RELATIONSHIP BETWEEN BRAIN NOREPINEPHRINE AND BARBITAL ACTIVITY IN STRESSED AND NON-STRESSED RATS

A Thesis

Presented to

The Faculty of the College of Pharmacy

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Elizabeth Jane Samson Dial

December 1972

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ABSTRACT

Chronic and acute stress is known to affect barbiturate activity. The effects of 14 day restriction-of-movement stress and hindleg ligation stress, singly and in combination, were observed on the duration of hypnosis of barbital (200 mg/kg, I.P.). Tolerance to barbital was observed when it was administered beginning at 26, 50, 74 hours after removal from chronic stress(without acute stress; however, it was significant only in males). Administration of acute stress at 50 hours after removal from chronic stress resulted in elimination of tolerance to barbital. A subsequent injection 24 hours later produced tolerance to barbital.

Proadifen (50 mg/kg, IP) prolonged sleeping time with the second dose of barbital, but had no effect on the first dose in chronically and acutely stressed animals. Proadifen did lengthen sleeping time when the first dose of barbital was administered 50 hours after stress in chronically stressed rats.

The stress changes in barbital activity were studied by determination of the whole brain norepinephrine concentration. Chronic stress resulted in a

greater brain concentration of norepinephrine than that seen in non-stressed rats. Barbital hypnosis increased the brain norepinephrine concentration in non-stressed rats, but not in stressed rats. Stressed animals exhibited a shorter sleeping time than non-stressed animals.

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INTRODUCTION

Barbital or diethylbarbituric acid is a long-acting barbiturate that is no longer commonly used clinically, but serves as an experimental drug. Barbital has the lowest partition coefficient of the barbiturates (1) and the lowest degree of binding to plasma protein; because of these factors it is slowly absorbed. To increase the rate of absorption, it is usually used as the more soluble sodium salt. Even so, the latent period, or induction time, can be fifteen minutes for an intravenous (IV) injection.

Dille *et al.* (2) found a uniform bodily distribution of barbital, with the drug being taken up more rapidly by the organs at smaller doses. Also, the brain contained less drug than the other organs, probably due to barbital's low lipid solubility. Koppanyi *et al.* (3) found a lower concentration of drug in the medulla of the brain than in other parts of the brain. Maynert and van Dyke (4), using ^{15}N-labelled barbital, studied central nervous system (CNS) distribution. There was no localization in any particular region of the brain

or in the spinal cord after using several different dose levels.

Barbital is not significantly metabolized in the body and is primarily excreted unchanged. It has been shown (4) by isotope dilution studies of urine from dogs that over 99% of the original undegraded barbital could be recovered. Dorfman and Goldbaum (5) also found no metabolism of barbital in kidney or liver slices *in vitro*. Only if whole body plus excreta were analyzed, could as much as 10% degradation of the drug be found. Goldschmidt and Wehr (6) demonstrated that 2-5% of the drug is metabolized *in vivo*. Ebert *et al*. (7) also found the same three metabolites as the previous authors. These metabolites were 5-ethylbutyl barbituric acid, 5 ethyl-5-beta-hydroxy-ethyl barbituric acid, and the glucuronide of the latter.

It has been shown that the action of barbital is primarily terminated by its excretion through the kidney (5). Either physical or chemical nephrectomy results in a prolonged duration of action of the drug. Since it has been shown *in vitro* that no metabolism of barbital occurs in the kidney, it is the diffusion of the drug which terminates its action.

Barbital is excreted slowly in the urine in man. About 70% is excreted in the first five

days, but excretion may continue for two to three weeks (8). In animals it is excreted more rapidly, about 50% in the first two days, and the remainder in the following week.

The exact mechanism of action of the barbiturates is unknown. Although these drugs have many direct depressant actions on nerve and muscle tissue, such as inhibition of oxidative metabolism in cell-free preparations of liver and brain mitachondria, there has been no clear elucidation of the mechanism or site of action. Anderson et al. (9) studied the rise in brain 5-hydroxytryptamine (5-HT) levels associated with barbiturate hypnosis and found that the rise in 5-HT followed CNS depression and so could not be a cause of the depression. It was also noted that the level of elevation of brain 5-HT corresponded to the degree of hypnosis.

Recent work by Erwin et al. (10) has led to a theory for barbiturate mechanism. An NADPH-linked aldehyde reductase (Enzyme Commission 1.1.1.2) which catalyzes the reduction of some brain biogenic aldehydes to their alcohol derivatives has been isolated. The barbiturates which have hypnotic properties inhibit this enzyme and cause a buildup of the aldehydes. Those barbiturates not having hypnotic properties, such as barbituric acid, do

not inhibit the enzyme. It is suggested that the CNS depressant effects of the barbiturates may be related to an inhibition of this enzyme, which causes an increase in brain biogenic aldehydes. Some biogenic aldehydes, such as 5-hydroxyindoleacetaldehyde and indoleacetaldehyde, have CNS depressant characteristics (67).

Lamson et al. (11) first described the "glucose effect" on barbiturate hypnosis. It was observed that an IV injection of glucose, or its intermediary metabolites, administered to dogs awakening from barbital-induced hypnosis, prolonged the hypnotic state. It was suggested that the rate of entry of barbiturate into the brain was affected. Later, Strother (12) showed that the increased sleeping times of short-acting barbiturates were caused by a decreased elimination rate of the drug. This rate change was due to an inhibition of drug metabolism. Peters and Strother (13) have presented evidence that glucose inhibits NADPH oxidase and several other enzymes of the electron transport chain. The mixed type of inhibition produced partially accounts for the decreased barbiturate metabolism and prolonged duration of action. The authors add that other factors besides enzyme inhibition may play a role in the "glucose effect".

Tolerance to a drug is an unusual resistance

to the normal dose of the drug. Tolerance can be divided into two types according to mechanism. The first is dispositional tolerance (14). This is a change in drug absorption, distribution, excretion, or metabolism which might cause a reduction in pharmacologic activity. The other type of tolerance is functional tolerance; that is, changes in the properties or functions of the target tissue make it less sensitive to the drug.

Barbital is a relatively non-metabolized drug to which tolerance has been reported to develop only over long periods of time (15). Ebert et al. (7) developed a shorter method for producing barbital tolerance. Male weanling rats were given daily injections of barbital for 13 days. After the thirteenth dose there was a three day rest period to allow for complete excretion of the drug. Then a challenging dose of barbital was administered. The tolerance found was manifested as greatly reduced sleep times as compared to non-pretreated controls. Also, drug distribution studies using ^{14}C-barbital were performed and there were no significant differences in activity at any time between the tolerant and control animals in brain, plasma, or urine. The slight metabolic degradation of barbitalwas followed but no difference was found in the percentage of metabolites from tolerant and control

rats. It was concluded that dispositional tolerance did not play a role in the mechanism of barbital tolerance, but rather that it was a functional tolerance due to an altered sensitivity of the cellular drug receptor site.

The duration of action of a drug can be terminated in several ways. It can be removed by the kidneys, redistributed from its primary site of action, or metabolized by enzymes, primarily in the liver. The concentration of enzymes may help determine the duration of drug action. While Remmer (16) was studying mechanisms of barbiturate tolerance, the stimulatory effect of barbiturates and other drugs on liver microsomal enzymes was discovered. When the enzyme that acts on a drug is induced, the drug is metabolized more rapidly, and the metabolite is formed more quickly. In the case of barbital which is not appreciably metabolized by enzymes, no change in duration of action is seen after enzyme induction.

Commey et al. (17) treated rats with a number of drugs and found an increase in the activity of various drug-metabolizing enzymes in the liver microsomes. With phenobarbital there was a stimulation of liver protein synthesis. Later (18) it was shown that while pretreatment with phenobarbital

or barbital could produce cross-tolerance to pentobarbital and hexobarbital, the pretreatment produced no tolerance in the action of barbital.

There may be more mechanisms involved in cross-tolerance than simple enzyme induction. Stohman and Loh (20) produced cross-tolerance to pentobarbital with barbital, but could show that it was not due to enzyme induction. Kroner et al. (21) have shown a certain enzyme inhibition by barbital. Venkatesan et al. (19) found that phenobarbital was not only an inducer, but also a repressor of certain enzyme systems.

Cross tolerance to barbiturates has also been produced by Turnbull and Stevenson (22). Brain homogenates from barbital - dependent animals were injected into control animals. This caused a tolerance to the effects of hexobarbital. This work, however, suggests that the tolerance was due to increased enzymatic activity.

There are several proposed theories which describe the development of functional tolerance to a drug. The enzyme induction theory of Goldstein and Goldstein (23) states that the drug which is producing tolerance inhibits the synthesis of a substance necessary for neuronal function. The reduced concentration of this substance causes derepression of the enzymes which synthesize it and

thus causes tolerance. Withdrawal allows excessive synthesis of the substance and thereby produces neuronal overactivity. This theory is similar to the denervation supersensitivity hypothesis (24). In this theory the drug blocks the release or action of some neurotransmitter and produces oversensitivity of the receptors for that drug. Another theory (25) is based on the concept of redundancy. While multiple neuronal pathways may serve the same function, probably only one is the main active pathway. The others are non-operating or redundant. If the main pathway is blocked by a drug, the redundant pathway function, producing tolerance. At withdrawal, all pathways are again operable, leading to overstimulation and a withdrawal reaction.

In 1936 Selye (26) described a general phenomenon which was called the "general adaptation syndrome". In rats subjected to noxious agents or stresses, a three stage syndrome was seen. Stresses such as cold, surgery, exercise, and drugs such as morphine and formaldehyde all caused these three stages. The first stage or "alarm reaction" occurred in the first 48 hours after treatment. It was characterized by loss of weight in the thymus, spleen, lymphnodes, liver, and fat tissue. Formation of petecheae in the GI tract, fall in body temperature,

loss of muscle tone, and edema were other symptoms noted in the first stage of Selye. The second stage started about 48 hours after the treatment. It was characterized by enlarged adrenals and thyroid, lack of body growth, atrophic gonads, and production by the pituitary of thyrotrophic and adrenotrophic hormones. If the stress were continued, but in a lessened degree, the animals could develop a resistance or "adaptation" to it, and their bodies would return to a near-normal condition. However, if the rather severe stress were continued, the animal would ultimately develop symptoms similar to those seen at first. This was the final stage of "exhaustion".

Until recently, the effects of stress on drug action were thought to be mainly due to an increase in drug metabolism. The stress response caused the pituitary gland to ACTH which stimulated the adrenal gland to produce glucocorticoid hormones. The glucocorticoids, then stimulated the synthesis of certain drug-metabolizing enzymes. Thus, the pituitary-adrenal axis was necessary for the changes in drug action seen after stress.

Bousquet et al. (27), using acute hindleg ligation of rats, found decreased sleep times with pentobarbital and hexobarbital, but not with barbital or phenobarbital. It was suggested that the

decreased sleep times were due to increased metabolism brought about by the stress effects. Since drug action could not be altered in either adrenalectomized or hypophysectomized animals, both glands were necessary for the stress effect.

Wei and Wilson (28) showed evidence of a different mechanism. Using an acute stress there was no change in hexobarbital metabolism immediately after stress, but there was a decrease in metabolism 48 hours after stress. This change occurred in adrenalectomized, but not in hypophysectomized animals. The effect could also be produced in non-stressed animals given an injection of somatotrophin (STH) 48 hours before measuring drug metabolism. Thus, a hypothalmus-pituitary axis was proposed as necessary for the stress effects.

Since stress, as well as repeated administration of barbiturates, is responsible for hepatic enzyme induction, the use of enzyme inhibitors has been applied to the study of barbiturate tolerance.

One inhibitor used was SKF-525A (Proadifen)[1], an inhibitor of hepatic microsomal enzymes. Its administration prior to the injection of a metabolizable drug resulted in a prolonged action of that drug due to its decreased metabolism. Hudson and

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[1]Available from Smith, Kline and French Laboratories, Philadelphia, Penn., USA.

