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Comparison of Hypothalamic Serotonergic Activity in Relation to Feeding and Strain Differences in Dietary Obesity Susceptible (OM) and Resistant (S5B/Pl) Rats

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COMPARISON OF HYPOTHALAMIC SEROTONERGIC ACTIVITY IN RELATION TO FEEDING AND STRAIN DIFFERENCES IN DIETARY OBESITY SUSCEPTIBLE (OM) AND RESISTANT (S5B/PL) RATS

By

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ABSTRACT

COMPARISON OF HYPOTHALAMIC SEROTONERGIC ACTIVITY IN RELATION TO FEEDING AND STRAIN DIFFERENCES IN DIETARY OBESITY SUSCEPTIBLE (OM) AND RESISTANT (S5B/PL) RATS

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Serotonergic stimulation has been linked with reduced energy intake in rats. Whether hypothalamic serotonergic activity measured by 5-HT production is greater in S5B/Pl (S) rats (dietary obesity resistant) than Osborne-Mendell (OM) rats (non-dietary obesity resistant) was tested. Each strain was divided into three groups: Fed Drug (pargyline, a MAO inhibitor), Non-Fed and Fed Sham. All rats were fed an energy-dense, high-fat diet and were adapted to eat a 2-hour meal every 24 hours. 5-HT accumulation was measured at 0, 20, 40, and 60 minutes post drug or sham injection to calculate 5-HT accumulation rate (b_1). S rats ate less food than the OM during a 20 minute period prior to killing ($p < 0.05$). Greater serotonergic activity was indicated in S Non-Fed rats (23.86 ng/g/min) than S Fed (12.08 ng/g/min) ($p < 0.10$), while the OM Non-Fed vs. Fed showed no difference (15.94 and 12.87 ng/g/min respectively) thus giving qualified support to the hypothesis.

Dedicated to

my parents.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Reports of the role of serotonin as a neurotransmitter that mediates satiety have become more common in the scientific and lay press recently. The relationship of serotonergic activity and food intake has been studied through two major routes: pharmacological manipulation of serotonergic activity and the effect of diet on serotonin (5-HT) levels in the brain. Before discussing these two aspects, basic 5-HT metabolism will be reviewed.

LITERATURE REVIEW

5-HT Metabolism

The precursor to serotonin, tryptophan (Trp), an essential amino acid, crosses the blood brain barrier by means of active transport (see Figure 1). Trp competes with other neutral amino acids (naa) for this transport so the rate at which it is carried into the neuron is dependent on the Trp:naa ratio (Wurtman et al., '76; Curson '81; Boadle-Biber, '82). Inside the neuron trptophan hydroxylase (TH) catalyzes the first step in the production of 5-HT by hydroxylation of Trp to form 5-HTP. Then an aromatic amino acid decarboxylase catalyzes the step to 5-hydroxytryptamine (5-HT). The step catalyzed by tryptophan hydroxylase is

Figure 1: Diagram of the Metabolic Pathways of 5-Hydroxytryptamine (5-HT)

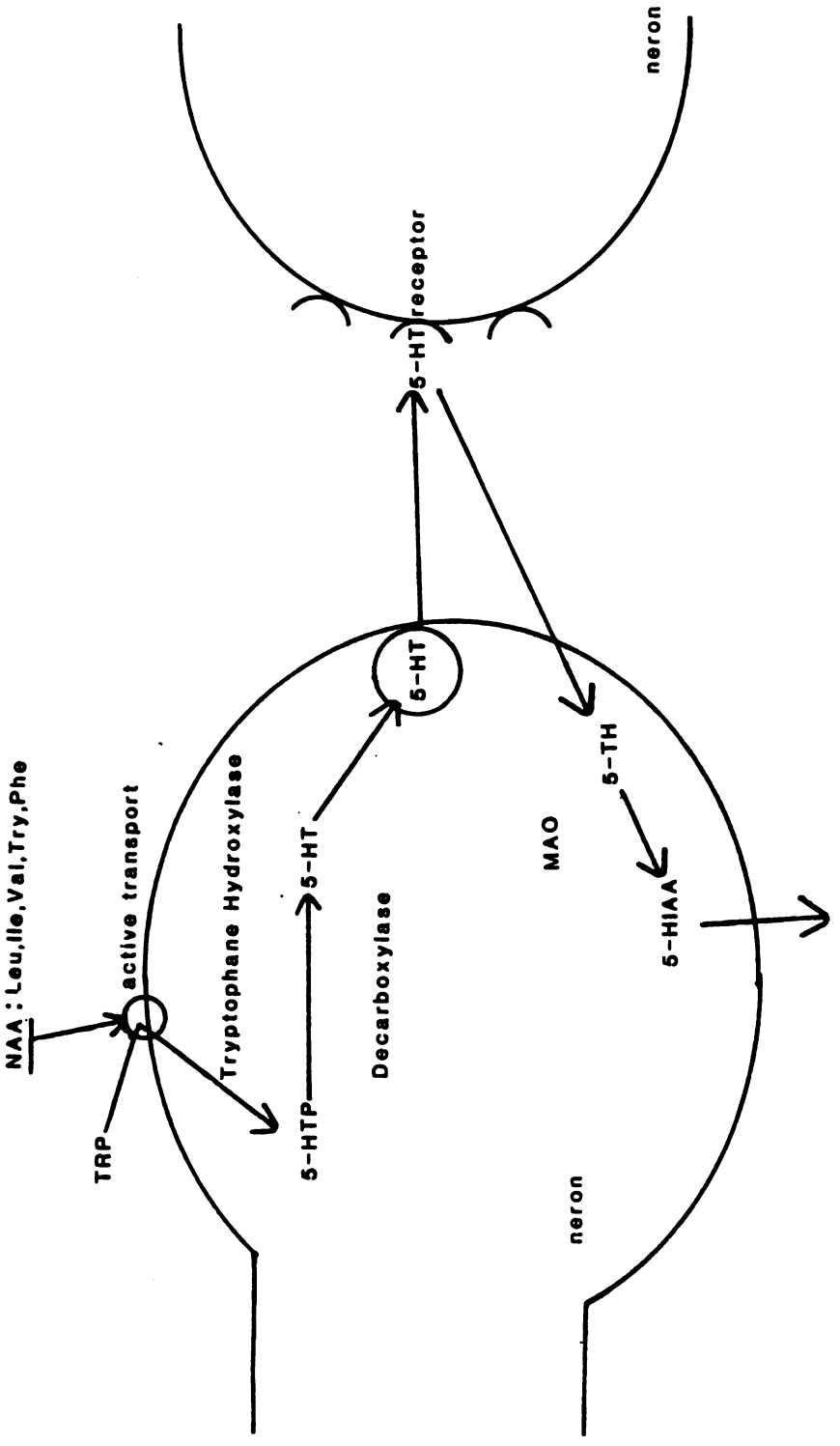
neron
5-HT receptor
5-HT
5-TH
MAO
5-HIAA
5-HT
Tryptophane Hydroxylase
5-HTP
Decarboxylase
active transport
NAA : Leu,Ile,Val,Try,Phe
TRP
neron

Figure 1

thought to be the rate limiting step and the synthesis rate is dependent on two factors: 1) substrate availability since the enzyme is not saturated at physiological levels, and 2) neuronal activity which can induce the tryptophan hydroxylase intrinsic activity (Hamond et al., '81; Boadle-Biber, '82). The 5-HT is transported to the neuron membrane where it is released upon depolarization into the synapse where it binds its receptor on an adjacent neuron. After 5-HT dissociates from the receptor, most of it is taken up again by the neuron and transformed by Monoamine Oxidase (MAO) to 5-hydroxyindole acetic acid (5-HIAA) which is then transported out of the neuron and away from the central nervous system.

Drug Studies

Fenfluramine, a prototypic serotonergic drug, meta-chlorophenylpiperazine and quipazine have a high affinity for 5-HT receptors and will cause dose dependent anorexia (Samanin et al., '80). Pretreatment with a 5-HT antagonist, methergoline, will reduce the effects of these drugs. Comparison of fenfluramine with amphetamine, which causes anorexia through catecholaminergic pathways, has indicated that two pathways may mediate satiety (Garattini, '81). Lesions of noradrenergic nerve terminals in the hypothalamus will block most of the effects of amphetamine but not those of fenfluramine (Garattini, '81). The reverse is true when serotonergic neurons are destroyed by raphe lesions. A comparison of amphetamine and fenfluramine anorexia by

Blundel et al. ('76) indicated that amphetamine affects hunger or the motivation to begin eating, while fenfluramine affects satiety or the motivation to discontinue feeding.

Blundel et al. ('76) measured the rate and frequency of food intake by rats given amphetamine or fenfluramine. Amphetamine delayed the onset of the first meal, while with fenfluramine, food intake began as usual but the meal size was reduced. Amphetamine-induced-delay of meal onset is interpreted as an effect on the initiation of feeding while fenfluramine induced decrease in meal size is interpreted as an effect on satiety. Burton et al. ('81) replicated the temporal pattern of fenfluramine with an apparatus similar to Blundel et al. ('76) that recorded the number of pellets consumed per minute and correlated observed feeding behavior changes with decreased intake. While Blundel et al. ('76) found that food intake decreased toward the end of the meal, they reported that food intake was decreased throughout the meal.

Grinker et al. ('79), in testing whether the Zucker obese rat has less precise control over food intake than the lean Zucker rat, found that amphetamine tended to reduce food intake by decreasing meal frequency, while fenfluramine tended to decrease meal size, but the differences were not significant because both drugs had some effect on each meal parameter.

Altogether, the studies on the temporal effects of fenfluramine vs. amphetamine provide a strong indication that serotonergic pathways may mediate food intake through

cessation of feeding or satiety, while catacholaminergic pathways may mediate food intake through feeding onset.

