer, 1971). The assumption of compound symmetry tests the hypothesis that the sample covariance matrix has a specified form. Since the assumption of compound symmetry was not violated, it can be assumed that the sample matrix belongs to a population of matrices with a specified form (Winer, 1971).

The possibility exists that under different conditions electrical stimulation of the anterior or medial hypothalamus could result in significant changes in the serum concentrations of IgG. Factors such as time of measurement, frequency, intensity, duration, location, inter-stimulus interval, or time of day of electrical stimulation may be important variables which could differentially effect the serum concentration of IgG. It is possible that anterior or medial hypothalamic stimulation significantly alters the concentration of another class of immunoglobulins (IgE, IgD, IgA, etc.). Although there are studies which have reported negative findings with respect to

hypothalamic involvement in immunity (Ado & Goldstein, 1973; Thrasher, Bernadis, & Cohen, 1971), the vast majority of research indicates that hypothalamic structures are involved in some aspects of the immune system. The present investigation differed in comparison to previous studies in that this research attempted to identify a particular antibody that may be affected by brain stimulation. Earlier investigations used techniques which may not have necessarily identified a particular class of antibody, however these investigations established that some aspect of neural activity influences the immune system. Only continued research in the areas of hypothalamic involvement in immunity will further clarify the role of central nervous system structures in immunity. Such research would enable scientists to begin to understand the complex relationships in the area of psychosomatic illness.

Appendix A

Unadjusted Means and Standard Deviations for Anterior, Medial, and Control Animals

Means

Group	Baseline	3	6	12	24
Anterior	7.09	5.58	5.82	6.62	6.72
Medial	7.85	6.49	6.91	7.72	7.81
Control	7.71	6.95	6.93	7.81	8.05

Standard Deviations

Group	Baseline	3	6	12	24
Anterior	2.56	1.56	1.41	2.27	1.90
Medial	1.81	1.45	1.52	1.75	1.80
Control	2.34	1.73	1.74	3.01	2.28

Appendix B

Table 1

Summary Table of 2-Way ANCOVA with Baseline Immunoglobulin G as the Covariate

Source of Variation	SS	df	MS	f	p
Between subjects	122.22	34	3.6		
Groups	16.54	2	8.3	2.5	0.097
Subjects With Groups	105.69	32	3.3		
Within Subjects	196.46	107	1.84		
Intervals	7.21	3	3.40	1.28	0.290
Interaction	5.53	6	0.92	0.49	0.810
Col x (Subj. w rows)	187.72	98	1.87		
Total	318.67	141			

Appendix C

Table 2

Summary Table of 2-Way ANCOVA for Sessions (Non-Counterbalanced)

Source of Variation	SS	df	MS	f	p
Between Subjects	122.21	34	3.6		
Groups	16.53	2	8.3	2.5	0.097
Subj. w. Groups	105.68	32	3.3		
Within Subjects	196.43	107	1.8		
Intervals	34.07	3	11.35	6.9	0.0003
Interaction	1.21	6	0.20	0.1	0.99
Total	381.64	141			

Appendix D

Table 3

Unadjusted Means and Standard Deviations for Lateral and Control Animals

Group	Baseline	3	6	12	24
			Means		
Lateral	9.80	8.70	9.68	8.39	8.44
Control	8.52	8.14	7.14	7.57	7.53
			Standard Deviations		
Lateral	1.22	2.43	2.34	2.13	2.46
Control	1.01	1.75	.75	1.26	1.79

Appendix E

Raw Scores for Lateral and Control Animals

Group	S#	Baseline	3	6	12	24
Lateral						
	L1	8.37	10.37	11.53	10.35	12.29
	L2	5.15	7.80	4.63	6.12	4.38
	L3	7.34	6.50	7.80	7.70	7.33
	L4	6.15	7.01	5.65	8.54	7.38
	L5	6.72	6.96	7.59	8.48	9.73
	L6	8.01	14.84	10.70	12.24	10.88
	L7	5.88	6.46	6.67	7.54	8.15
	L8	8.37	9.63	6.92	5.81	7.36
Control						
	S1	6.15	6.28	7.01	6.50	5.94
	S2	6.15	9.36	5.97	6.70	5.03
	S3	5.63	6.50	7.80	7.70	8.40
	S4	5.88	7.33	6.50	9.57	7.38
	S5	8.01	8.53	6.89	7.08	8.86
	S6	5.39	7.52	8.33	7.23	6.32
	S7	7.67	7.93	7.52	9.43	10.61
	S8	5.39	11.67	7.07	6.38	7.73
$\overline{X}$						
SD						

Appendix F

Immunoglobulin G. Concentrations

Group	Animal #	Baseline	3	6	12	24
Anterior						
	A1	7.29	5.65	4.97	7.80	6.74
	A2	4.39	6.00	4.37	3.29	6.04
	A3	9.43	8.97	9.61	5.53	6.59
	A4	7.00	7.22	6.47	7.45	4.93
	A5	4.19	3.79	4.17	3.68	3.98
	A6	4.48	7.24	4.80	4.71	4.65
	A7	6.50	3.87	4.96	5.67	6.82
	A8	4.82	4.77	4.15	3.87	5.17
	A9	5.82	5.84	6.35	5.78	5.63
	A10	8.74	9.46	9.02	5.90	9.77
	A11	10.54	9.22	8.10	7.27	7.69
	A11	11.83	7.40	9.10	7.65	8.24
Medial						
	M1	6.35	6.35	5.45	7.73	7.40
	M2	6.80	8.79	4.68	4.54	7.21
	M3	5.20	6.67	7.45	3.81	4.83
	M4	9.43	8.90	9.87	11.64	5.66
	M5	7.06	6.76	7.33	7.99	7.24
	M6	6.91	9.24	5.87	7.33	6.46
	M7	6.27	5.03	5.72	6.32	8.61
	M8	10.08	5.45	4.76	4.79	6.47

Group	Animal E	Baseline	3	6	12	24
	M9	7.40	8.05	8.36	8.92	8.43
	M10	7.95	8.83	8.80	6.41	9.91
	M11	9.52	8.89	6.27	6.75	7.77
	M12	11.19	7.95	9.22	7.93	8.36
Control						
	C1	6.65	5.27	5.45	7.21	8.19
	C2	11.03	12.06	8.41	8.77	12.58
	C3	9.22	9.87	10.18	6.51	9.67
	C4	5.83	7.06	7.53	7.45	4.70
	C5	7.06	6.45	6.76	7.37	6.92
	C6	4.34	3.95	8.15	4.40	3.95
	C7	6.55	3.96	8.28	6.78	5.75
	C8	4.87	4.99	3.91	4.79	5.24
	C9	6.47	5.93	5.36	7.96	6.56
	C10	11.21	10.61	10.99	8.28	13.03
	C11	9.52	8.12	8.73	6.56	8.76
	C12	9.74	7.95	8.23	7.93	7.38

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