Clay (29) used SKF-525A in a study of stress effects on barbital action. After subjecting animals to a chronic restriction-of-movement stress SKF-525A was administered prior to two doses of barbital at 2 and 26 hours after removal from stress. There was no change in sleeping time after the injection at 2 hours after stress, but greatly increased sleeping time after the injection at 26 hours after stress. These changes were not due to alterations in metalolism (59). Since barbital is not significantly metabolized, the action of the enzyme inhibitor should have had no effect on barbital duration of action. However, SKF-525A has other actions besides that of enzyme inhibitor. It has a natriuretic effect on the kidney (30), a hypocholesterolemic affect (31), and an inhibitory effect on isolated muscle contraction (32). SKF-525A causes a lowered level of hepatic glycogen (33), an MAO and an AChE inhibition (34,35), and a stimulation of tryptophan pyrrolase (36).

Another enzyme inhibitor used in the study of barbiturate tolerance was Actinomycin D. It is an inhibitor of protein synthesis and thus of enzymes as well. It acts by blocking DNA-dependent messenger RNA synthesis in the nucleus (37). Driever and Bousquet (38) used it in a study of the effects of acute stress on pentobarbital action. It was found that Actinomycin D prolonged the sleep time of

pentobarbital immediately after stress, but had no effect upon barbital sleep time after stress.

Stress itself produces various physiological changes, such as depletion of ascorbic acid from the adrenal gland. Smookler and Buckley (39) stressed rats chronically for 12-20 weeks with a combination of auditory stress, flashing lights, and shaking stress. During that time, changes in corticosterone were measured and found to increase three-fold until the fourth week, but then return to normal levels in the fifth week. The blood pressure also rose to a maximum at eight weeks. Brain norepinephrine levels were depleted in the first five weeks, but then returned to normal. Brain dopamine was unaffected. Another study involving chronic stress (69), has reported an increase in brain norephinephrine concentration after stress. Kvetnansky and Mikulaj (40), using acute immobilization stress daily for five weeks to a year, reported that adrenal epinephrine levels decreased at first, but later (after 5.5 weeks) returned to normal. Concurrently, adrenal norepinephrine levels were at first unaffected, but then increased and urinary excretion of epinephrine and norepinephrine was increased at all times after removal from stress. Thus, it was suggested that the "adaptation" to stress by the adrenal medulla is due to increased production and excretion of the

catecholamine, rather than to a decreased release. A further study of levels of rate-limiting enzymes in the synthesis of catecholamines showed increases in these enzymes during stress with return to normal levels after stress (41). Taylor and Snyder (42) also studied effects of restraint stress and concurrently, cold stress. It was shown that stress could decrease brain histamine levels and increase its formation in the hypothalmus.

Other studies on stress have shown that a decrease in brain norepinephrine depends on the type of stress used. Welch and Welch (43) showed that housing mice in groups of 20 caused a reduction in brain norepinephrine in comparison with mice in isolation. Also, swimming, running on a wheel, and electric grid shock (44,45) caused a decrease in brain norepinephrine, while they caused a rise in brain serotonin. Levels of norepinephrine returned to normal in one to six hours after stress. The stress-depletion of norepinephrine could be prevented by phenobarbital or an MAO inhibitor (45), and enhanced by amphetamine (46). Foot shock in rats (48) caused increased rates of anabolism and catabolism of norepinephrine, dopamine, and serotonin. However, dopamine and serotonin could be resynthesized at the same rate as their metabolism, while norepinephrine could not. Norepinephrine and 5-HT were

localized in subcellular nerve-ending particles (47).

Some studies have been done on the control of the hypothalamus over responses to stress. Hiroshige and Sato (49) showed that corticotrophin releasing factor is not produced rhythmically until the fourteenth day of postnatal development. Responses to stress, however, develop by the seventh day. Thus, the initiation of ACTH secretion either by stress or natural rhythm is induced by two different mechanisms. Previously, Davidson et al. (50) had studied the "feedback" control of ACTH secretion in rats. By implanting various glucocorticoids in the hypothalamus the resulting responsiveness to ether and electric shock stresses could be measured. Hydrocortisone acetate, and not corticosterone or ACTH implantation, decreased responses to stress. However, the phenomenon could not be ascribed to decreased adrenal sensitivity as the effect was seen within four hours after implantation, before systemic concentration could be an adequate concentration to account for the effects seen.

Studies have also been done on hormones of the pituitary, other than ACTH, and their effects on stress responses. Growth hormone is secreted in the anterior pituitary of the rat at the same rate of synthesis by both sexes as weanlings (51). However, as they age, males increase their rate of

synthesis of two to three times that of females. Growth hormone causes the suppression of synthesis of some liver enzymes (52,53). The effects of stress on pituitary GH content are variable. Some stresses, such as cold and fasting, cause a depletion, while others, such as auditory stimulation cause an increase (54).

The adrenal gaand also undergoes many changes during stress. Paul et al. (55) measured the concentration of cyclic AMP in the adrenal after acute immobiliaation stress. There were significant increases in adrenocortical levels, but not medullary levels. Hypophysectomy blocked the rise, while denervation of the gland resulted in a rise to only 50% of the previous high. Thus, it was suggested that either the splanchnic nerve or the adrenal medulla via the nerse might be releasing some factor which regulated adrenocortical cyclic AMP.

Furner and Stitzel (56), using cold stress and phenobarbital and hydrocortisone treatments have shown that varying effects to stress can be obtained in adrenalectomized animals. It is suggested that extra-adrenal steroids (such as the sex steroids) may be involved.

The status of the thyroid can also affect drug metabolism. In rats, hyperthyroidism and hypothyroidism can produce similar responses to

pentobarbital, seemingly without affecting CNS sensitivity (57).

If stress could produce changes in drug distribution it would provide a possible mechanism. Ebert et al. (7) found no changes in barbital distribution in tolerant and non-tolerant rats. Similarly, Huang (58) found no differences in tissue barbital distribution or excretion rate between chronically stressed and non-stressed rats. Therefore, a change in drug distribution or metabolism did not seem to be the cause of the stress effects on barbital drug action.

Stress also affects barbital tolerance. Hudson and Clay (29) produced tolerance to barbital after one dose by using chronically stressed rats. Animals were subjected to 14 days of restriction-of-movement stress and then administered two consecutive daily doses of barbital. Tolerance was observed with the second dose. This phenomenon has been repeated several times (59,60,61) and with heat (29) and cold (61) stress as well.

Another phenomenon was observed by Aston (62,63) in regard to barbiturate action. Hypersusceptibility (seen as prolonged sleeping time) to pentobarbital and barbital was found after a 28 day period following two consecutive daily doses of the drug. The effect was not due to an alteration

in metabolism of either drug. Lee (60) produced another form of hypersusceptibility by subjecting rats to chronic restriction stress and administering two consecutive daily doses of barbital. The animals were acutely stressed on the third day and administered a third dose of barbital. With this third dose hypersusceptibility was observed; a fourth daily dose resulted in tolerance.

CHAPTER I

MATERIALS AND METHODS

I. Experimental Animals

Animals used in this study were bred and maintained in the air-conditioned animal quarters of the University of Houston College of Pharmacy.[1] The room temperature was maintained at 22°C. ±1°C., and the relative humidity was 30±2%. An attempt was made to keep noise to a minimum, and dim, diffuse lighting was used. Diet consisted of Purina Laboratory Chow[2] and water, supplied to all rats ad libitum. These conditions applied at all times, unless otherwise indicated.

A. Breeding and Raising of Animals. Mature male and female rats were selected for breeding purposes. A breeding group consisted of four to six females and two male rats. Each group was placed in a large

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[1] Wistar strain rats were obtained from Harlan Industries, Incorporated, Cumberland, Indiana. Sprague-Dawley strain rats used in the norepihephrine assays were obtained from Texas Inbred Mice Company, Houston, Texas 77047.

[2] Ralston Purina Company, Checkerboard Square, St. Louis, Missouri.

stainless steel cage with a wire mesh top. The cages measured 56x51x23 centimeters. The bottoms of the cages were covered with Iso-dri bedding[3] during the breeding procedures. Clean cages and fresh bedding were supplied three times weekly. Extreme caution was used in transferring female animals from one cage to another in order to minimize possible injury to gravid animals.

The breeding period lasted for ten days. After this time the female animals were placed in individual cages in which they later delivered and raised their litters. The breeding procedure was carried out in a room containing only the breeding animals. Another "stress-free" room was maintained for the gravid females, females with litters, recently weaned animals, and control "non-stressed" animals.

Gravid females were housed in opaque white plastic cages with removable metal covers. The cages measured 46x26x21 centimeters. The bottoms of the cages were covered with processed white pine shavings.[4]

Two to four days after birth, the animals in each litter were counted and the number of nursing

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[3]Carworth, Division of Becton, Dickinson and Company, New York City, New York 10956.

[4]Ab-Sorb-Dri bedding, Michael Wood Products, Incorporated, Garfield, New Jersey.

rats in each litter was limited to eight. Excess rats were transferred to a smaller litter of the same age. Any animals left over at this point were sacrificed. At 22 to 24 days following birth the young rats were weaned. Breeder female rats were returned to the breeding room and allowed to recuperate for at least 14 days before being bred again.

After weaning, the young rats were separated according to sex. They were placed in cages with no more than four animals per cage. They remained in the "stress-free" room until large enough for experimentation. Rats used in the experiment were 31-42 days old at the beginning of treatment and their weights ranged from 120-180 grams. Each experimental group contained equal numbers of males and females unless otherwise indicated.

B. Restriction of Movement at Room Temperature. The rats were weighed and placed in individual restriction cages made from wire mesh. The cages were 18x8x5 centimeters. They were small enough so that the animal could turn around only with difficulty. Food and water were placed at opposite ends of the cage. The restricted rats were then placed in small opaque plastic cages, measuring 29x18x13 centimeters. The bottom of each cage was

covered with pine shavings.[5]

The restricted animals were maintained in a semidark room at 22°C. ±1°C. for 14 days. During this time food and water were replenished daily and clean cages and shavings were supplied three times a week. At the fifteenth day of stress the animals were weighed and placed in individual opaque plastic cages which allowed complete freedom of movement.

For control rats were maintained in the "stress-free" room for 14 days. After this period, they were removed from the "stress-free" room, weighed, and placed in individual plastic cages.

C. Hindleg Ligation. Each rat was weighed, and wrapped securely in cloth towels for easier handling. A five centimeter long rubber band was wound around the right hindleg four to six times and placed on the upper thigh. The animal was returned to its individual plastic cage for a 2.5 hour period. At the end of that time the rubber band was removed as gently as possible. The animal was then placed in its cage for 45 minutes before further experimental procedures.

II. Administration of Drugs

A. Preparation of Drugs. A solution of barbital was prepared from powdered barbital sodium[6] with sterile

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[5]Ab-Sorb-Dri bedding, Michael Wood Products, Incorporated, Garfield, New Jersey.

saline as the solvent. To simplify volume calculations, the concentration of the solution in mg/ml was adjusted to be exactly one-half the dose in mg/kg of body weight. The concentration of the solution was made to 100 mg/ml. With this dilution the weight of the animal in grams could be multiplied by two and divided by 1000 to give the volume of the dose in milliliters.

A solution of Proadifen (2-diethylaminoethyl-2,2-diphenylvalerate, hydrochloride)[7] was prepared by the same procedure as for barbital. The concentration was adjusted to 25 mg/ml. Each solution was freshly prepared and kept under refrigeration.

B. Injection Procedures. Two hours after removal from restriction of movement stress or removal from the "stress-free" room, the rats were injected with barbital (200mg/kg) by the intraperitoneal (I.P.) route. A one milliliter tuberculin syringe with a 27 gauge, 3/8 inch hypodermic needle was used. The barbital was injected again after 24,48, or 72 hours. Starting 3.25 hours before the 48 hour injection, the animals were subjected to the hindleg ligation stress for 2.5 hours.