Brain Lesions or 5-HT Depletion. Depletion of brain 5-HT by use of drugs such as parachlorophenylalamine (p-CPA), 5-6-Dihydroxytryptophane (5-6-DHT), or 5-7-Dihydroxytryptophane (5-7-DHT) or by lesions can cause a reduction in satiety or hyperphagia in rats (Blundel, et al., '79) through these methods often show inconsistent results. For example, Ashley et al. ('79) used 5-7-DHT, p-CPA or thermal raphe lesions in rats and found that, given a choice of high or low protein intake, protein intake decreased with each treatment but energy intake was not affected. However, Waldbillig et al. ('81) gave microinfusions of D-7-DHT into the ventralateral hypothalamus as opposed to ventricular injections into the brain used by Anderson ('79) and found that rats consumed more kcals and gained more weight compared to controls when fed a high fat diet. Two possible reasons for the differences between these studies are 1) the 5-7-DHT was administered to a more specific area in the Waldbillig study and 2) the different diets given may have affected foods intake. Possibly, serotonergic pathways mediating energy intake were less able to respond to the calorie-dense diet used by Waldbillig while the less calorie-dense diet used by Ashley, et al. ('79) would not have challenged the pathways.

Serotonergic Stimulation and Food Choices. Wurtman and Wurtman ('77) allowed Sprague-Dawley rats to select food intake from two isocaloric diets that were high or low in

protein with equal amounts of fat. When these animals were given the drug fenfluramine, which enhances or stimulates serotonergic transmission, the rats decreased their energy intake by decreasing the food consumed from the high carbohydrate food cup while protein intake was maintained.

In a series of trials, the rats were presented with two food cups and the proportions of fat, protein and carbohydrate varied with each trial. When given fenfluramine, the rats always chose to decrease energy intake by decreasing intake from the food cups high in carbohydrate and maintained intake from the food cups high in protein regardless of the proportions (Wurtman & Wurtman, '79). When total grams of protein and carbohydrate and Kcals consumed from all cups in a trial were calculated, the protein intake was similar to control while energy intake and grams of carbohydrate decreased significantly in rats given fenfluramine. Changes in fat were not clearly reported and were implied to be incidental to carbohydrate and protein choices since the diets were made isocaloric by adjusting fat content. Furthermore, when the animals were given a choice between three cups where the fat level varied (1--high protein, high carbohydrate, moderate fat; 2--low protein, high carbohydrate, moderate fat; 3--low protein, low carbohydrate, high fat) they still decreased carbohydrate and maintained protein intake when given fenfluramine.

However, diet choices were not quite the same when Sprague-Dawley rats were presented with three food cups with pure rations of fat, protein and carbohydrate (each with an

appropriate amount of vitamins and minerals) and were given fenfluramine (Orthen-Gambill & Kanereck, '82). When the three rations were isocaloric, fenfluramine caused a reduction in energy intake by decreasing fat intake while carbohydrate and protein were not significantly reduced compared to controls. When the fat component was made more energy dense, energy intake was reduced by decreasing fat and protein intake. One other difference in the design of these two studies is that Orthen-Gambill gave three doses of fenfluramine, 1.5, 3.0, and 6.0 mg/kg, and averaged the response while Wurtman et al. ('79) only observed the effects of one dose at 2.5 mg/kg. The responses at higher doses in the Orthen-Gambill study showed the difference between the studies most distinctly.

These drug studies indicate that fenfluramine produces an anorectic effect through serotonergic pathways. However, whether the anorexia is mediated through an effect on fat, protein or carbohydrate selection is not clear and seems contingent on the types of diet presented. Studies which use dietary methods to affect serotonin levels to get a measure of serotonergic activity, present a different picture of serotonin's role in feeding.

Diet Studies

Correlation of plasma Trp to neutral naa ratio and brain 5-HT with feeding has indicated that brain levels of 5-HT are inversely related to protein intake while there is no relationship with energy intake (Anderson, '79). Woodger et

al. ('79) reported that when streptozotocin-induced diabetic rats and control rats were offered a choice of 10 and 60% casein diets and individual groups had 0, 1.45, and 4.55% Trp added to the diet, the protein intake of both diabetic and control rats was inversely related to plasma Trp:naa ratio, brain Trp, and brain 5-HT while no relationship was seen with energy intake. Therefore, increasing Trp consumption was associated with increased brain 5-HT and Trp, and decreased protein intake. It appears from this study that insulin sufficiency, which has an effect on Trp:naa is not essential for affecting changes in brain Trp and 5-HT from dietary influences. Woodger et al. concluded that the plasma amino acid ratio of Trp:naa plays a major role in affecting 5-HT synthesis although the results cannot be taken as a cause and effect relationship.

In a study of self-selected meal composition on the relation of circadian rhythms to plasma and brain Trp, and brain 5-HT, Li et al. ('82) found that plasma Trp:naa and brain Trp showed significant circadian rhythms. Comparison of these rhythms to corresponding food circadian choices indicated an inverse correlation to protein intake, while carbohydrate and energy consumption were not found to be correlated. In the second part of this study, the animals were sacrificed 20 minutes after a meal, plasma Trp:naa, and 5 hydroxyindole (the whole brain 5-HT and 5 HIAA quantities combined) were inversely related to protein intake. Li, et al. ('82) concluded that plasma Trp:naa ratio, brain Trp and 5 hydroxyindole concentrations are under continual influence

of food choice and may act as part of the feedback mechanism that regulates short-term food selection but not energy intake.

Studies that are similar to the above studies of Woodger et al. ('79) and Li et al. ('82) have not always produced consistent results. When there was an alteration in the amount of dietary fat, lean and obese (ob/ob) mice consumed different amounts of protein. Decreased plasma levels of Trp:naa was associated with increased protein intake (Chee et al., '81). In an extension of this study, Romsos et al. ('82) reported that, with a similar feeding regime, the different levels of long-term protein intake were not related to changes in brain 5-HT in the obese and lean (ob/ob) mice. When the diet was supplemented with Trp, no significant changes in protein and energy intake were observed. In a similar study by Peters & Harper ('81), where Sprague-Dawley rats were allowed to choose between isocaloric high and low protein diets, no correlation between long-term protein intake and brain 5-HT levels was found.

The reason for these inconsistent results within diet studies is hard to explain. Since all the studies assayed whole brain levels of Trp and 5-HT, it may be necessary to examine more discrete brain regions because it is possible that different nerve bundles may have different responses to the same stimuli.

Conclusions

The pharmacological studies and the dietary studies indicate that 5-HT has some role in the mediation of energy and macro nutrient intake. The inconsistencies within and between them point to the complex nature of the CNS and that the role of serotonergic pathways in food intake still needs to be clarified.

The two types of studies may produce inconsistent results because they have very different approaches, each with its own weakness. Drug studies show gross effects of serotonergic stimulation or depletion which may produce an unphysiological state, while diet studies only show a correlation of dietary intake and brain levels of 5-HT, plasma Trp:naa ratio and brain Trp levels. Basic physiological theory suggests that brain levels of a neurotransmitter or its precursor are not reliable indicators of neuron activity or of the amount of transmitter released into the synapse (Henry & Ternaux, '81; Boadle-Biber, '82; Curzon, '81). Wistar rats injected with p-chlorophenylalanine (p-CPA), which inhibits the synthesis of 5-HT, were found one month later to have the same resting brain brain 5-HT levels as control animals (Alexander, et al., '80). When the rats were injected with a MAO inhibitor, the accumulation of brain 5-HT for rats injected with p-CPA was much less than controls. This could indicate that, even with different synthesis rates, the neuron maintains a certain level of neurotransmitter and the same amount of 5-HT would be released into the synapse. To present conclusive findings on

the role of serotonin in food intake, a more direct measure of neuron activity than base 5-HT levels is needed.

AN APPROACH TO ASSESSING SEROTONERGIC ACTIVITY

Serotonergic Activity and Feeding

No study reviewed to date has measured the effect of feeding on serotonergic neuron activity which is the key to linking a neuro-pathway to a physical event. Two studies comparing 5-HT levels in laboratory animals with different food intakes have been reviewed.

Different 5-HT levels in obese Zucker rats, which greater or smaller were hyperphagic, and non-obese Zucker rats were found in the mesencephalon but not in the hypothalamus, cortex, hippocampus, corpus striatum, remaining diencephalon, pons-medulla or cerebellum of ____ Zucker (Finkelstein et al., '82). Trp was decreased in the cortex, hippocampus, corpus striatum, hypothalamus and diencephalon. Also, the free plasma Trp was reduced in the obese rat and was cited as a possible cause for the reduction in brain Trp. The association of decreased 5-HT levels in the mesencephalon, the Trp in several brain areas in the obese Zucker rat indicates little about actual serotonergic activity.

Genetically obese hyperglycemic mice had increased whole brain 5-HT, total plasma Trp and total plasma-free Trp (Garthwaite, et al., '79). These findings are hard to compare with the findings of Finkelstein, et al. ('79) because 5-HT was assayed in the whole brain and

hyperglycemic mice were used as opposed to rats. Also, food intakes for the mice were not reported in the Garthwaite study. This study, like Finkelstein's et al. ('82), can only associate 5-HT levels with obesity and differences in food intake.

The Dietary Model of Obesity

The dietary obesity model (Mickelsen et al., 1955) was used here to determine if serotonergic activity has any relation to energy intake. Evaluation of several strains of rats for obesity has provided two strains, the Osborne Mendel (OM) and the S 5B/P1 (S) which respond differently to a high fat diet (Schemmel et al., '70). When fed a high fat diet, OM rats consumed more kcals, retained more energy per kcal consumed and gained more weight than S rats (Schemmel et al., '72; Schemmel & Mickelsen, '73). When both strains were fed a high carbohydrate diet--less calorically dense than the high fat diet--the differences in food intake and weight gain were much less (Schemmel, '70). In view of serotonin's role in appetite control, the different response to caloric density between strains presents the question: Do the two strains have different serotonergic activity that can be associated with their different food intakes?