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[6]American Pharmaceutical Company, Incorporated, New York, New York.

[7]Smith, Kline, and French Labs., Philadelphia, Pennsylvania.

Proadifen (50 mg/kg) was injected I.P. four hours before the 48 and 72 hour barbital injections in selected groups of rats.'

III. Norepinephrine Determination

Animals were sacrificed by decapitation using a Harvard decapitator.[8] The whole brain was removed and frozen and assayed. A modified version of the Anton and Sayre (63,65) assay for brain norepinephrine was used. The oxidation step was that of Laverty and Taylor (64).

A. Preparation of Reagents. Perchloric acid was made in a 0.4 N concentration by measuring into a bottle a 12.02 ml aliquot of 70% $HClO_4$ and diluting it to 500 ml with glass distilled water. Then 2.5 grams of sodium metabisulfite were dissolved in the solution.

A 3 M Tris (hydroxymethyl) amine methene buffer solution, pH 8.3, was prepared by dissolving 181.7 grams of Tris in 500 ml of distilled water. The pH was adjusted to 8.3 with NaOH.

A 0.2 M sodium phosphate buffer was made by dissolving 7.6 grams of Na_3PO_4 in 100 ml water and adjusting the pH tp 6.5.

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[8]Harvard Apparatus Company, Incorporated, Dover, Massachusetts.

A 0.02 N iodine solution was made by dissolving 5.0 grams of sodium iodide in 95 ml of distilled water, adding 254 mg of iodine, and storing in a brown bottle in the refrigerator.

A solution of 2.5% sodium sulfite in 2.5 N NaOH containing 1% EDTA was prepared. To 40 ml of 2.5 N NaOH were added 400 mg of EDTA and then one gram of Na_2SO_3.

A stock solution of norepinephrine was made by dissolving 1.99 mg of norepinephrine in 100 ml of 0.05 N HCl. This yielded a solution of 10 mg/ml. On the day of the test, 1.0 ml of the stock was diluted to 10 ml with 0.2 N HCl/

The alumina oxide[9] was prepared for use by soaking in 0.1 M EDTA. The moisture was evaporated by heating in an oven until dry. The washed alumina was stored in a desiccator.

B. Adsorption and Elution of Catecholamines on Alumina. The whole rat brain was weighed and then homogenized for 45 seconds in four volumes of 0.4 N perchloric acid using a Teflon mortar and glass pestle. The homogenate was transferred to a 10 ml polyethylene tube and centrifuged at 30,000 x g for ten minutes at 4°C.

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[9]Woelm Neutral Activity Grade 1, Alupharm Chemicals, New Orleans, Louisiana.

Three tubes with known concentrations of norepinephrine were prepared to act as standards for the final calculation of results. Another tube was to be used as a blank. Using the freshly diluted stock norepinephrine solution, the three tubes received respectively, 0.1, 0.2, and 0.4 mg of norepinephrine. These were diluted to 4.5 ml with 0.4 N perchloric acid. The blank tube contained only perchloric acid. Thereafter, the standard tubes and the blank were treated identically to the extracted tissues.

A 4.5 ml aliquot of the clear supernatant fluid from the homogenate was transferred to a 35 ml conical tube. The pH was adjusted to 8.3 using 3 M Tris buffer and pH paper. This step required approximately 0.75 ml of Tris buffer. Then 500 mg of alumina oxide were added to the 35 ml centrifuge tube.

The tightly stoppered tube was shaken gently for 15 minutes and then centrifuged at 2000 rpm for five minutes. The supernatant fluid was aspirated. The alumina in the tube was washed once by addition of 3.0 ml water, then shaken gently for five minutes, and centrifuged again at 2000 rpm. The superantant fluid was then aspirated.

To the alumina tube were added 3.0 ml of 0.2 N HCl acid. The tube was shaken for 15 minutes and

then centrifuged at 2000 rpm. Aliquots of 1.0 ml of the final aqueous phase were pipetted into test tubes for oxidation of norepinephrine.

C. Oxidation of Norepinephrine. To the 1.0 ml of aqueous phase was added approximately 0.3 ml of 0.2 M sodium phosphate buffer, to adjust the final pH to 6.5. Oxidation then occurred with the addition of 0.3 ml of iodine solution. The standard and blank tubes received only 0.2 ml of iodine solution. After three minutes, 1.0 ml of alkaline sulfite reagent was added. Five minutes later 0.3 ml of glacial acetic acid was added to bring the solution to a final pH of about 4.8.

After a 20 minute waiting period at room temperature the % transmission of the sample was read on an Aminco-Bowman spectrofluorimeter[10] at an activation wavelength of 380 and a fluorescence wavelength of 480. The multiplier setting was 0.03, and split arrangement was #5A.

D. Calculation of Results. The value of the blank reading was subtracted from all other readings. A new standard curve for norepinephrine was prepared for each assay. It was drawn by plotting the % Transmission values of the standards against their concentrations in mg.

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[10]Aminco-Bowman, Silver Springs, Maryland.

Then each experimental value was located on the graph and its corresponding concentration determined. This value was then used in the following formula:

mg norepinephrine/g. brain = (conc. from graph)(total vol. of homogenate/4.5 ml)(2.9 ml/2.8 ml)(1/brain weight).

This formula could be further reduced to :

mg norepinephrine/g. brain = (conc. from graph)(1.15).

IV. Experimental Design

A. Study I: These experiments were designed to show the effects of both chronic and acute stress on duration of barbital sleeping time.

Study I-1: Rats were subjected to chronic restriction of movement stress for 14 days and then given injections of barbital at 2, 26, 50, and 74 hours after removal from stress. At 46, 75 hours after removal from stress, prior to the third injection, they were subjected to acute hindleg ligation stress for 2.5 hours. Control non-stressed animals were raised in a "stress-free" room and then were injected and acutely stressed at the same times as for pre-stressed animals.

Study I-2: In order to determine the effects of the barbital injections themselves, the dose on day 1 was omitted.

Study I-3: Doses 1 and 2 were omitted.

Study I-4: Doses 1, 2, and 3 were omitted.

B. Study II: These experiments show the effect of the chronic restriction stress only on duration of barbital sleeping time.

Study II-1: The pre-stressed and non-stressed animals were injected at 2, 26, 50, and 74 hours after removal from stress or stress-free conditions.

Study II-2: Doses on days 1 and 2 were omitted.

C. Study III: These experiments show the effect of an enzyme inhibitor, SKF-525A, on duration of barbital sleeping time in chronically stressed rats, with and without the acute stress. Doses on days 1 and 2 after removal from chronic stress were omitted.

Study III-1: SKF-525A was administered 45 minutes before the acute stress, and 24 hours later.

Study III-2: SKF-525A was administered at the same time as in study III-1, and 24 hours later; acute stress was not applied.

D. Study IV: These experiments show the effects of chronic stress and barbital on total norepinephrine concentration in the rat brain.

Study IV-1: The total brain norepinephrine content was determined for non-stressed animals, and chronically stressed animals at 2 hours and 26 hours after removal from stress.

Study IV-2: The norepinephrine concentration was determined for pre-stressed and non-stressed animals at the time of regaining of the righting reflex after one dose of barbital.

Study IV-3: The norepinephrine concentration was measured on pre-stressed rats at 26 hours

after removal from stress after they had received one dose of barbital at 2 hours after stress.

Study IV-4: The norepinephrine concentration was determined for pre-stressed animals after two doses of barbital.

All data were analyzed by the Student "t" test. Confidence limits were set at 95%. ($p < 0.05$).

CHAPTER II

RESULTS

The following tables contain the results from this study. They are arranged so that a summary table accompanies each individual study.

The induction time was defined as the amount of time elapsed between the injection of the drug and the loss of the righting reflex. The duration of hypnosis was the length of sleeping time, or amount of time between the loss and regaining of the righting reflex.

TABLE 1. INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/Kg.,I.P.) IN RATS AFTER REMOVAL FROM 14 DAY RESTRICTION OF MOVEMENT STRESS. ANIMALS SUBJECTED TO 2.5 HOURS OF HINDLEG LIGATION STRESS PRIOR TO DOSE III

Rat No.	Sex	Body Weight (grams)			Induction Time (min.)				Duration of Hypnosis (min.)			
		D-1[2]	D-15[3]	Change	I	II	III	IV	I	II	III	IV
1	M	150	254	+104	112.5	42.3	34.9	81.7	167.2	131.5	248.8	70.9
2	M	145	208	+ 63	91.9	65.4	66.1	57.6	116.7	171.0	145.0	127.5
3	F	124	178	+ 54	59.6	55.8	34.9	38.5	181.1	216.5	203.9	170.0
4.	F	130	216	+ 86	53.0	86.1	62.2	106.6	161.3	142.2	147.3	99.3
5	F	110	162	+ 52	55.7	42.1	62.3	55.2	153.9	134.0	138.7	90.9
6	F	134	198	+ 64	49.7	85.1	59.6	no LRR[1]	262.1	141.5	237.8	no LRR[1]
7	F	135	170	+ 35	64.1	49.3	58.1	102.0	242.8	236.3	213.3	41.0
8.	F	110	146	+ 36	67.9	84.4	57.4	no LRR[1]	135.2	118.3	137.6	no LRR[1]
9	F	134	190	+ 56	70.6	32.5	35.0	42.4	271.4	222.3	229.3	185.3
Mean (T)		130	191	+ 61	69.4	60.3	52.3	69.1	188.0	168.2	189.1	112.1
S.D.[4](T)				22.2	20.4	20.8	13.4	17.5	56.6	45.1	46.4	52.3
S.E.[5](T)				7.4	6.8	6.9	4.5	6.6	18.9	15.1	15.5	19.8

[1]Animal did not lose the righting reflex. Rat was close to the loss of righting reflex and did not move around.

[2]First day of restriction-of-movement stress.

[3]Day of removal from restriction stress

[4]Standard deviation.

[5]Standard error of the mean

TABLE 2. INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/Kg., I.P.) IN RATS AFTER REMOVAL FROM MAINTAINANCE IN THE "STRESS-FREE" ROOM FOR 14 DAYS. ANIMALS SUBJECTED TO 2.5 HOURS OF HINDLEG LIGATION STRESS PRIOR TO DOSE III.

Rat No.	Sex	Body Weight (grams)			Induction Time (min.)				Duration of Hypnosis (min.)			
		D-1	D-15	Change	I	II	III	IV	I	II	III	IV
1	M	158	260	+102	15.8	76.8	57.6	49.3	313.9	161.8	144.6	166.1
2	M	166	268	+102	75.5	no LRR[1]	63.0	58.4	146.0	no LRR[1]	127.6	79.7
3	M	126	240	+114	47.9	42.1	39.6	65.8	282.0	227.6	293.6	77.8
4	F	140	190	+ 50	59.5	71.0	49.4	49.9	61.4	86.5	150.8	88.4
5	F	145	220	+ 75	59.5	32.2	70.1[2]	32.9[2]	186.1	122.6	49.6[2]	107.1[2]
6	F	108	210	+102	115.5	53.6	56.7	42.6	75.2	95.2	95.5	92.6
7	F	128	210	+ 82	94.3	67.5	45.2	53.8	139.2	91.3	117.7	83.5
Mean (t)		137	228	+ 90	71.1	57.2	51.9	53.3	172.0	130.8	155.0	98.0
S.D. (T)				22.0	25.8	17.6	8.7	8.1	96.2	55.1	70.7	42.0
S.E. (T)				8.3	9.7	7.2	3.6	3.3	36.4	22.6	28.9	17.2

[1]Animal did not lose the righting reflex.

[2]Injection was incomplete; data was not used in the calculations.

TABLE 3. SUMMARY OF INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.). ANIMALS SUBJECTED TO 2.5 HOURS OF HINDLEG LIGATION STRESS PRIOR TO DOSE III. (Study I-1).

Treatment	Number of Animals	Induction Time (min.)				Duration of Hypnosis (min.)			
		I	II	III	IV	I	II	III	IV
Stress	T (9)	69.4[1] (6.8)	60.3 (6.9)	52.3 (4.5)	69.1 (6.6)	188.0 (18.9)	168.2 (15.1)	189.1 (15.5)	112.1 (19.8)
Control	T (7)	71.1 (9.7)	57.2 (7.2)	51.9 (3.6)	53.3 (3.3)	172.0 (36.4)	130.8 (22.6)	155.0 (28.9)	98.0 (17.2)

[1]Mean (± standard error in parenthesis).

TABLE 4. INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.) IN RATS AFTER REMOVAL FROM 14 DAY RESTRICTION OF MOVEMENT STRESS. SNIMALS SUBJECTED TO 2.5 HOURS OF HINDLEG LIGATION STRESS PRIOR TO DOSE III. INITIAL DOSE GIVEN AT 26 HOURS AFTER RESTRICTION STRESS.

Rat No.	Sex	Body Weight (grams)			Induction Time (min.)				Duration of Hypnosis (min.)			
		D-1	D-15	Change	I	II	III	IV	I	II	III	IV
1	M	210	210	0	-	127.1	89.2	44.1	-	110.0	60.5	96.4
2	M	170	220	+50	-	no LRR[1]	46.4	65.5	-	no LRR[1]	150.8	98.2
3	M	200	236	+36	-	no LRR[1]	34.8	54.6	-	no LRR[1]	230.0	112.3
4	M	230	215	-15	-	60.2	45.4	72.2	-	122.6	280.1	64.4
Mean	(M)	203	220	+24	-	93.7	54.0	27.1	-	116.3	180.4	92.8
S.D.	(M)			34.2		14.9	24.1	12.4		8.9	96.0	20.2
S.E.	(M)			19.8		10.6	12.1	6.2		6.3	48.0	10.1

TABLE 4 - Continued

Rat No.	Sex	Body Weight (grams) D-1	D-15	Change	Induction Time (min.) I	II	III	IV	Duration of Hypnosis (min.) I	II	III	IV
1	F	150	186	+36	-	58.4	42.0	52.4	-	146.6	212.6	87.5
2	F	155	190	+35	-	62.3	41.9	70.1	-	138.8	126.0	47.0
3	F	152	195	+43	-	63.4	41.2	60.0	-	115.9	226.8	82.0
Mean	(F)	152	190	+38	-	58.3	42.0	52.4	-	146.6	212.6	87.8
S.D.	(F)			4.4		7.2	0.8	22.4		35.3	80.4	44.0
S.E.	(F)			2.5		4.2	0.5	13.0		20.4	46.4	25.4

[1]Animal did not lose the righting reflex.

TABLE 5. INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.) IN RATS AFTER REMOVAL FROM MAINTENANCE IN THE "STRESS-FREE" ROOM FOR 14 DAYS. ANIMALS SUBJECTED TO 2.5 HOURS OF HINDLEG LIGATION PRIOR TO DOSE III. INITIAL DOSE GIVEN AT 26 HOURS AFTER REMOVAL FROM STRESS-FREE ROOM.

Rat No.	Sex	Body Weight (grams) D-1	D-15	Change	Induction Time (min.) I	II	III	IV	Duration of Hypnosis (min.) I	II	III	IV
1	M	204	348	+144	- 90.6	47.8	94.9		- 60.5	222.3	93.5	
2	M	180	322	+142	- 60.1	27.8	34.6		- 138.2	299.4	155.7	
3	M	196	318	+122	- 46.6	37.8	33.5		- 68.0	186.9	118.8	
4	M	184	295	+111	- 56.9	78.0	55.3		- 166.7	264.5	161.8	
Mean	(M)	191	321	+130	- 63.6	47.9	54.6		- 108.4	243.3	132.5	
S.D.	(M)			17.0	18.9	21.7	28.7		52.3	49.0	32.2	
S.E.	(M)			8.5	9.5	10.9	14.3		26.2	24.5	16.1	
1	F	140	212	+ 72	- 75.4	36.8	31.6		- 147.8	276.3	118.6	
2	F	148	216	+ 68	- 56.4	35.8	31.3		- 233.5	318.5	180.1	
3	F	140	214	+ 74	- no LRR[1]	46.0	82.6		- no LRR[1]	106.8	97.3	
Mean	(F)	143	214	+ 71	- 65.9	39.5	48.5		- 190.7	233.9	132.0	
S.D.	(F)			3.1	13.5	5.1	29.5		60.6	112.2	32.0	
S.E.	(F)			1.8	9.5	2.9	17.0		42.9	64.8	24.8	

[1]Animal did not lose the righting reflex.

TABLE 6. SUMMARY OF INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.). ANIMALS SUBJECTED TO 2.5 HOURS OF HINDLEG LIGATION STRESS PRIOR TO DOSE III. INITIAL DOSE GIVEN AT 26 HOURS AFTER REMOVAL FROM STRESS OR "STRESS-FREE" ROOM. (STUDY I-2).

Treatment	Sex and No. of Animals	Induction Time (min.) I	II	III	IV	Duration of Hypnosis (min.) I	II	III	IV
Stress	M (4)	-	93.7[1] (10.6)	54.0 (12.1)	59.1 (6.2)	-	116.3 (6.3)	180.4 (48.0)	92.8 (10.1)
Stress	F (3)	-	58.4 (4.2)	42.0 (0.5)	52.4 (13.1)	-	146.6 (20.4)	212.6 (46.4)	87.8 (25.4)
Control	M (4)	-	63.6 (9.5)	47.9 (10.9)	54.6 (14.4)	-	108.4 (26.2)	243.3 (24.5)	132.5 (16.1)
Control	F (3)	-	65.9 (9.5)	39.5 (2.9)	48.5 (17.0)	-	190.7 (42.9)	233.9 (64.8)	132.0 (24.8)

[1]Mean (± standard error in parenthesis).

TABLE 7. INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.) IN RATS AFTER REMOVAL FROM 14 DAY RESTRICTION OF MOVEMENT STRESS. ANIMALS SUBJECTED TO 2.5 HOURS OF HINDLEG LIGATION STRESS PRIOR TO DOSE III. INITIAL DOSE GIVEN AT 50 HOURS AFTER REMOVAL FROM RESTRICTION STRESS.

Rat No.	Sex	Body Weight (grams)			Induction Time (min.)				Duration of Hypnosis (min.)			
		D-1	D-15	Change	I	II	III	IV	I	II	III	IV
1	M	190	260	+70	-	-	no Lrr[1]	76.6	-	-	no LRR[1]	117.1
2	M	165	230	+65	-	-	62.8	62.9	-	-	119.7	73.4
3	M	144	235	+91	-	-	81.0	40.1	-	-	115.0	148.4
4	M	174	225	+51	-	-	49.4	50.9	-	-	291.0	133.1
5	M	150	230	+80	-	-	59.0	72.0	-	-	237.6	4.6
6	M	160	190	+30	-	-	47.0	58.8	-	-	80.6	152.8
Mean	(M)	164	228	+65	-	-	59.8	60.2	-	-	168.8	104.9
S.D.	(M)			21.6	-	-	13.5	13.5			89.2	55.1
S.E.	(M)			8.8			6.0	5.5			39.9	22.5
1	F	144	185	+41	-	-	45.7	46.7	-	-	92.0	101.0
2	F	150	200	+50	-	-	44.7	35.4	-	-	154.1	84.6
3	FF	148	176	+28	-	-	61.0	53.6	-	-	127.6	211.5
4	F	166	160	- 6	-	-	114.4	76.2	-	-	155.9	78.7
5	F	145	194	+49	-	-	78.4	51.3	-	-	249.5	104.1
6	F	146	200	+54	-	-	48.2	61.9	-	-	161.7	131.2
Mean	(F)	150	186	+36	-	-	65.4	54.2	-	-	156.8	118.5
S.D.	(F)			22.5			27.2	13.9			52.3	48.0
S.E.	(F)			9.2			11.1	5.7			21.4	19.6
Mean	(T)	157	207	+50	-	-	62.9	57.2	-	-	162.2	111.7
S.D.	(T)			27.5			21.2	13.4			68.5	51.2
S.E.	(T)			8.0			6.4	5.5			20.7	14.8

[1]Animal did not lose the righting reflex.

TABLE 8. INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.) IN RATS AFTER REMOVAL FROM MAINTENANCE IN THE "STRESS-FREE" ROOM FOR 14 DAYS. ANIMALS SUBJECTED TO 2.5 HOURS OF HINDLEG LIGATION STRESS PRIOR TO DOSE III. INITIAL DOSE GIVEN AT 50 HOURS AFTER REMOVAL FROM RESTRICTION STRESS.

Rat No.	Sex	Body Weight (grams)			Induction Time (min.)				Duration of Hypnosis (min.)			
		D-1	D-15	Change	I	II	III	IV	I	II	III	IV
1	M	176	348	+172	-	-	53.3	100.8	-	-	253.4	248.8
2	M	150	295	+145	-	-	50.6	51.9	-	-	257.1	151.4
3	M	128	276	+148	-	-	119.3	no LRR[1]	-	-	217.7	no LRR[1]
4	M	140	295	+155	-	-	62.7	54.8	-	-	338.7	272.9
5	M	180	345	+165	-	-	76.6	no LRR[1]	-	-	140.3	no LRR[1]
6	M	156	310	+154	-	-	60.7	62.2	-	-	164.4	123.8
Mean	(M)	155	312	+157	-	-	70.5	67.4	-	-	228.6	199.2
S.D.	(M)			10.2			25.6	22.6			71.6	72.6
S.E.	(M)			4.2			10.5	11.3			29.2	36.3
1	F	170	330	+160	-	-	113.3	51.7	-	-	180.2	289.3
2	F	120	210	+ 90	-	-	71.9	50.7	-	-	289.9	166.4
3	F	140	232	+ 92	-	-	62.9	49.9	-	-	203.1	131.5
4	F	140	175	+ 35	-	-	48.2	48.8	-	-	220.0	132.6
5	F	110	200	+ 90	-	-	73.3	65.5	-	-	100.0	71.9
6	F	130	202	+ 72	-	-	32.8	54.3	-	-	355.0	250.4
Mean	(F)	135	225	+ 90	-	-	67.1	53.5	-	-	224.7	173.7
S.D.	(F)			40.6			25.2	6.2			88.5	81.5
S.E.	(F)			16.6			10.3	2.5			36.1,	33.2
Mean	(T)	145	268	+123	-	-	68.8	59.1	-	-	226.7	183.9
S.D.	(T)			44.9			25.4	15.6			76.7	74.1
S.E.	(T)			13.0			7.3	4.9			22.1	23.4

[1]Animal did not lose the righting reflex.

TABLE 9. SUMMARY OF INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.) ANIMALS SUBJECTED TO 2.5 HOURS OF HINDLEG LIGATION STRESS PRIOR TO DOSE III. INITIAL DOSE GIVEN AT 50 HOURS AFTER REMOVAL FROM STRESS OR "STRESS-FREE" CONDITIONS. (STUDY I-3).

Treatment	Sex and No. of Animals	Induction Time (min.) I	II	III	IV	Duration of Hypnosis (min.) I	II	III	IV
Stress	M (6)	-	-	59.8[1] (6.0)	60.2 (5.5)	-	-	168.8 (39.9)	104.9 (22.5)
Stress	F (6)	-	-	65.4 (11.1)	54.2 (5.7)	-	-	156.8 (21.4)	118.5 (19.6)
Stress	T (12)	-	-	62.9 (6.4)	57.2 (5.5)	-	-	162.2 (20.7)	111.7 (14.8)
Control	M (6)	-	-	70.5 (10.5)	67.4 (11.3)	-	-	228.6 (29.2)	199.2 (36.3)
Control	F (6)	-	-	67.1 (10.3)	53.5 (2.5)	-	-	224.7 (36.1)	173.7 (33.2)
Control	T (12)	-	-	68.8 (7.3)	59.1 (4.9)	-	-	226.7 (22.1)	183.9 (23.4)

[1]Mean (± standard error in parenthesis).