Measuring Serotonergic Activity

All the studies reviewed here, which examined the relation of serotonin to some aspect of food intake, used either indirect approaches to measure serotonergic activity or observed feeding behavior under the influence of

pharmacologic serotonergic stimulation. More direct methods for measuring neuron activity, which involve steady state and non-steady state techniques, are now being used. Steady state methods use radioactive labeling of 5-HT, 5-HTP, or Trp, or a stable isotope of O_2 to follow the production of 5-HT and 5-HIAA (Neckers, '82). Non-steady state methods involve inhibition of a metabolic step in 5-HT production or catabolism (see Figure 1) and use the relative rate of increase or decrease of a 5-HT metabolite as an indicator of production or breakdown which are in part related to serotonergic activity (Hamon, et al., '81).

In this study, MAO inhibition by pargyline was chosen as the method to assess serotonergic activity because it is as accurate a measure of 5-HT production as other non-steady state methods (Morot-Gaundry et al. '74). Comparison of pargyline with pheniprazine, another MAO inhibitor, produced similar rates of 5-HT accumulation in the mouse brain 10 minutes after drug injection. Morot-Gaundry et al. ('74) concluded that the two drugs produced the same response and that, though the drugs tested may not provide an accurate estimate of actual 5-HT turnover, they can be used to estimate relative changes in 5-HT turnover in various treatment conditions.

MAO inhibition with pargyline is rapid and causes a linear accumulation of 5-HT for at least the first 60 minutes after injection (Johnson & Crowley, '82; Morot-Gaundry et al., '74). Using another inhibitor that acts on decarboxylase production of 5-HT from 5-HTP (see Figure 1) would

produce a linear accumulation of 5-HTP at a rate similar to MAO inhibition. However, Morot-Gaundry et al. ('74) found that the response stays linear for only 30 minutes.

VanLoon et al. ('81) compared four methods of 5-HT turnover estimation in the hypothalamus: MAO inhibition induced increase of 5-HT and decrease of 5-HIAA, 5-HTP accumulation after decarboxylase inhibition with m-hydroxybenzylhydrazine and accumulation of 5-HIAA after probenecid induced block of 5-HIAA transport at one time point post injection. The four methods showed very similar estimates, though 5-HIAA accumulation from probenecid transport inhibitor was lowest but consistent with the other accumulation of disappearance rates. Though no linear accumulation measure was one, this suggests that MAO inhibition is a useful and a state of the art tool for assessing 5-HT accumulation.

So MAO inhibition by pargyline was used in this study since it provides a longer 5-HT accumulation time which would help to show relative differences in accumulation rates, as well as allow for easier sample collection and since it is as effective as pheniprazine. The major assumptions in using pargyline as an MAO inhibitor are that complete and rapid inhibition of MAO occurs; that accumulated 5-HT does not diffuse away from the CNS; that 5-HTP decarboxylation is not significantly inhibited; and that significant end product inhibition does not occur within 60 minutes.

A relative indication of serotonergic activity can be detected through a measure of 5-HT production since there is good evidence that tryptophan hydroxylase (TH) activity and possibly tryptophan uptake can be induced with increased serotonergic activity (Hamon, et al., '81; Henry & Ternaux, '81). Since TH is not saturated at physiological levels, the intrinsic activity of the carrier could play a role in 5-HT synthesis. Hamon et al. ('81) notes that changes in 5-HT synthesis, due to changes in brain Trp levels, may happen independently of changes in plasma Trp:naa because the carrier rate can be induced by a drug, dibutyrylcyclic-AMP, and because normal circadian fluctuation in 5-HT synthesis observed using brain slices are associated with fluctuations in the intrinsic activity of the carrier. TH activity is thought to be induced from nerve depolarization which allows Ca^2 influx resulting in activation of a protein kinase. The protein kinase may then activate the enzyme or a cofactor (Hamon, et al., '81; Boadle-Biber, '82). Whether the Trp uptake or TH is stimulated, the net result would be observed in 5-HT accumulation. One possible disadvantage of relying on 5-HT production to indicate neuron activity is that the uptake and TH intrinsic rates may not always be induced in the same direction, though there are indications that the Trp carrier activity and TH intrinsic rate may be induced simultaneously. Hamon et al. ('81) found that the same substance which stimulates the Trp carrier also stimulates TH. This gives support to the hypothesis that TH and the carrier may be affected in the same manner under a given

condition. So, 5-HT production is used here as a relative measure of TH activity which in turn reflects neuron activity.

The hypothalamus was chosen as the region to assay for 5-HT activity since it has been shown to have major control over feeding behavior and has a high amount of serotonergic nerve terminals (Leibowitz, '80).

Objectives

The overall objective of this study was to examine the relationship of serotonergic activity to satiety and food, or energy, intake. The major hypothesis to be tested was that when fed an energy dense (i.e., high fat) diet, the S rat will eat less and have a greater serotonergic activity in response to feeding than the OM rat. A secondary hypothesis was that serotonergic activity will increase in response to feeding.

MATERIALS AND METHODS

The rats were male Osborne-Mendell (OM) and S5B/P1 (S) that were 7 to 8 weeks of age at the time of sacrifice. Both strains of rats were obtained from a breeding colony at MSU, FSHN. After weaning, at 21-24 days, the animals were housed in suspended individual metal cages and kept in an animal room lighted from 0700 to 1900. All animals had chow available while in litters, then at 21-24 days they were weaned and fed a high fat diet (Table 1) (Schemmel, et al. '82). After one week of ad libitum feeding of the high fat diet, the food cups were placed in the cages each day at 1900 and removed from the cages each day at 2100 to give a two-hour feeding period at the beginning of the dark cycle. The rats were meal fed for approximately 2 1/2 weeks before killing and 5-HT assay.

Experimental Design

Three factors were involved in the experimental design: Strain, Fed State and Drug, which results in eight possible groups (Figure 2). The Fed rats were given their food cups for the first 20 minutes of their usual feeding period. Then, they were injected with vehicle or pargyline within 10 minutes after feeding. The Non-Fed rats were not allowed to feed prior to injection. Thus, they were killed after having been not fed for the customary 22 hours.

TABLE 1

Composition of the high fat diet

High Fat Diet	Wt/100 gm
casein[a]	32.0
vitamin mix[b]	1.5
cellulose[c]	3.0
mineral mix[d]	6.0
dl methionine	0.4
cerelose[e]	7.1
corn oil	5.0
hydrogenated shortening	45.0[f]
6.06 kcal/g	

	% of kcal
pro	21
CHO	5
fat	74

[a]High protein casein, purchased from Teklad Test Diets, Madison, WI.
[b]A.O.A.C. purchased from Tekled Test Diets, Madison, WI.
[c]Purchased from Teklad Test Diets, Madison, WI.
[d]AIN-76, purchased from Teklad Test Diets, Madison, WI.
[e]Purchased from Corn Industry Division of CPC International Co., Englewood, NJ.
[f]Crisco, purchased from Procter and Gamble, Cincinnati, OH.

Figure 2: Illustration of Cross Classified Factors in Experimental Design

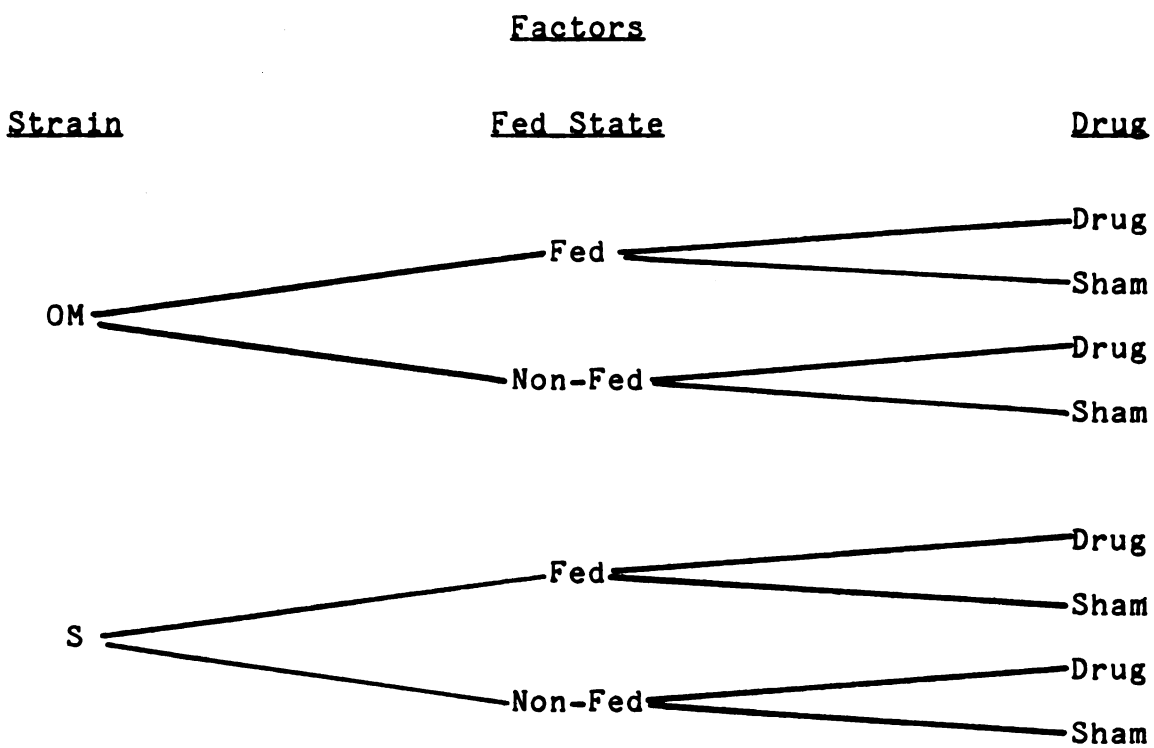
Factors
Strain
Fed State
Drug
OM
Fed
Drug
Sham
Non-Fed
Drug
Sham
S
Fed
Drug
Sham
Non-Fed
Drug
Sham

Figure 2

Food Intake

Each animal was assign a food cup. The cups were weighted at least twice a week and whenever diet was added to the cups. Food intake was measured in terms of grams of food removed from the cup. Due to the solid nature of the high fat diet, diet was not lost due to spillage. Average food intake per day for an individual rat was calculated by averaging its food intake over a 4 to 8 day period before killing. The individual daily average was used to calculate the overall daily average for each strain. The four Fed test groups were given their food cups at 1900 hours and the cups were removed after 20 minutes. To calculate the 20 minute meal size, the cups were weighed before and after the meal.

Injection and Sacrifice

At the time of sample collection, a group of 8 rats--4 from each strain in a given treatment group (i.e., Fed Sham, Fed Drug, Non-Fed Drug, etc.)--were injected with pargyline or vehicle, then sacrificed at 0, 20, 40, or 60 minutes post injection. The rats were injected in reverse chronological order (that is, the 60 minute rat was injected first) so that the 0 time animal was injected last and killed within 30 seconds after injection. Each strain was injected this way so that the sacrifice times were staggered. The injections were i.p. and were either 0.9% saline (vehicle) or pargyline (Sigma Corp.) at 75 mg/kg in 0.9% saline. Approximately 40 mg of pargyline were dissolved in 1.0 ml of 0.9%

saline and rats were injected with different volumes but no volume exceeded 0.5 ml. The animals were injected and sacrificed by decapitation in the animal room under red lights between 1900 and 2030, the first hour 1 1/2 of the usual feeding period.

Dissection

After decapitation, the brain was immediately removed and the hypothalamus was dissected out using the procedure described by Holman et al. ('76). The hypothalamus was then placed in a pretared plastic weighing boat, frozen on dry ice and was held for up to 1 1/2 hours while sample collection was being completed. The time between decapitation and hypothalamus freezing was rarely greater than 3 minutes.

Sample Analysis

The process for 5-HT analysis used are very similar to those described by Reinhard et al. ('80), Mefford ('80, Mefford & Barchas ('80), and Shum et al. ('82). The hypothalami were weighed frozen in the preweighed container and then transferred to a glass homogenization tube that had 200 ul 0.1N perchloric acid and 50 ul of 1.75 ng/ul n-methyl 5-HT (nm-5HT), the internal standard. After hand homogenizing for about 60 seconds, the homogenization tube and pestle were washed down with 100 ul of 0.1N perchloric acid and the tube was covered with parafilm. The samples were homogenized in random order each time and were held at 40°F for 5-45 minutes until the rest of the sample were homoginized. The homogenization tubes were centrifuged at 5,000 rpm in a

refrigerated Sorval RC-5 centrifuge for 10-12 minutes at 0°-4°C. The supernatants were then decanted into a microfilter centrifuge tube (B.A.S.) and centrifuged through a 0.2 um microfilter for 1-2 minutes. The collected samples were then pipetted into a disposable 20 x 20 cm culture tube, placed on dry ice, stored at -40°C and assayed for 5-HT and 5-HIAA within 12 hours.

Due to high overall sample loss in the first half of the samples, 80, and the second half of 80 samples received slightly different processing. The second half was processed as described above except 25 ul of 0.5% sodium bisulfate and 25 ul of 0.2M EDTA were added to the homogenization tube to reduce sample loss. Also, after the samples were pipetted into the disposable culture tube, they were placed on crushed ice and assayed for 5-HT and 5-HIAA within 3 hours without being frozen. The supernatant yield was estimated to be 80-190 ul.

All samples were assayed for 5-HT and 5-HIAA by high performance liquid chromatography with electrochemical detection (LCEC) which has been shown to be an accurate and efficient means of 5-HT quantification (Warsh et al., '80). The LCEC system consisted of a B.A.S. LC 4 amperometric detector with a Watman reverse phase column (OD 5-3). The mobile phase consisted of monochloroacetic acid buffer (0.1M, pH, 3.0) containing 0.012% sodiumoctyl sulfate and 7-9% acetonitrile. The flow rate was set at approximately 7.5 ml/minute and the column was operated at ambient temperature. The electrochemical detector was set at 2nA/volt with

a potential of 0.56 volts with the recorder set to give a full scale deflection with one volt.

The 5-HIAA, 5-HT, and N-M-5HT (N-Methyl-5HT) standards were diluted in 0.1N perchloric acid to a concentration of 0.1 ng/ul. Curves for all standards had an R^2 value of at least .97. Due to rapid decay of the 5-HIAA standard it was mixed frequently.

For each sample, the ng of 5-HT and 5-HIAA/gm hypothalamus was calculated by solving the regression equation ($Y = b_o + b_1(X)$) for X, multiplying by ul dilution of total sample/ul injected, adding the % loss indicated by nm-5HT recovery, and dividing by the hypothalamus weight. The ng/gram of hypothalamus at each time point was used to calculate the slope (b_1) of 5-HT accumulation and 5-HIAA decline.

Statistics

The data were evaluated by two methods. A Bonferoni T test for multiple comparisons was used because it could incorporate the SS_e for each slope into the test statistic. Since the first and second set of animals received slightly different processing, the slopes and variances of each test group from each set were calculated, then the average means and variances were averaged together to allow for the possible different influences of the processing differences. A two-way analysis of variance on the slopes derived from each sample collection was done to compare results with the Bonferoni T.

To determine if any differences between base levels in any of the groups existed, a two-way ANOVA was done on the 0 time value. A student's T test and a test of linearity was done on each drug group 5-HT slope to test whether it was different than 0 and to see whether a linear response was produced.

RESULTS

Body Weights

During the final week before killing, and after rats had adjusted for at least 1 1/2 weeks to the meal-feeding schedule, S rats gained 31 ± 1 gms per week while OM rats gained 32 ± 1 gms per week. When they were killed, mean weights ± SEM were 163 ± 3 for the S and 181 ± 3 for the Osborne-Mendell rats.

Food Intakes

Average food intake for the 2-hour feeding period was 8.8 ± 0.2 SEM gm/day for S rats and 9.5 ± 0.2 for OM rats. The average 20 minute meal for the Fed test groups was 6.9 ± 0.96 gms for S rats and 7.8 ± 0.4 for OM rats. OM rats ate significantly more in both the 2-hour and the 20-minute meal ($P < 0.05$) than S rats.

5-HT Accumulation

Comparisons of 5-HT accumulation between the 2 strains of rats and between the various treatments are presented in Table 2 as average slopes ± SEM and Figures 3-8 as graphs of each comparison. In the Fed vs. Non-Fed Drug comparisons (see Figures 3 & 4) the S Non-Fed slope was 11.78 ng/g min. greater than the S Fed slope ($P < 0.10$) while the OM Non-Fed was 3.07 ng/gm min. greater than the OM Fed ($P > 0.10$).

TABLE 2

Comparison of 5-HT Accumulation Over Time in OM and S Rats with Different Fed & Drug States

Drug Treatment	Min Post Inj	Number of rats at each time point		Accumulation of 5-HT or Slope (b_1=ng/g/min) State of Feeding		
		Fed	Non-Fed	Fed	Non-Fed	Significance
				S		
Drug	0	6	6	12.08±3.60	23.86±3.60	P<0.10**
	20	6	7			
	40	6	6			
	60	6	6			
Sham	0	5	-	3.80±2.25	Not analyzed	--
	20	4	-			
	40	6	-			
	60	6	-			
				OM		
Drug	0	6	6	12.87±2.90	15.94±3.43	NS
	20	6	7			
	40	7	6			
	60	7	5			
Sham	0	6	-	1.31±2.13	Not analyzed	--
	20	5	-			
	40	6	-			
	60	5	-			

*Values are ± S.E.M.

**Bonferoni T test $T_{B/2}$ M=7, V=118.

***No difference was found between strains when Fed and injection states were constant.

Figure 3: Fed vs. Non-Fed Comparison of 5-HT Accumulation in the S Rat After Injection of Pargyline.

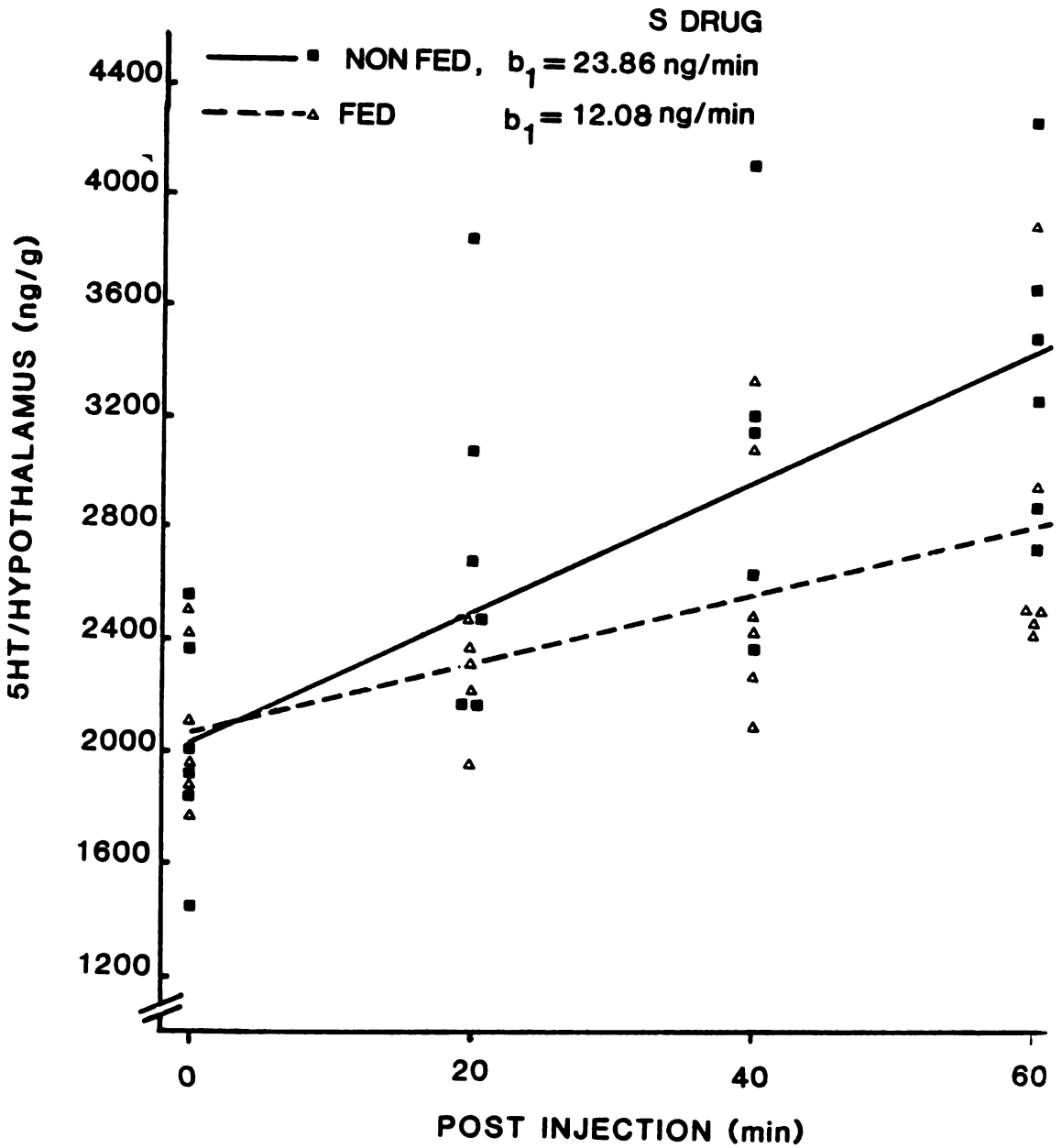
S DRUG
NON FED, b_1 = 23.86 ng/min
FED b_1 = 12.08 ng/min
5HT/HYPOTHALAMUS (ng/g)
4400
4000
3600
3200
2800
2400
2000
1600
1200
0
20
40
60
POST INJECTION (min)