TABLE 10. INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.) IN RATS AFTER REMOVAL FROM 14 DAY RESTRICTION OF MOVEMENT STRESS. ANIMALS SUBJECTED TO 2.5 HOURS OF HINDLEG LIGATION STRESS PRIOR TO DOSE III. INITIAL DOSE GIVEN AT 74 HOURS AFTER RESTRICTION STRESS.

Rat No.	Sex	Body Weight (grams)			Induction Time (min.)					Duration of Hypnosis (min.)				
		D-1	D-15	Change	I	II	III	IV	V	I	II	III	IV	V
1	M	140	196	+56	-	-	-	no LRR[1]	50.7	-	-	-	no LRR[1]	82.0
2	M	150	200	+50	-	-	-	no LRR[1]	no LRR[1]	-	-	-	no LRR[1]	no LRR[1]
3	M	130	230	+100	-	-	-	84.6	47.0	-	-	-	139.6	21.6
Mean (M)		140	209	+69	-	-	-	no LRR[1]	48.9	-	-	-	no LRR[1]	51.8
S.D. (M)				27.3					2.6					42.7
S.E. (M)				15.8					1.9					30.2
1	F	125	165	+40	-	-	-	50.7	34.7	-	-	-	90.9	31.4
2	F	145	200	+55	-	-	-	no LRR[1]	no LRR[1]	-	-	-	no LRR[1]	no LRR[1]
3	F	114	180	+66	-	-	-	70.1	33.1	-	-	-	201.5	77.6
4	F	100	152	+52	-	-	-	38.4	40.6	-	-	-	134.0	19.9
Mean (F)		121	174	+53	-	-	-	53.1	36.1	-	-	-	142.1	43.0
S.D. (F)				10.7				16.0	3.9				55.6	30.6
S.E. (F)				5.9				9.2	2.3				32.2	17.7
Mean (T)		129	189	+60	-	-	-	61.0	41.2	-	-	-	141.5	46.5
S.D. (T)				19.3				23.7	7.6				45.5	30.8
S.E. (T)				7.3				11.9	3.4				22.8	13.8

[1]Animal did not lose the righting reflex.

TABLE 11. INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.) IN RATS AFTER REMOVAL FROM MAINTENANCE IN THE "STRESS-FREE" ROOM FOR 14 DAYS. ANIMALS SUBJECTED TO 2.5 HOURS OF HINDLEG LIGATION STRESS PRIOR TO DOSE III. INITIAL DOSE GIVEN AT 74 HOURS AFTER REMOVAL FROM THE "STRESS-FREE" ROOM.

Rat No.	Sex	Body Weight (grams)			Induction Time (min.)					Duration of Hypnosis (min.)				
		D-1	D-15	Change	I	II	III	IV	V	I	II	III	IV	V
1	M	150	170	+ 20	-	-	-	no LRR[1]	60.4	-	-	-	no LRR[1]	31.8
2	M	120	244	+124	-	-	-	57.5	59.4	-	-	-	98.2	48.9
3	M	132	245	+113	-	-	-	97.6	no LRR	-	-	-	83.0	no LRR[1]
4	M	118	242	+124	-	-	-	56.1	36.3	-	-	-	137.4	115.2
Mean (M)		130	223	+ 95	-	-	-	70.4	52.0	-	-	-	106.2	65.3
S.D. (M)				49.7				23.6	13.6				28.1	37.8
S.E. (M)				24.9				13.6	7.9				16.2	21.9
1	F	105	190	+ 85	-	-	-	35.6	no LRR	-	-	-	148.1	no LRR[1]
2	F	125	196	+ 71	-	-	-	52.4	43.4	-	-	-	125.9	175.8
3	F	98	162	+ 64	-	-	-	60.2	50.6	-	-	-	115.9	117.6
4	F	110	178	+ 68	-	-	-	40.8	49.6	-	-	-	121.4	86.5
Mean (F)		110	182	+ 72	-	-	-	47.3	47.9	-	-	-	127.8	126.6
S.D. (F)				9.4				11.1	3.9				14.1	45.4
S.E. (F)				4.7				5.6	2.3				7.6	26.2
Mean (T)		120	203	+ 84	-	-	-	57.2	50.0	-	-	-	118.6	96.0
S.D. (T)				35.8				14.0	9.3				12.3	52.2
S.E. (T)				12.7				5.3	3.8				8.4	21.4

[1]Animal did not lose the righting reflex.

TABLE 12. SUMMARY OF INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.) ANIMALS SUBJECTED TO 2.5 HOURS OF HINDLEG LIGATION STRESS PRIOR TO DOSE III. INITIAL DOSE GIVEN AT 74 HOURS AFTER REMOVAL FROM STRESS OR "STRESS-FREE" ROOM. (STUDY I-4).

Treatment	Sex and No. of Animals	Induction Time (min.) I	II	III	IV	V	Duration of Hypnosis (min.) I	II	III	IV	V
Stress	M (3)	-	-	-	no LRR[1]	48.9[2] (1.9)	-	-	-	no LRR[1]	51.8 (30.2)
Stress	F (4)	-	-	-	53.1 (9.2)	36.1 (2.3)	-	-	-	142.1 (42.2)	43.0 (17.7)
Stress	T (7)	-	-	-	61.0 (11.9)	41.2 (3.4)	-	-	-	141.5 (22.8)	46.5 (13.8)
Control	M (4)	-	-	-	70.4 (13.6)	52.0 (7.9)	-	-	-	106.2 (16.2)	65.3 (21.9)
Control	F (4)	-	-	-	47.3 (5.6)	47.9 (2.3)	-	-	-	127.8 (7.6)	126.6 (26.2)
Control	T (8)	-	-	-	57.2 (5.3)	50.0 (3.8)	-	-	-	118.6 (8.4)	96.0 (21.4)

[1]Animal did not lose righting reflex.

[2]Mean (± standard error in parenthesis).

TABLE 13. INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.) IN RATS AFTER REMOVAL FROM 14 DAY RESTRICTION OF MOVEMENT STRESS.

Rat No.	Sex	Body Weight (grams)			Induction Time (min.)				Duration of Hypnosis (min.)			
		D-1	D-15	Change	I	II	III	IV	I	II	III	IV
1	M	155	210	+55	59.1	no LRR[1]	77.5	52.4	155.7	no LRR[1]	60.8	73.1
2	M	160	230	+70	43.8	30.3	66.8	45.4	206.3	141.7	147.8	60.3
3	M	166	218	+52	75.6	30.3	37.8	no LRR[1]	105.2	54.2	129.5	no LRR[1]
4	M	122	170	±48	no LRR[1]	58.6	38.7	50.0	no LRR[1]	105.0	154.0	115.4
Mean (M)		151	207	+54	59.5	39.7	55.2	49.3	155.7	100.3	123.3	82.9
S.D. (M)				10.0	16.2	16.3	20.1	3.6	10.0	43.9	42.8	28.8
S.E. (M)				5.0	9.4	9.4	10.0	2.1	9.2	25.4	21.4	16.7
1	F	130	185	+55	33.5	35.4	38.1	33.7	259.4	256.8	170.2	165.4
2	F	137	198	+61	37.3	28.7	38.2	37.8	439.8	199.5	113.2	34.2
3	F	145	184	+39	70.5	57.1	no LRR[1]	46.7	97.7	85.4	no LRR[1]	150.9
4	F	114	150	+36	37.9	28.1	no LRR[1]	no LRR[1]	162.0	no LRR[1]	104.2	no LRR[1]
Mean (F)		132	179	+48	44.8	37.3	38.2	39.4	239.7	161.5	141.7	116.8
S.D. (F)				12.2	17.2	13.6	0.1	6.7	110.5	80.9	40.3	71.9
S.E. (F)				6.1	8.6	6.8	0.07	3.8	55.3	40.5	28.6	41.5

[1] Animal did not lose the righting reflex.

TABLE 14. INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM 9200 mg/kg.) IN RATS AFTER REMOVAL FROM MAINTENANCE IN THE "STRESS-FREE" ROOM FOR 14 DAYS.

Rat No.	Sex	Body Weight (grams) D-1	D-15	Change	Induction Time (min.) I	II	III	IV	Duration of Hypnosis (min.) I	II	III	IV
1	M	125	104	- 21	no LRR[1]	no LRR[1]	55.1	46.3	no LRR[1]	no LRR[1]	63.9	89.7
2	M	140	260	+120	45.3	31.9	53.3	78.4	243.6	269.8	90.8	35.9
3	M	150	240	+ 90	45.6	43.0	no LRR[1]	no LRR[1]	183.9	164.6	no LRR[1]	no LRR[1]
4	M	118	232	+114	56.7	55.2	50.7	45.0	283.9	138.3	95.2	145.6
Mean (M)		133	209	+ 76	49.2	43.4	53.0	56.6	237.1	190.0	83.3	90.4
S.D. (M)				65.8	6.5	11.7	2.2	18.9	50.3	69.5	17.0	54.9
S.E. (M)				32.9	3.8	6.7	1.3	10.9	29.1	40.2	9.8	31.7
1	F	215	172	- 43	43.4	30.0	49.0	44.6	183.4	195.6	147.4	146.4
2	F	148	186	+ 38	no LRR[1]	no LRR[1]	61.5	43.6	no LRR[1]	no LRR[1]	76.4	92.4
3	F	112	178	+ 66	30.1	44.7	54.2	no LRR[1]	108.4	115.0	62.5	no LRR[1]
4	F	126	208	+ 82	no LRR[1]	51.3	no LRR[1]	42.7	no LRR[1]	123.4	no LRR[1]	132.5
Mean (F)		150	186	+ 36	36.8	42.0	54.9	43.6	145.9	144.7	95.4	123.8
S.D. (F)				55.5	9.4	10.9	6.3	0.9	53.0	34.3	45.9	28.0
S.E. (F)				27.8	6.7	7.7	3.6	0.5	37.6	25.6	26.5	16.2

[1]Animal did not lose the righting reflex.

TABLE 15. SUMMARY OF INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200mg/kg., I.P.). (STUDY II-1).

Treatment	Sex and No. of Animals	Induction Time (min.) I	II	III	IV	Duration of Hypnosis (min.) I	II	III	IV
Stress	M (4)	59.5[1] (9.4)	39.7 (9.4)	55.2 (10.0)	49.3 (2.1)	155.7 (9.2)	100.3 (25.4)	123.3 (21.4	82.9 (16.7)
Stress	F (4)	44.8 (8.6)	37.3 (6.8)	38.2 (0.07)	39.4 (3.8)	239.7 (55.3)	161.5 (40.5)	141.7 (28.6)	116.8 (41.5)
Control	M (4)	49.2 (3.8)	43.4 (6.7)	53.0 (1.3)	56.6 (10.9)	237.1 (29.1)	190.9 (40.2)	83.3 (9.8)	90.4 (31.7)
Control	F (4)	36.8 (6.7)	42.0 (7.7)	54.9 (3.6)	43.6 (0.5)	145.9 (37.6)	144.7 (25.6)	95.4 (26.5)	123.8 (16.2)

[1]Mean (± standard error in parenthesis).

TABLE 16. INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.) IN RATS AFTER REMOVAL FROM 14 DAY RESTRICTION OF MOVEMENT STRESS. INITIAL DOSE GIVEN AT 50 HOURS AFTER REMOVAL FROM RESTRICTION STRESS.