Figure 3

Figure 4: Fed vs. Non-Fed Comparison of 5-HT Accumulation Rate in the OM Rat After Injection of Pargyline.

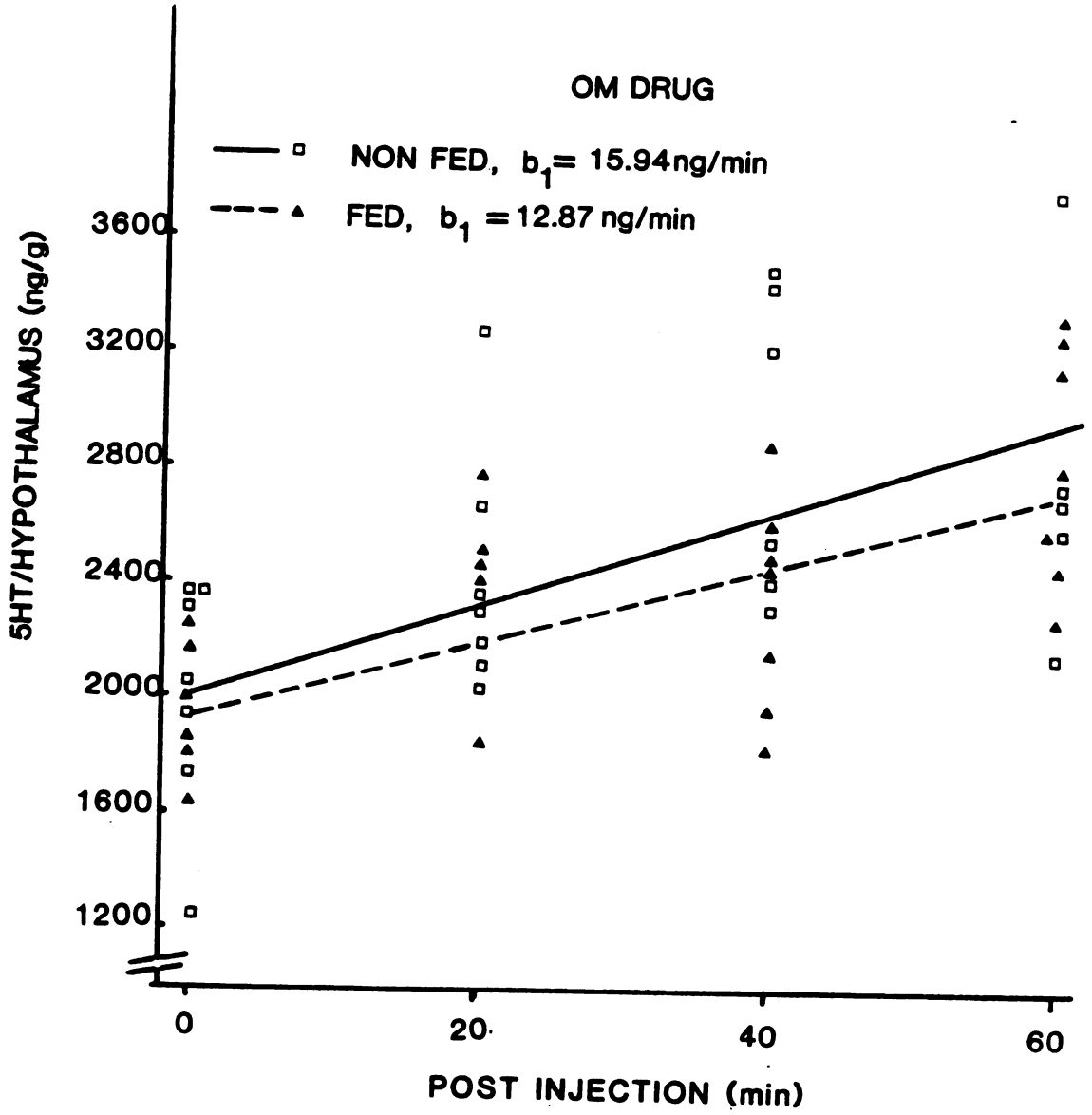
OM DRUG
NON FED, b_1= 15.94ng/min
FED, b_1 =12.87 ng/min
5HT/HYPOTHALAMUS (ng/g)
3600
3200
2800
2400
2000
1600
1200
0
20
40
60
POST INJECTION (min)

Figure 4

Figure 5: Strain Comparison of 5-HT Accumulation Rate in the Fed State After Injection of Pargyline.

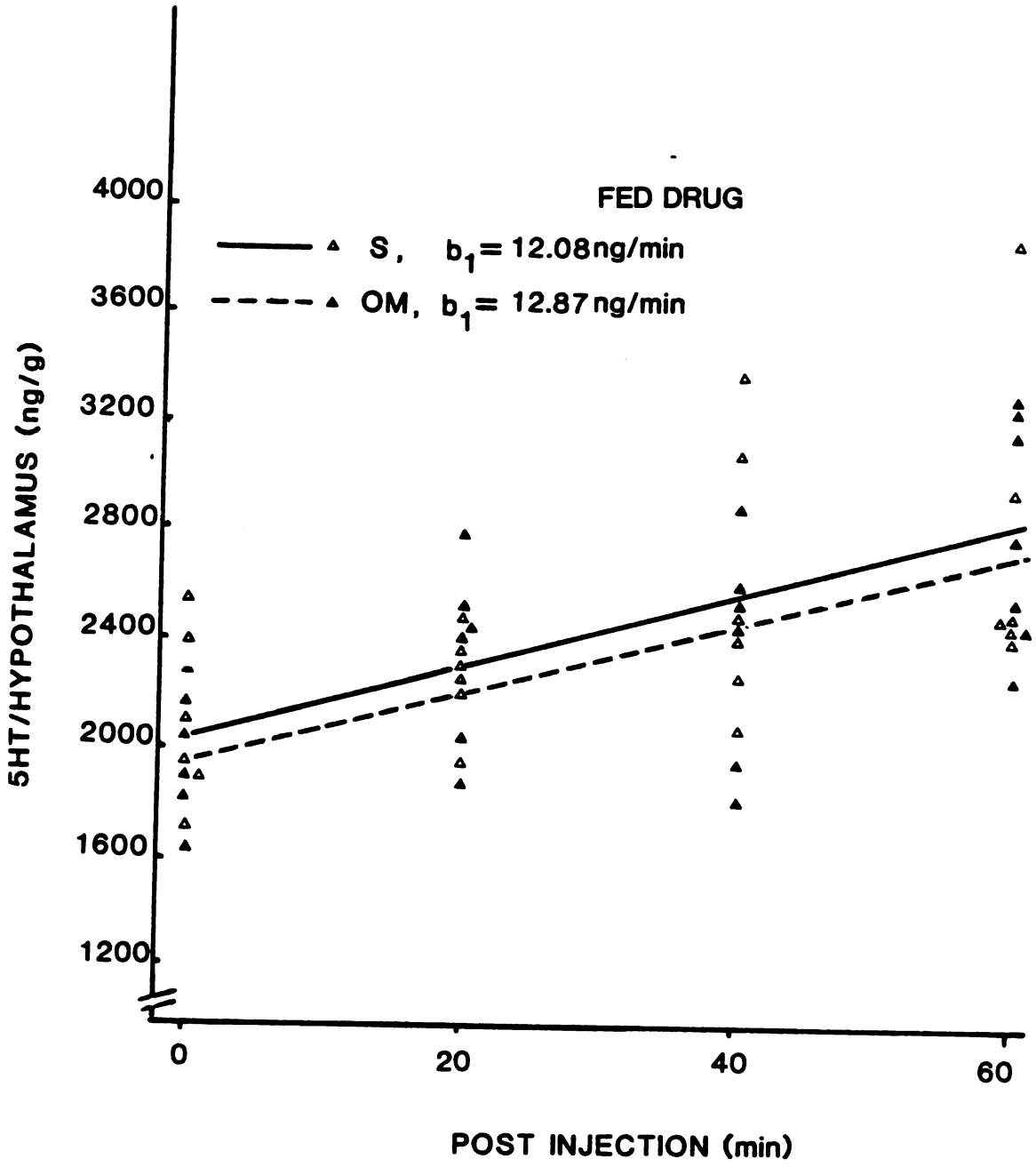
FED DRUG
S, b_1= 12.08ng/min
OM, b_1= 12.87ng/min
5HT/HYPOTHALAMUS (ng/g)
4000
3600
3200
2800
2400
2000
1600
1200
0
20
40
60
POST INJECTION (min)

Figure 5

Figure 6: Strain Comparison of 5-HT Accumulation Rate in the Fed State After Injection of Vehicle.