Rat No.	Sex	Body Weight (grams) D-1	D-15	Change	Induction Time (min.) I	II	III	IV	Duration of Hypnosis (min.) I	II	III	IV
1	M	174	-	-	-	-	75.7	75.1	-	-	79.3	66.9
2	M	182	196	+14	-	-	63.1	no LRR[1]	-	-	123.9	no LRR[1]
3	M	148	226	+78	-	-	81.6	no LRR[1]	-	-	61.7	no LRR[1]
4	M	156	254	+98	-	-	60.6	57.2	-	-	142.9	126.8
5	M	160	250	+90	-	-	59.8	68.9	-	-	104.4	97.5
6	M	172	238	+66	-	-	40.6	55.2	-	-	163.6	117.4
Mean	(M)	165	233	+69	-	-	63.6	64.1	-	-	112.6	102.2
S.D.	(M)			33.1			14.3	9.5			38.0	26.5
S.E.	(M)			14.8			5.8	4.8			15.5	13.3
1	F	154	200	+46	-	-	57.1	42.7	-	-	205.7	162.5
2	F	160	205	+35	-	-	no LRR[1]	41.7	-	-	no LRR[1]	105.6
3	F	146	100	+54	-	-	42.8	51.4	-	-	206.8	226.5
4	F	155	208	+43	-	-	41.4	50.7	-	-	133.1	169.9
5	F	154	174	+20	-	-	59.9	64.3	-	-	35.7	53.9
6	F	127	180	+53	-	-	48.0	36.4	-	-	35.7	53.9
Mean	(F)	149	195	+42	-	-	49.8	47.9	-	-	158.0	143.1
S.D.	(F)			12.8			8.3	9.9			75.5	59.0
S.E.	(F)			5.2			3.7	4.0			33.8	24.1

[1]Animal did not lose the righting reflex.

TABLE 17. INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.) IN RATS AFTER REMOVAL FROM MAINTENANCE IN THE STRESS-FREE ROOM FOR 14 DAYS. INITIAL DOSE GIVEN AT 50 HOURS AFTER REMOVAL FROM "STRESS-FREE" ROOM.

Rat No.	Sex	Body Weight (grams)			Induction Time (min.)				Duration of Hypnosis (min.)			
		D-1	D-15	Change	I	II	III	IV	I	II	III	IV
1	M	132	285	+153	-	-	53.7	no LRR[1]	-	-	225.5	no LRR[1]
2	M	134	284	+150	-	-	44.6	105.2	-	-	120.9	72.7
3	M	150	310	+160	-	-	50.6	no LRR[1]	-	-	320.8	no LRR[1]
4	M	150	284	+134	-	-	59.7	49.5	-	-	135.2	175.6
5	M	160	282	+122	-	-	55.4	48.8	-	-	129.4	80.9
6	M	138	290	+152	-	-	37.7	38.5	-	-	318.3	250.0
Mean	(M)	144	289	+145	-	-	50.3	60.5	-	-	208.4	144.8
S.D.	(M)			14.2			8.0	25.4			94.0	84.4
S.E.	(M)			5.8			3.3	12.7			38.4	42.2
1	F	130	220	+ 90	-	-	48.3	54.3	-	-	314.6	139.9
2	F	138	232	+ 94	-	-	41.6	*	-	-	259.4	*
3	F	114	208	+ 94	-	-	41.0	37.4	-	-	239.7	238.8
4	F	120	208	+ 88	-	-	49.5	no LRR[1]	-	-	239.4	no LRR[1]
5	F	140	252	+112	-	-	42.4	35.2	-	-	153.6	164.5
6	F	125	260	+135	-	-	52.9	80.8	-	-	292.1	71.8
Mean	(F)	128	230	+102	-	-	46.0	51.9	-	-	253.1	153.8
S.D.	(F)			18.2			5.0	21.0			55.6	69.0
S.E.	(F)			7.4			2.0	10.5			22.7	34.5

[1]Animal did not lose the righting reflex.

[2]Animal escaped.

TABLE 18. SUMMARY OF INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.). INITIAL DOSE GIVEN AT 50 HOURS AFTER REMOVAL FROM STRESS OR "STRESS-FREE" ROOM. (STUDY II-2).

Treatment	Sex and No. of Animals	Induction Time (min.) I	II	III	IV	Duration of Hypnosis (min.) I	II	III	IV
Stress	M (6)	-	-	63.6[1] (5.8)	64.1 (4.8)	-	-	112.6 (15.5)	102.2 (13.3)
Stress	F (6)	-	-	49.8 (3.7)	47.9 (4.0)	-	-	158.0 (33.8)	143.1 (24.1)
Control	M (6)	-	-	50.3 (3.3)	60.5 (12.7)	-	-	208.4 (38.4)	144.8 (42.2)
Control	F (6)	-	-	46.0 (2.0)	51.9 (10.5)	-	-	253.1 (22.7)	153.8 (34.5)

[1]Mean (± standard error in parenthesis).

TABLE 19. INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.) IN RATS AFTER REMOVAL FROM 14 DAY RESTRICTION OF MOVEMENT STRESS. ANIMALS SUBJECTED TO 2.5 HOURS OF HINDLEG LIGATION STRESS PRIOR TO DOSE III. INITIAL DOSE GIVEN AT 50 HOURS AFTER REMOVAL FROM RESTRICTION STRESS. SKF-525A (50 mg/kg ., I.P.) ADMINISTERED 4 HOURS BEFORE BOTH BARBITAL INJECTIONS. (STUDY III-1).

Rat No.	Sex	Body Weight (grams) D-1	D-15	Change	Induction Time (min.) I	II	III	IV	Duration of Hypnosis (min.) I	II	III	IV
1	M	160	241	+81	-	-	42.2	38.3	-	-	154.4	269.8
2	M	140	218	+78	-	-	62.4	34.8	-	-	112.0	289.8
3	M	184	244	+60	-	-	66.5	27.5	-	-	209.5	202.8
4	M	234	276	+42	-	-	42.5	19.0	-	-	258.3	450.6
5	M	194	278	+84	-	-	41.6	18.4	-	-	326.9	450.1
6	M	222	228	+66	-	-	41.0	34.4	-	-	136.6	189.6
7	M	194	238	+44	-	-	75.5	37.3	-	-	155.2	322.4
Mean	(M)	190	255	+65	-	-	53.1	30.0	-	-	193.3	310.7
S.D.	(M)			17.2			14.6	8.4			76.5	106.0
S.E.	(M)			6.5			5.5	3.2			29.0	40.1
1	F	145	204	+ 59	-	-	39.4	26.4	-	-	223.3	266.5
2	F	148	204	+ 56	-	-	38.9	25.5	-	-	159.6	414.7
3	F	134	172	+ 38	-	-	31.8	47.1	-	-	128.3	70.1
4	F	128	188	+ 60	-	-	30.9	23.4	-	-	122.2	189.7
5	F	114	194	+ 70	-	-	35.4	22.6	-	-	239.6	398.2
Mean	(F)	136	192	+ 57	-	-	35.3	29.0	-	-	174.6	267.8
S.D.	(F)			11.7			3.9	10.2			54.1	144.5
S.E.	(F)			5.3			1.8	4.6			24.2	64.4
Mean	(T)	167	229	+ 62	-	-	45.7	29.6	-	-	185.5	292.9
S.D.	(T)			15.2			14.4	8.8			66.0	119.3
S.E.	(T)			4.4			4.2	2.5			19.1	34.5

TABLE 20. INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.) IN RATS AFTER REMOVAL FROM 14 DAY RESTRICTION OF MOVEMENT STRESS. INITIAL DOSE GIVEN AT 50 HOURS AFTER REMOVAL FROM RESTRICTION STRESS. SKF-525A (50 mg/kg., I.P.) ADMINISTERED 4 HOURS BEFORE BOTH BARBITAL INJECTIONS. (STUDY III-2).

Rat No.	Sex	Body Weight (grams)			Induction Time (min.)				Duration of Hypnosis (min.)			
		D-1	D-15	Change	I	II	III	IV	I	II	III	IV
1	M	160	234	+ 74	-	-	52.3	24.9	-	-	219.0	427.3
2	M	160	248	+ 88	-	-	39.2	28.1	-	-	107.0	393.5
3	M	164	222	+ 58	-	-	104.1	24.3	-	-	225.7	262.6
4	M	160	238	+ 78	-	-	49.7	23.2	-	-	257.5	253.2
Mean	(M)	161	236	+ 75	-	-	61.3	25.1	-	-	202.3	334.2
S.D.	(M)			12.5	-	-	29.1	2.1	-	-	65.7	89.1
S.E.	(M)			6.3	-	-	14.6	1.1	-	-	32.9	44.6
1	F	162	214	+ 52	-	-	43.7	19.8	-	-	137.0	415.8
2	F	148	296	+148	-	-	42.8	19.1	-	-	171.0	290.6
3	F	118	166	+ 48	-	-	32.8	22.4	-	-	290.6	145.2
4	F	124	176	+ 52	-	-	25.8	19.0	-	-	139.3	329.7
5	F	138	180	+ 42	-	-	38.8	47.5	-	-	219.6	175.3
Mean	(F)	138	206	+ 68	-	-	36.8	25.6	-	-	191.5	271.3
S.D.	(F)			44.7			7.5	12.4			64.6	111.5
S.E.	(F)			19.9			3.4	5.5			28.9	49.9
Mean	(T)	148	219	+ 71	-	-	47.7	25.4	-	-	196.3	299.2
S.D.	(T)			32.9			22.6	8.8			61.1	100.3
S.E.	(T)			10.9			7.5	2.9			20.4	33.5

TABLE 21. NOREPINEPHRINE LEVELS IN RATS MAINTAINED IN "NON-STRESS" CONDITIONS.

Rat No.	Sex	Body Weight (grams)	Brain Weight (grams)	Norepinephrine (ug/g.br)
1	M	220	1.814	0.339
2	M	220	1.645	0.278
3	M	220	1.606	0.299
4	M	225	1.614	0.306
5	M	230	1.666	0.339
6	M	235	1.629	0.353
Mean	(M)	225	1.663	0.319
S.D.	(M)		0.077	0.032
S.E.	(M)		0.032	0.013
1	F	260	1.647	0.328
2	F	205	1.700	0.340
3	F	205	1.619	0.322
Mean	(F)	223	1.655	0.330
S.D.	(F)		0.046	0.009
S.E.	(F)		0.026	0.005
Mean	(T)	224	1.660	0.323
S.D.	(T)		0.065	0.023
S.E.	(T)		0.022	0.008

TABLE 22. NOREPINEPHRINE LEVELS IN RATS SUBJECTED TO 14 DAYS RESTRICTION OF MOVEMENT STRESS. MEASURED AT 2 HOURS AFTER REMOVAL FROM STRESS.

Rat No.	Sex	Body Weight (grams) D-1	D-15	Change	Brain Weight (grams)	Norepinephrine (ug/g.brain)
1	M	152	220	+ 68	1.601	0.362
2	M	130	208	+ 78	1.562	0.378
3	M	128	230	+102	1.693	0.365
Mean	(M)	137	219	+ 83	1.619	0.368
S.D.	(M)				0.067	0.009
S.E.	(M)				0.039	0.005
1	F	128	170	+ 42	1.567	0.402
2	F	160	208	+ 48	1.494	0.402
3	F	145	186	+ 41	1.631	0.353
Mean	(F)	144	188	+ 44	1.564	0.396
S.D.	(F)				0.069	0.028
S.E.	(F)				0.039	0.016
Mean	(T)	141	204	+ 63	1.591	0.377
S.D.	(T)				0.068	0.021
S.E.	(T)				0.028	0.009

TABLE 23. NOREPINEPHRINE LEVELS IN RATS SUBJECTED TO 14 DAYS RESTRICTION OF MOVEMENT STRESS. MEASURED AT 26 HOURS AFTER REMOVAL FROM STRESS.

Rat No.	Sex	Body Weight (grams) D-1	D-15	Change	Brain Weight (grams)	Norepinephrine (ug/g.brain)
1	M	156	236	+ 80	1.572	0.431
2	M	130	190	+ 60	1.598	0.425
Mean	(M)	143	213	+ 70	1.585	0.428
S.D.	(M)				0.018	0.004
S.E.	(M)				0.013	0.003
1	F	134	188	+ 54	1.622	0.358
2	F	130	176	+ 46	1.572	0.392
Mean	(F)	132	182	+ 50	1.597	0.375
S.D.	(F)				0.035	0.024
S.E.	(F)				0.025	0.017
Mean	(T)	138	198	+ 60	1.591	0.402
S.D.	(T)				0.024	0.034
S.E.	(T)				0.012	0.017

TABLE 24. SUMMARY OF MEAN NOREPINEPHRINE CONCENTRATION IN "NON-STRESSED" RATS, AND STRESSED RATS AT 2 AND 26 HOURS AFTER REMOVAL FROM STRESS. (STUDY IV-1).

Rat Treatment	Brain Weight (grams)	Norepinephrine (ug/g.brain)
Non-stress		
M (6)	1.663	0.319 (0.013)[1]
F (3)	1.655	0.330 (0.005)
T (9)	1.660	0.323 (0.008)
Stress (2 hours)		
M (3)	1.619	0.368 (0.005)
F (3)	1.564	0.386 (0.016)
T (6)	1.591	0.377 (0.009)
Stress (26 hours)		
M (2)	1.585	0.428 (0.003)
F (2)	1.597	0.375 (0.017)
T (4)	1.591	0.402 (0.017)

[1]Mean (± standard error)

TABLE 25. INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.) IN RATS MAINTAINED IN "NON-STRESS" CONDITIONS. NOREPINEPHRINE LEVELS WERE MEASURED AT THE TIME OF REGAINING OF THE RIGHTING REFLEX.

Rat No.	Sex	Body Weight (grams)	Induction Time (min.)	Duration of Hypnosis (min.)	Brain Weight (grams)	Norepinephrine (ug/g.brain)
1	M	220	40	220	1.641	0.380
2	M	214	38	200	1.723	0.304
3	M	222	51	241	1.658	0.380
4	M	220	37	167	1.664	0.380
5	M	210	47	132	1.753	0.367
Mean	(M)	217	42.6	190.0	1.688	0.362
S.D.	(M)		6.2	43.2	0.048	0.033
S.E.	(M)		2.8	19.4	0.021	0.015
1	F	200	48	156	1.620	0.375
2	F	218	42	183	1.857	0.399
3	F	206	42	196	1.763	0.345
4	F	208	44	275	1.649	0.405
5	F	208	33	249		
Mean	(F)	208	43.8	211.8	1.722	0.381
S.D.	(F)		2.5	48.9	0.109	0.027
S.E.	(F)		1.1	21.9	0.055	0.014
Mean	(T)	213	43.2	201.9	1.705	0.371
S.D.	(T)		4.5	44.8	0.077	0.030
S.E.	(T)		1.4	14.2	0.026	0.010

TABLE 26. INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.) IN RATS SUBJECTED TO 14 DAYS RESTRICTION OF MOVEMENT STRESS. ANIMALS INJECTED 2 HOURS AFTER REMOVAL FROM STRESS. NOREPINEPHRINE LEVELS MEASURED AT THE TIME OF REGAINING OF THE RIGHTING REFLEX.

Rat No.	Sex	Body Weight (g.) D-1	D-15	Change	Induction Time (min.)	Duration of Hypnosis (min.)	Brain Weight (grams)	Norepinephrine (ug/g.brain)
1	M	122	218	+ 96	55.1	96.6	1.740	0.399
2	M	135	238	+103	44.0	130.6	1.579	0.399
3	M	152	216	+ 64	73.1	81.7	1.724	0.360
Mean	(M)	136	224	+ 88	57.4	103.0	1.681	0.386
S.D.	(M)				14.5	25.0	0.089	0.023
S.E.	(M)				8.4	14.4	0.051	0.013
1	F	136	202	+ 66	47.6	158.1	1.672	0.455
2	F	120	150	+ 30	57.9	119.0	1.442	0.420
3	F	128	182	+ 54	51.3	131.0	1.644	0.362
Mean	(F)	128	178	+ 50	52.3	136.0	1.586	0.412
S.D.	(F)				5.2	20.1	0.126	0.047
S.E.	(F)				3.0	11.6	0.072	0.027
Mean	(T)	132	201	+ 69	54.8	119.5	1.634	0.399
S.D.	(T)				9.2	27.2	0.110	0.036
S.E.	(T)				3.8	11.1	0.045	0.015

TABLE 27. INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.) IN RATS SUBJECTED TO 14 DAYS RESTRICTION OF MOVEMENT STRESS. ANIMALS INJECTED 2 HOURS AFTER REMOVAL FROM STRESS. NOREPINEPHRINE LEVELS MEASURED AT 26 HOURS AFTER STRESS.

Rat No.	Sex	Body Weight (g.) D-1	D-15	Change	Induction Time (min.)	Duration of Hypnosis (min.)	Brain Weight (grams)	Norepinephrine (ug/g.brain)
1	M	126	153	+ 27	59.9	271.8	1.482	0.477
2	M	150	230	+ 80	68.9	77.0	1.516	0.368
3	M	120	210	+ 90	67.6	40.9	1.740	0.345
Mean	(M)	132	198	+ 66	65.8	129.9	1.579	0.397
S.D.	(M)				4.9	124.5	0.140	0.051
S.E.	(M)				2.4	71.6	0.081	0.029
1	F	130	178	+ 48	57.6	154.8	1.622	0.362
2	F	132	190	+ 58	57.3	91.8	1.422	0.438
3	F	132	170	+ 38	42.3	243.3	1.657	0.391
Mean	(F)	131	179	+ 48	52.4	163.3	1.567	0.397
S.D.	(F)				8.6	76.0	0.174	0.038
S.E.	(F)				5.0	43.8	0.100	0.022
Mean	(T)	132	189	+ 59	146.6	146.6	1.573	0.397
S.D.	(T)			9.6		94.0	0.120	0.051
S.E.	(T)			3.9		38.4	0.049	0.021

TABLE 28. INDUCTION TIME AND DURATION OF HYPNOSIS OF BARBITAL SODIUM (200 mg/kg., I.P.) IN RATS SUBJECTED TO 14 DAYS RESTRICTION OF MOVEMENT STRESS. ANIMALS INJECTED AT 2 HOURS (I) AND 26 HOURS (II) AFTER STRESS. NOREPINEPHRINE LEVELS MEASURED AT REGAINING OF THE RIGHTING REFLEX OF DOSE II.

Rat No.	Sex	Body Weight (g.) D-1	D-15	Change	Induction Time I	II	Duration of Hypnosis I	II	Brain Weight (grams)	Norepinephrine (ug/g.brain)
1	M	125	214	+ 89	57.1	74.0	87.9	10.0	1.808	0.420
2	M	130	170	+ 40	56.5	73.0	105.9	10.0	1.562	0.415
3	M	130	220	+ 90	80.0	no LRR[1]	71.0	no LRR[1]	1.801	0.306
4	M	120	224	+104	44.7	72.0	67.9	61.0	1.687	0.389
Mean	(M)	126	207	+ 81	59.6	73.0	83.2	27.0	1.715	0.383
S.D.	(M)				14.8	1.0	17.1	29.4	0.116	0.064
S.E.	(M)				7.4	0.6	8.6	16.9	0.058	0.032
1	F	140	204	+ 64	45.4	47.0	105.0	64.0	1.766	0.437
2	F	135	164	+ 29	60.5	40.0	83.6	111.0	1.651	0.425
3	F	125	188	+ 63	54.3	49.0	89.7	28.0	1.448	0.354
4	F	140	166	+ 26	43.6	39.0	123.0	147.0	1.618	0.403
Mean	(F)	135	181	+ 46	51.0	44.0	106.3	81.5	1.621	0.405
S.D.	(F)				7.7	5.0	28.8	43.7	0.132	0.037
S.E.	(F)				3.9	2.5	14.4	21.9	0.066	0.018
Mean	(T)	131	194	+ 63	56.5	56.3	94.8	58.1	1.655	0.394
S.D.	(T)				12.0	16.1	25.2	45.8	0.126	0.044
S.E.	(T)				4.2	6.1	12.1	18.0	0.045	0.015

TABLE 29. SUMMARY OF NOREPINEPHRINE CONCENTRATIONS AT REGAINING OF THE RIGHTING REFLEX FOR "NON-STRESSES" RATS AFTER ONE DOSE OF BARBITAL (200 mg/kg., I.P.) AND FOR STRESSED RATS AFTER ONE AND TWO DOSES OF BARBITAL.

Rat Treatment	Brain Weight (g.)	Induction Time (min.) I	Induction Time (min.) II	Duration of Hypnosis I	Duration of Hypnosis II	Norepinephrine (ug/g.brain)
Non-stressed' (one dose)						
M (5)	1.688	42.6[1] (2.8)	-	190.0 (19.4)	-	0.362 (0.015)
F (4)	1.722	43.8 (1.1)	-	211.8 (21.9)	-	0.381 (0.014)
T (9)	1.705	43.2 (1.4)	-	201.9 (14.2)	-	0.371 (0.010)
Stressed (one dose)						
M (3)	1.681	57.4 (8.4)	-	103.0 (14.4)	-	0.386 (0.013)
F (3)	1.586	52.3 (3.0)	-	136.0 (11.6)	-	0.412 (0.027)
T (6)	1.634	54.8 (3.8)	-	119.5 (11.1)	-	0.399 (0.015)
Stressed (two doses)						
M (4)	1.715	59.6 (7.4)	73.0 (0.6)	83.2 (8.6)	27.0 (16.9)	0.383 (0.032)
F (4)	1.621	51.0 (3.9)	44.0 (2.5)	106.3 (14.4)	81.5 (21.9)	0.405 (0.018)
T (8)	1.655	56.5 (4.2)	56.3 (6.1)	94.8 (12.1)	58.1 (18.0)	0.394 (0.015)

[1]Mean (± standard error)

CHAPTER III

DISCUSSION OF RESULTS

Study I: Effects of chronic and acute stress on barbital activity in the rat.

Study I-1: Tables 1, 2, and 3.

In the chronically stressed animals the following comparisons of sleeping time can be made: dose 2 duration was smaller than that of dose 1, ie., tolerance was exhibited; dose 3 sleeping time after acute stress was greater than that of dose 2, ie., hypersusceptibility was observed; and dose 4 duration was smaller than that of dose 3, ie., tolerance was again seen. Only the difference between the last two values was significant ($p<0.02$ for Total). The same relative differences in sleeping time were seen in the non-stressed group. However, none of these differences were significant. Earlier work in this laboratory (29, 61) has shown that the differences between durations of dose 1 and 2 described above are significant with larger sample sizes. Lee (60) has also shown that differences do exist between the sleeping times of doses 3 and 4.

Bousquet et al. (27) have shown in normal laboratory rats that acute hindleg ligation stress produces tolerance to hexobarbital and pentobarbital, but not to phenobarbital or barbital. In the present study, dose 3 after acute hindleg ligation resulted in hypersusceptibility to barbital. Dose 2 tolerance to barbital after chronic stress has not been shown by other workers.

Sleeping time for dose 3 (after acute stress) was the same as that of dose 1. The sleeping time for dose 4 (after chronic and acute stress) was lower than that of dose 2 (after chronic stress). Dose 4 duration represented 59.7% of dose 1, compared to dose 2 duration that was 89.5% of dose 1. Thus, dose 4 sleeping time showed greater tolerance compared to dose 1 than did dose 2 sleeping time.

Study I-2: Tables 4, 5, and 6.