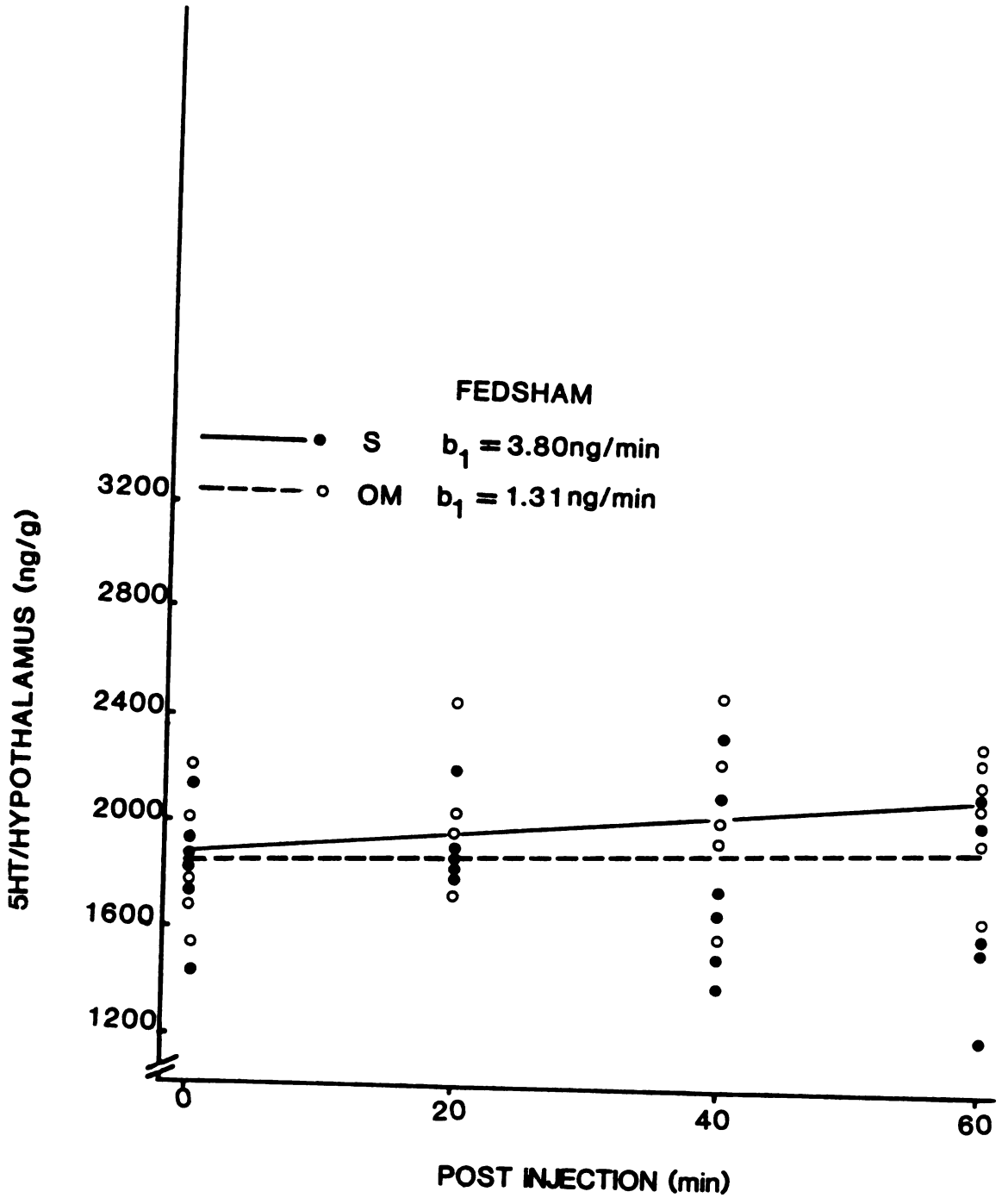
FEDSHAM
S $b_1 = 3.80$ng/min
OM $b_1 = 1.31$ng/min
5HT/HYPOTHALAMUS (ng/g)
3200
2800
2400
2000
1600
1200
0
20
40
60
POST INJECTION (min)

Figure 6

Figure 7: Strain Comparison of 5-HT Accumulation Rate in the Non-Fed State After Injection of Pargyline.

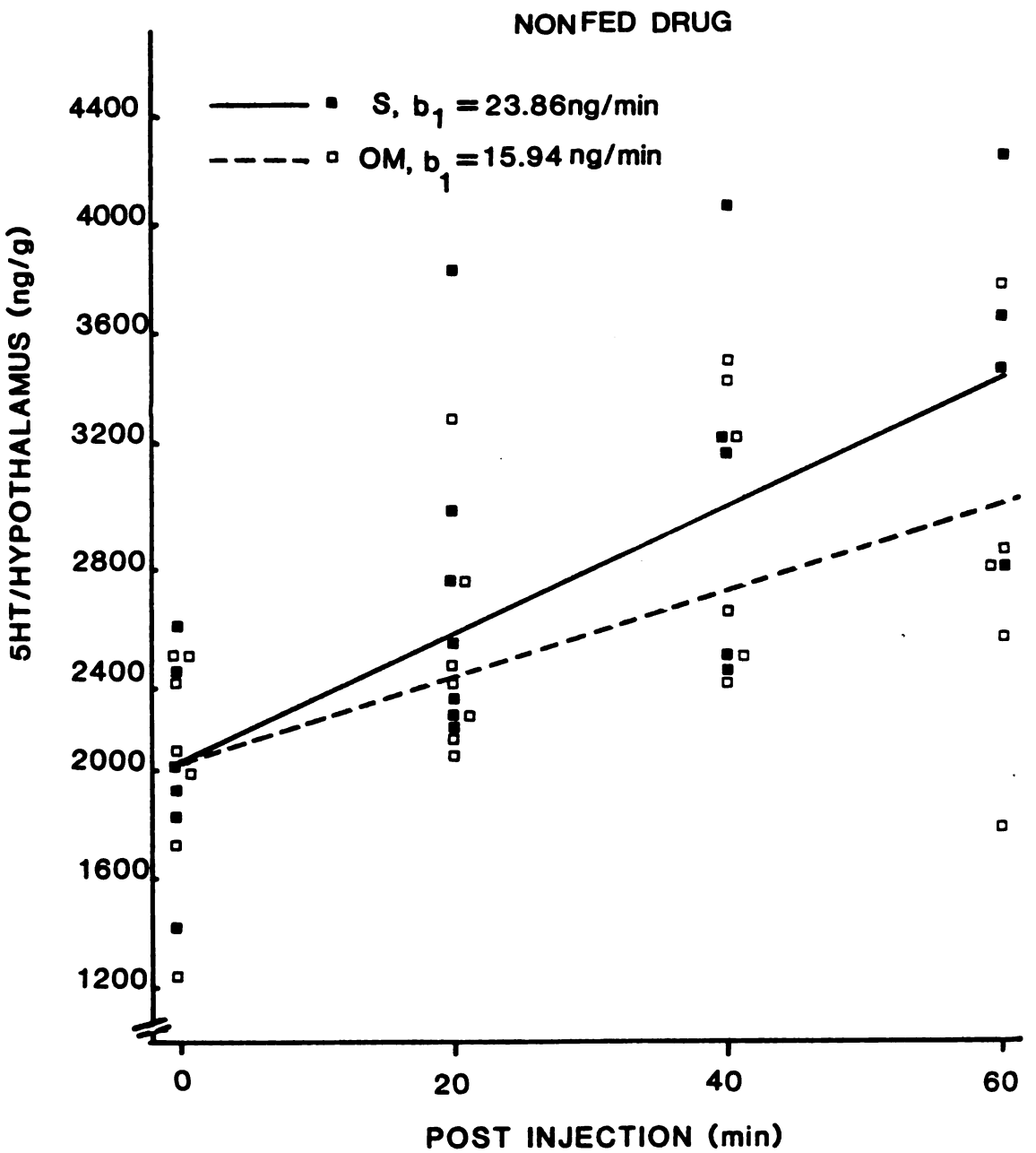
NONFED DRUG
S, b_1 = 23.86ng/min
OM, b_1 = 15.94 ng/min
5HT/HYPOTHALAMUS (ng/g)
4400
4000
3600
3200
2800
2400
2000
1600
1200
0
20
40
60
POST INJECTION (min)

Figure 7

Figure 8: Fed Drug vs. Fed Sham Comparison of 5-HT Accumulation Rate in the S Rat.