The first dose of barbital, given on the second day after removal from stress or the stress-free room, gave a shorter sleeping time in prestressed animals ($p<0.05$ for males) compared to the (Total) first dose sleeping time of barbital in study I-1. The sleeping time of the second dose (day 3, after the acute stress) was greater than that on the previous day (55% greater in stressed males; 45% greater in stressed females; 124% greater in male non-stressed, $p<0.01$; 23% greater in female non-stressed). The

sleeping time of the third dose (day 4, after acute stress) was lower than that of dose 2 in both stressed and non-stressed animals (at least 44% lower in all groups; male non-stressed, $p < 0.025$).

Tolerance was exhibited on the second day after chronic stress, even though there was no previous dose of barbital. Hypersusceptibility to barbital was seen after the acute stress on day 3.

Study I-3: Tables 7, 8, and 9.

The chronically stressed rats were not given a dose of barbital until the third day after removal from chronic stress, one hour after the acute stress. The resulting first sleeping time on day 3 was only slightly shorter than that for dose 1 (day 1 after chronic stress) in study I-1. The second dose (day 4 after chronic and acute stress) showed shorter sleeping times than with the first dose (p .02 for Total). In the chronically and acutely stressed rats the second dose on day 4 showed a sleeping time not different from that of dose 4 of study I-1.

In the non-stressed animals given their first dose of barbital after the acute stress (day 3), the sleeping time (Total) was greater than that of the prestressed rats ($p < 0.05$) and greater than that of the prestressed and non-stressed animals in study I-1 for dose 3 (20% greater than stressed, 46% greater than control). A second dose of barbital for the

non-stressed animals showed a 19% lower (Total) sleeping time.

Barbital doses on day 3 and 4 after chronic and then acute stress showed the two-day tolerance effect seen on days 1 and 2 after chronic stress, and days 3 and 4 after chronic and acute stress in study I-1. Thus, the acute stress can reverse the barbital tolerance previously seen after chronic stress.

Study I-4: Tables 10, 11, and 12.

The sleeping time (Total) of the first barbital dose given on day 4 (after chronic and acute stress) showed tolerance in both stressed and non-stressed animals compared with the first dose of barbital in study I-1 (25% less than stressed, 31% less than non-stressed). The prestressed males did not lose the righting reflex which is a sign of extreme tolerance. The barbital dose on day 5 gave a shorter sleeping time than that on day 4 for both stressed ($p<0.05$ for Total) and non-stressed animals.

A tolerance to barbital developed by the day after the acute stress (four days after chronic stress) even though no previous doses of barbital were given. Tolerance was previously shown to occur in these animals on the day after chronic stress with or without a prior dose of barbital.

Study II: Effects of chronic stress on barbital activity in the rat.

Study II-1: Tables 13, 14, and 15.

Each succeeding dose of barbital in both sexes of stressed and non-stressed animals exhibited greater tolerance (non-significant) every day, except for day 4 in the non-stressed animals and day 3 in the male stressed. There was a non-significant increase in sleeping time in the non-stressed animals on day 4 over that of day 3, but day 4 sleeping time was less than that of day 2 (males, $p<0.05$).

The greatest tolerance, compared to dose 1, was seen with sleeping time of the fourth dose of day 4 after chronic stress (males, $p<0.01$). The stressed males on day 3 had non-significantly greater sleeping time than on day 2. Chronic stress produced barbital tolerance after one dose and the tolerance increased when no acute stress was given.

In the stressed animals, the males showed somewhat lower sleeping time than the females every day. In the controls, this effect was not seen consistently. The third dose of barbital (day 3 after only chronic stress) of both sexes gave lower sleeping times than for the (Total) sleeping time of dose 3 (after acute stress) in study I-1 in prestressed animals ($p<0.05$ for males, 34% lower; 25% lower for females).

Non-stressed males and females with dose 3 also showed sleeping times lower than for the (Total) sleeping time of dose 3 (after acute stress) of non-

stressed animals in study I-1 (46% lower for males, $p<0.05$; 39% lower for females). Thus, the hypersusceptibility to barbital observed in prestressed rats given an acute stress on day 3 was due to the acute stress itself, since it was not seen if the acute stress was omitted.

Study II-2: Tables 16, 17, and 18.

Stressed rats of both sexes, given their first barbital dose on day 3 after chronic stress showed a lower sleeping time compared to the first dose (day 1 after chronic stress) in study II-1 (28% lower for males, $p<0.05$; 34% lower for females). The sleeping time was close to that seen with dose 3 (day 3 after chronic stress) of study II-1. The sleeping time of dose 2 on day 4 after chronic stress was slightly shorter than that of dose 1 (9% lower for males and females). On both days the males showed somewhat lower sleeping times than the females. In contrast to these observations, Wei and Wilson (28) showed an increase in sleeping time on day 3 after acute heat stress. However, that work was done with a different stress and with hexobarbital, which is metabolized by enzymes.

Non-stressed animals of both sexes had greater sleeping times than prestressed animals on the first dose (day 3) ($p<0.05$ for males and females). They

also showed lower sleeping times with dose 2 than with dose 1 (31% lower for males; 39% lower for females, $p<0.05$). Dose 1 sleeping time (day 3) was greater than dose 3 (day 3) sleeping time for non-stressed animals in study II-1 ($p<0.02$ for males, $p<0.005$ for females).

Tolerance to barbital was seen on day 3 after chronic stress, regardless of whether doses of barbital had been administered. Therefore, some action related to the stress itself caused the development of tolerance.

Study III: Effects of SKF-525 on barbital activity in chronically and acutely stressed rats.

Study III-1: Table 19.

In the prestressed animals given SKF-525A, the first dose of barbital (day 3 after chronic and acute stress) gave (Total) sleeping time greater, but not different from that of dose 1 (day 3 after chronic and acute stress) in study I-3. The second dose of SKF-525A and barbital gave a greater sleeping time ($p<0.01$ for Total, $p<0.05$ for males) and lower induction time ($p<0.005$ for Total, $p<0.01$ for males) compared to the previous dose. The sleeping time of dose 2 (day 4 after chronic and acute stress) in the SKF-525A pretreated animals was greater than that of dose 2 (day 4 after chronic and acute stress) in study I-3 ($p<0.001$ for Total, $p<0.05$ for males).

This increase in sleeping time after treatment with SKF-525A has been reported by Maxwell (68) to occur in non-stressed animals with pentobarbital and phenobarbital, but not with barbital. Previous work in this laboratory (29, 61, 58) has yielded the same increase in sleeping time as seen here, but with doses on days 1 and 2 after chronic stress.

Study III-2: Table 20.

The first dose of barbital (day 3 after chronic stress only in the SKF-525A pretreated animals gave a longer sleeping time than that of dose 1 (day 3 after chronic stress only) in study II-2 ($p<0.05$ for males). Tolerance to barbital was observed with this same treatment in study II-2, but SKF-525A has caused an increase in sleeping time here on the first dose.

Dose 2 of barbital and SKF-525A (day 4 after chronic stress) gave a greater sleeping time than that of dose 1 ($p<0.02$ for Total) and a shorter induction time ($p<0.02$ for Total). This second dose gave a greater sleeping time than that for dose 2 (day 4 after chronic stress) in study II-2 ($p<0.02$ for males). SKF-525A has been reported (68) to have no effect on barbital activity. Previously, it was shown that SKF-525A could affect the duration of the second dose of barbital. Here it is seen that the duration of the first dose can be prolonged.

Study IV: Effects of chronic stress and barbital hypnosis on norepinephrine concentration in the rat brain.

Study IV-1: Tables 21, 22, 23, and 24.

The (total) norepinephrine brain concentration of 0.377 mg norepinephrine/g brain in chronically stressed rats at 2 hours after removal from stress was greater than that of 0.323 mg norepinephrine/g brain in non-stressed rats ($p<0.005$ for Total, $p<0.02$ for males, $p<0.05$ for females). The level of 0.402 mg norepinephrine/g brain at 26 hours after chronic stress was also greater than that in the non-stressed animals ($p<0.02$ for Total, $p<0.001$ for males). The brain norepinephrine concentration at 26 hours after stress was greater than that at 2 hours after stress, in males only $9p<0.01$). It has been shown in this laboratory that at 26 hours after removal from chronic stress, the corticosterone level is greater in male rats than that during the chronic stress.

Several authors (43, 44, 45) have shown that acute stress will decrease rat brain norepinephrine concentration. Little work has been done on the effects of chronic stress on brain norepinephrine concentration, but an increase has been reported (69) after chronic stress. Chronic stress here resulted in an increase in brain norepinephrine concentration in rats. Kvetnansky (40) has shown that chronic

restraint stress results in an increased adrenal synthesis and excretion of epinephrine and norepinephrine. During stress the net blood levels of epinephrine increase and then decrease as the animals adapt to the stress. The animals in chronic stress had "adapted" to stress by increasing adrenal production and renal excretion of epinephrine.

Study IV-2: Tables 25 and 26.

The concentration of norepinephrine was 15% greater in non-stressed rats after one dose of barbital than that before the dose. The concentration was 0.323 ug norepinephrine/g brain at the time of injection, and was 0.371 ug norepinephrine/g brain at the time of regaining of the righting reflex (p 0.005 for Total, $p<.05$ for males, $p<0.05$ for females). In stressed animals norepinephrine concentration increased 6% (non-significant) after one dose of barbital. There was no difference in norepinephrine levels between stressed and non-stressed rats after one dose of barbital. These findings agree with those from others laboratories. Phenobarbital hypnosis has been shown (9, 45) to increase levels of norepinephrine and serotonin in the rat brain of normal laborators (non-stressed) animals.

The sleeping time of one non-stressed animals was longer than that of the stressed animals ($p<0.001$ for Total, $p<0.02$ for males, $p<0.05$ for females).

Study IV-3: Tables 24 and 27.

The stressed animals that had received one dose of barbital at 2 hours after stress and were sacrificed 26 hours after stress had the same level of norepinephrine as stressed animals sacrificed 2 hours after stress. The above barbital-treated-animals also had (total) norepinephrine levels equivalent to stressed (non-barbital-treated) animals 26 hours after stress.

Study IV-4: Tables 28 and 29.

Stressed animals that had received two doses of barbital had the same brain concentration of norepinephrine at the time of regaining of the righting reflex after dose 2 as stressed and non-stressed animals at the time of regaining of the righting reflex after one dose of barbital. A second dose of barbital in stressed rats did not raise the concentration of norepinephrine at the time of regaining of the righting reflex.

In the stressed animals, the sleeping time for dose 2 (day 2 after stress) was shorter than that for dose 1 in both sexes, but the difference was significant only in the males ($p<0.05$). The second day sleeping time in stressed animals was also lower than the sleeping time for dose 1 of the non-stressed animals ($p<0.001$ for Total, $p<0.005$ for males, $p<0.02$ for females). Tolerance to barbital was observed

with the second dose on day 2 after chronic stress.

In these studies of brain concentration of norepinephrine it was observed that the chronically stressed rats that had a greater level of norepinephrine than the non-stressed rats, at the time of barbital administration slept shorter lengths of time than the non-stressed rats.

CHAPTER IV

SUMMARY AND CONCLUSIONS

1. Tolerance to barbital was observed in male rats at 26, 50, and 74 hours after removal from chronic restriction stress. The tolerance was eliminated at 50 hours after chronic stress by application of acute hindleg ligation stress.

2. Two consecutive daily doses of Proadifen HCl and barbital produced hypersusceptibility to barbital on the second dose in both chronically stressed, and chronically and acutely stressed rats. Hypersusceptibility was seen with the first dose in chronically stressed male rats given a two day rest period after stress before injection.

3. Total brain norepinephrine concentration was greater in rats subjected to chronic restriction stress than in non-stressed rats. Total brain norepinephrine concentration was increased after barbital hypnosis in non-stressed rats. There was no change in norepinephrine concentration as a result of barbital hypnosis in chronically stressed rats. A

greater level of brain norepinephrine in the stressed rats resulted in a shorter sleeping time compared to non-stressed rats.

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