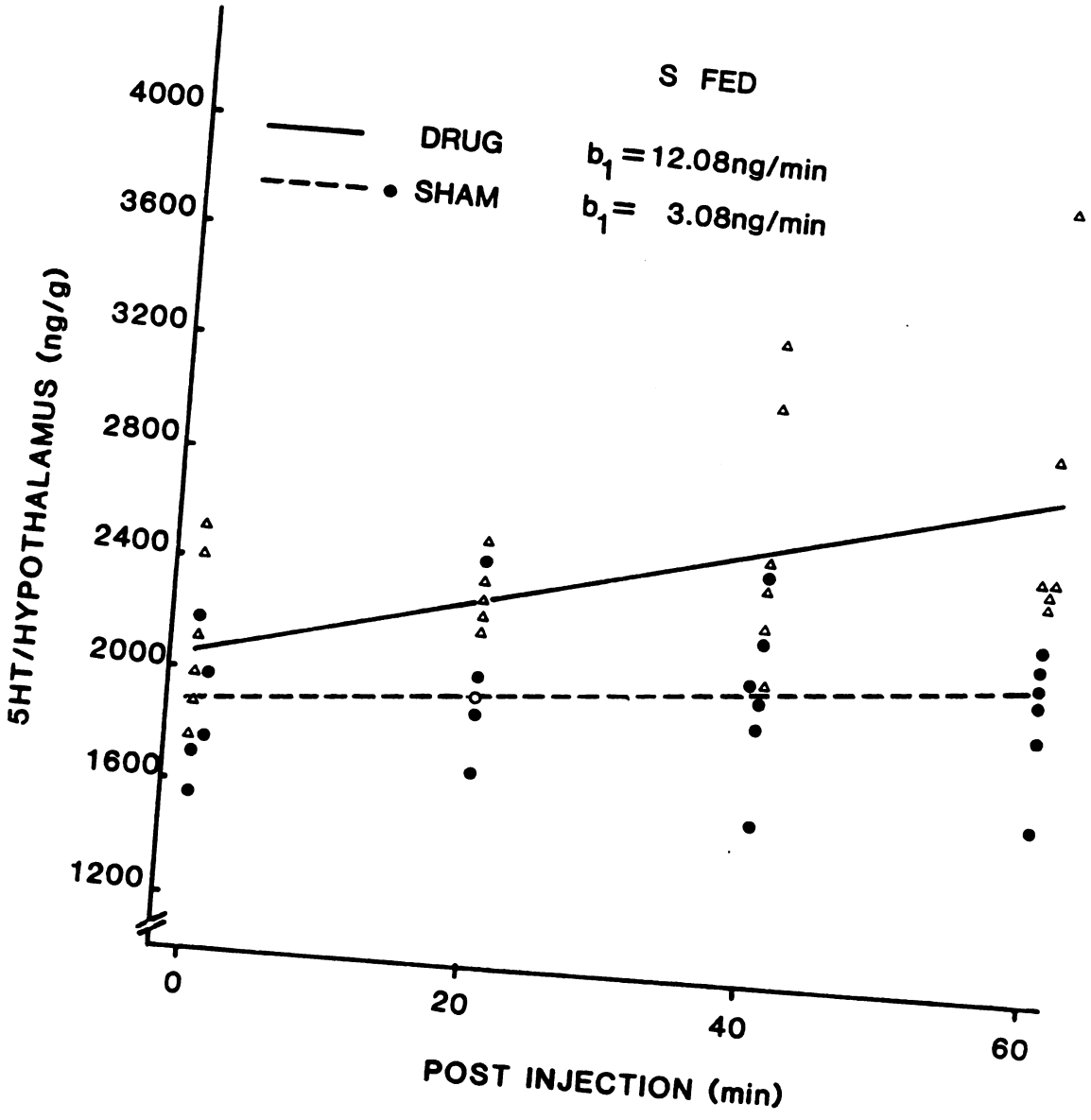
S FED
DRUG
SHAM
b_1 = 12.08ng/min
b_1 = 3.08ng/min
5HT/HYPOTHALAMUS (ng/g)
4000
3600
3200
2800
2400
2000
1600
1200
0
20
40
60
POST INJECTION (min)

Figure 8

In the Fed state the 5-HT accumulation rate was similar in the OM and S rat whether or not they were injected with pargyline (Figures 5 & 6, respectively).

In the Non-Fed drug state the slope of the S rat was 7.92 ng/g min. greater than the OM rat. However, due to high variability, the difference was not significant.

Comparison of Fed drug vs. sham injections in the Fed state within strains (Figure 8 for S, and Figure 9 for OM) produced a slope in the drug group that was 8.28 ng/g min. greater and 11.56 ng/g min. greater in the S and OM rat, respectively. These differences in the 5-HT accumulation rate were not significant.

The analysis of variance on individual slopes is presented in Table 3. The F value indicated a significant variation in treatments. The Bonferoni T for nonorthoganal multiple comparisons on the average b_1 for a test group, did not show a difference in any of the comparisons that were done on slopes calculated for a whole test group. Only the Non-Fed drug groups from both strains combined vs. the Fed Sham groups from both strains were able to produce a significant difference ($p < 0.05$).

5-HIAA Decline

Comparisons of 5-HIAA decline expressed as slope are presented in Table 4. No comparison of strain within treatment (Fed Drug, Non-Fed Drug, or Fed Sham) produced a difference with the Bonferoni T test. Nor did the slopes appear to have any relation to their 5-HT counterparts which

Figure 9: Fed State Drug vs. Sham Comparison of 5-HT Accumulation Rate in OM Rats.

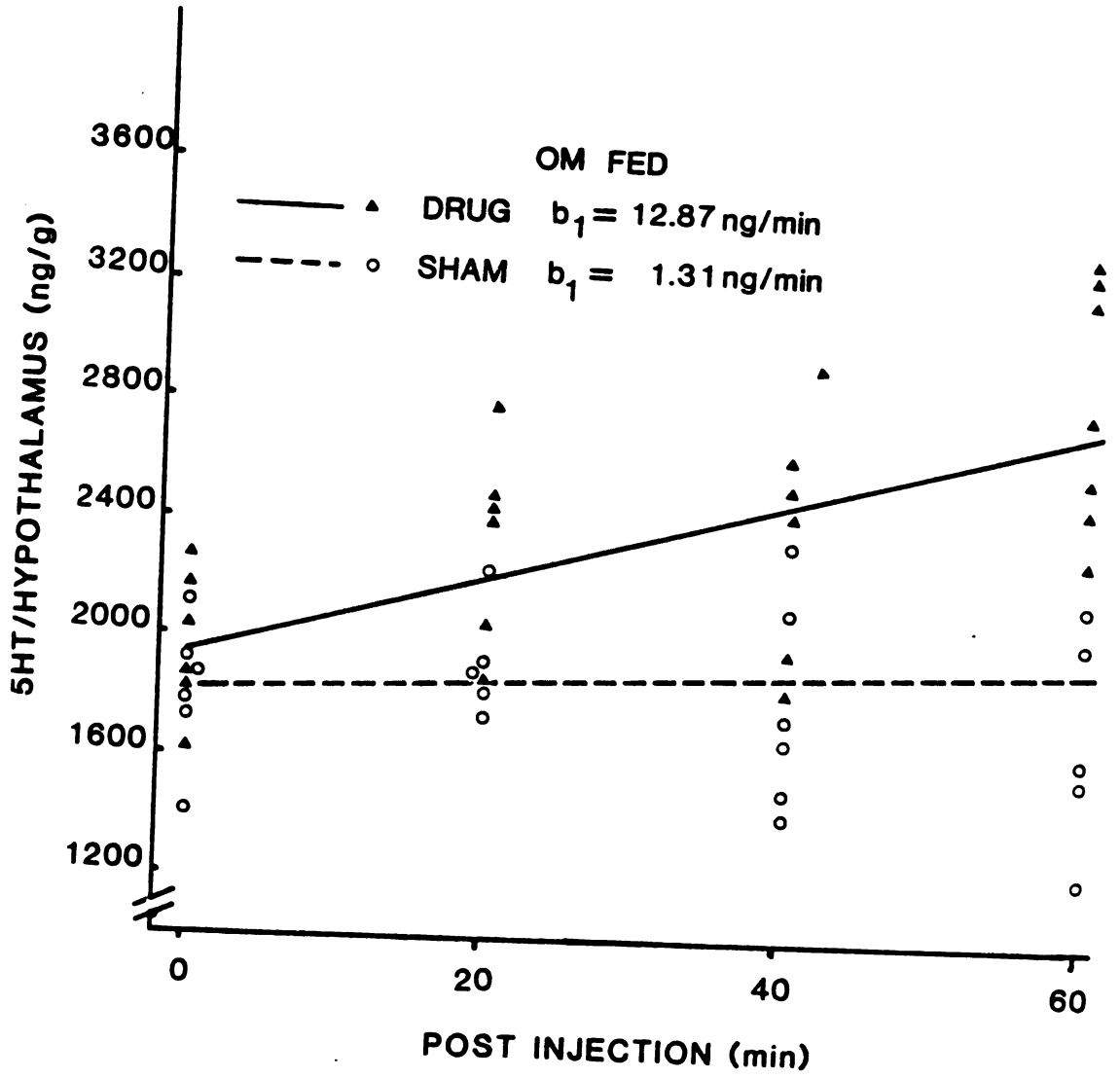
OM FED
DRUG b_1 = 12.87 ng/min
SHAM b_1 = 1.31 ng/min
5HT/HYPOTHALAMUS (ng/g)
3600
3200
2800
2400
2000
1600
1200
0
20
40
60
POST INJECTION (min)

Figure 9

TABLE 3

ANOVA for Estimations of b_1 or 5-HT Accumulation Rate Based on One Sample Per Time Point

Source of Variation	df	MS	F
Strain	1	74.46	.3560
Treatments	2	1200.32	5.739*
Interaction	2	214.00	1.0236
Error	30	209.14	

TABLE 4

Comparison of Mean 5-HIAA Decline Over Time or b_1 in OM and S Rats with Different Fed and Drug States

Drug Treatment	Min Post Inj	Number of rats at each time point		Decline or slope (b_1=ng/g/min) State of Feeding		Significance
		Fed	Non-Fed	Fed	Non-Fed	
				S 5B/PL		
Drug	0	7	6	-3.89±2.02	-5.22±1.20	NS
	20	4	6			
	40	6	5			
	60	6	6			
Sham	0	7	-	0.94±2.99	Not analyzed	--
	20	3	-			
	40	7	-			
	60	7	-			
				OM		
Drug	0	7	6	-7.45±1.31	-6.42±1.08	NS
	20	6	6			
	40	7	4			
	60	7	5			
Sham	0	7	-	-1.36±1.43	Not analyzed	--
	20	5	-			
	40	7	-			
	60	6	-			

*Values are ±S.E.M.

**Bonferoni T test $T_{B/2}$, M=7, V=118.

***No difference was found between strains when fed and drug states were constant.

may be due to the large standard error in relation to the absolute values of the slopes.

The Fed Drug vs. Non-Fed Drug treatment comparison within strains shows no differences and do not appear related to their 5-HT counterparts for reasons indicated above.

The Fed Drug vs. Fed Sham comparison did not show any difference though the declines appear to be greater in the drug group.

Hypothalamus Weights

The S 5B/P1 rat had an average hypothalamus weight of 47.1 ± 0.9 mg while the OM rat had 50.4 ± 1.1 mg. The differences were significant with 95% confidence (n = 154).

Mean Level At Each Time Point

The mean 5-HT levels at each time point in each group ± the standard deviation are presented in Figure 10.

0-Time Values. The mean amounts ± S.E.M. of 5-HT and 5-HIAA and ANOVA are presented in Tables 5 and 6, respectively. ANOVA results indicate that the values were similar in each group for both 5-HT and 5-HIAA.

60 Min. Values. The mean 60 minute post injection values and the ANOVA is presented in Table 7. The F value for treatments was significantly different. A Bonferoni T test for the following four non-orthoganal comparisons was done: Fed vs. Non-Fed strains combined, Fed Drug vs. Fed Sham strains combined, S Fed Drug vs S Non-Fed Drug, and OM Fed Drug vs. OM Non-Fed Drug. The Fed Drug vs. Fed Sham comparison was the only one that was significant ($P < 0.01$)

Figure 10. Bar Graph of Mean ± S.D. 5-HT Accumulated in Each Treatment Group at Each Time Point.

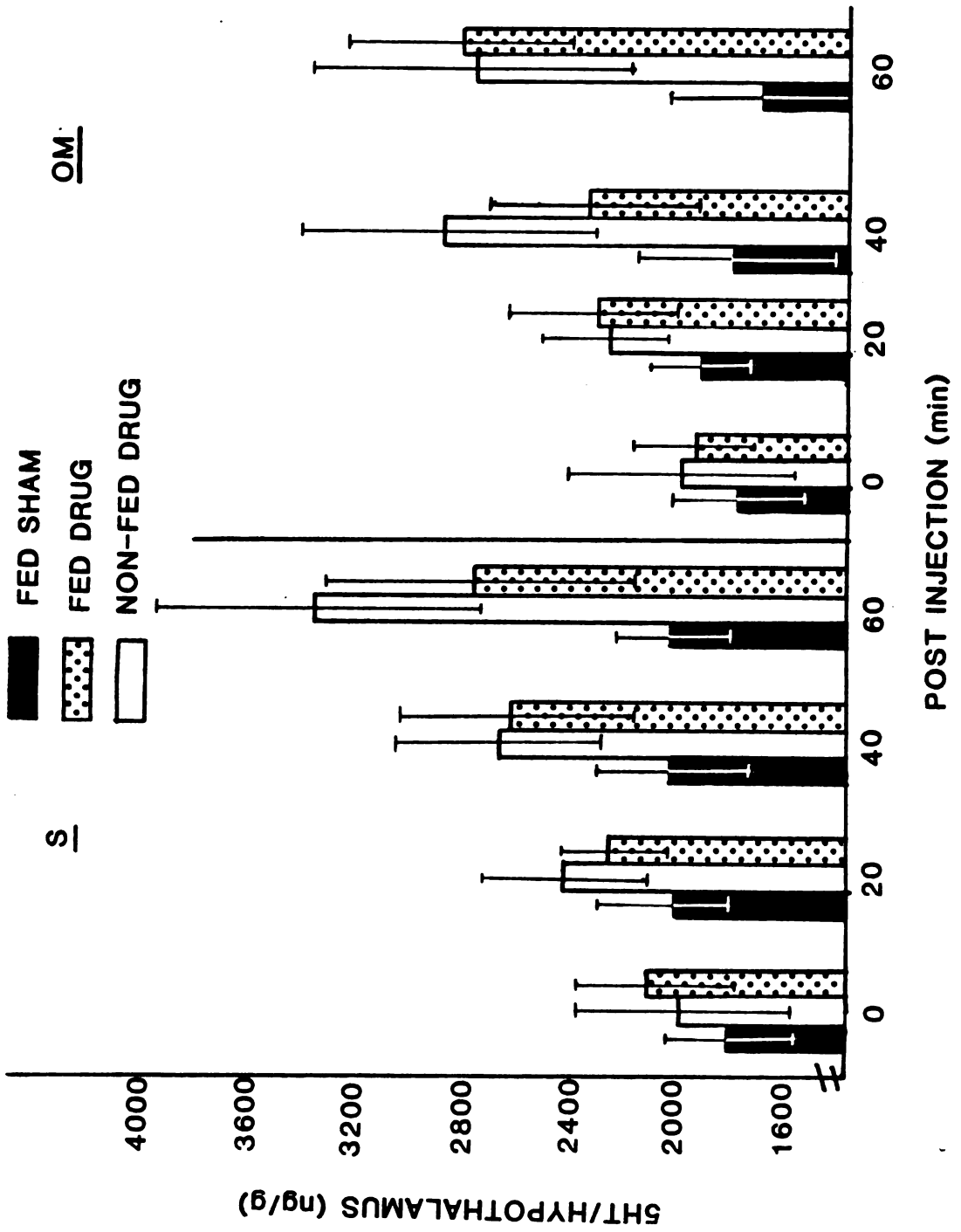
FED SHAM
FED DRUG
NON-FED DRUG
OM
S
5HT/HYPOTHALAMUS (ng/g)
4000
3600
3200
2800
2400
2000
1600
0
20
40
60
0
20
40
60
POST INJECTION (min)

Figure 10

TABLE 5

Amount of 5-HT* in the Hypothalamus at Zero Time in OM & S Rats

Treatment	5-HT (Ng/g) OM	S
Fed Drug	1967±93*	2114±118
Non-Fed Drug	2009±156	2016±164
Fed Sham	1807±96	1833±109

*Mean ± S.E.M.

ANOVA for 0 Time 5-HT Values for All Treatment Groups

Source of Variation	df	MS	F
Strain	1	1354	.0133
Treatments	2	9102.5	.0898
Interaction	2	796.3	.0079
Error	30	101,403	

TABLE 6

Amount of 5-HIAA in the Hypothalamus at Zero Time in the OM & S Rats

	5-HIAA in hypothalamus at 0 Time (Ng/g)	
Treatment	OM	S
Fed Drug	847.±346	884.±113
Non-Fed Drug	763.±145.3	740.±147.5
Fed Sham	708.±108.7	745.±83.6

ANOVA for 0 Time 5-HIAA Values for All Treatment Groups

Source of Variation	df	MS	F
Strain	1	2440.4	.00378
Treatments	2	6542.8	.10145
Interaction	2	3402.9	.00527
Error	30	645,157	

TABLE 7

Amount of 5-HIAA in the Hypothalamus
at 60 Min. Post Injection
in OM & S Rats

	OM	S
Fed Drug	2832±156	2792±232
Non-Fed Drug	2799±265	3387±240
Fed Sham	1719±164	2049±94

ANOVA of 60 Min. Post Injection 5-HT
Values in All Treatment Groups

Source of variation	df	MS	F
Strain	1	388176	1.772
Treatments	2	3202928	14.618*
Interaction	2	195641	0.893
Error	30	219113	

f , 2, 30

.05 = 3.32

while the S Fed vs. Non-Fed was only close to the critical value.

Effect of Pargyline

The four drug-injected groups had slopes significantly greater than 0 ($P < 0.05$) and no line was shown to be non-linear ($P < 0.05$).

DISCUSSION

Compared to a previous study (Schemmel unpublished data) the average daily food intake for S rats was 93% of ad libitum while OM intake was 69% of ad libitum. The S rat consumed more diet per gram of body weight than the OM in this study which is consistent with ad libitum feeding. In the Fed test groups the average 20 minute meal intake was 74% and 68% of the two hour meal for the OM and S rats respectively. This indicated that a considerable amount of food had been consumed for both groups at the time of sample collection.

The pattern of weight gain was also different when compared to ad libitum weight gain. While both strains continued to grow after adjusting to the meal feeding schedule, the S and OM rats were 86% and 59% of their respective ad libitum weights at 7 1/2 weeks of age.

The results can lend qualified support to the overall hypothesis that, when sustained on a high (calorie dense) diet, the S rat will consume less food and have greater serotonergic activity in response to feeding than the OM rat. First, the S Non-Fed 5-HT accumulation tended to be significantly greater than the S Fed ($p < 0.10$). Second, the OM Non-Fed accumulation was also greater than the OM Fed but the difference was less than the S Non-Fed vs. Fed

comparison and was not significant. Also, of equal importance, the S rat ate 0.7 gms less food per day in the 2-hour feeding and 0.9 gms less during the 20-minute meal periods than the OM rat ($p < 0.05$). The conclusion that the S rat had a greater serotonergic activity is qualified because the strain comparison in the Non-Fed state indicated that though there was a difference in the S accumulation rate (23.86 ng/g/min) and the OM rate (15.94 ng/g/min), it was not significant.

The secondary hypothesis is rejected by these results. They suggest that serotonergic activity, as measured by 5-HT production, decreases with food intake. If both hypotheses were true, a greater activity would be expected in the Fed state of the strain that ate less. Since a greater pre-meal serotonergic activity was associated with the strain that consumed less diet, this could indicate that the greater the serotonergic activity before a meal the smaller the meal will be. This conclusion is inconsistent with the findings of Blundel et al. ('76) which through examination of the temporal effect of fenfluramine, suggested that serotonergic activity increased after the onset of feeding. So the results associate a greater serotonergic activity with the animal that consumed less food and suggests that increased serotonergic activity before feeding, instead of in response to feeding, would cause decreased food intake.

It may be postulated that since the S rat had a greater difference in 5-HT activity between the Fed vs. Non-Fed state than the OM and ate less than the OM rat, the S rat

may be more responsive to energy or food intake than the OM rat. These points may explain part of the mechanism which enables the S rat to maintain the same energy intake when fed a high fat--calorie dense--diet.

The lack of difference between the Fed Sham vs. Fed Drug groups in each strain (Figures 8 and 9) is inconsistent with the expected drug effects (Morot-Gaundry et al. '74, VanLoon et al. '81) but does not necessarily indicate lack of drug effectiveness. What the statistics imply here is that the variance of the slopes is great enough that they could be parallel. However, the observed slope trends indicate that they are different and the Bonferoni comparison of the levels of 5-HT at the 60 minute time point shows that the Fed Drug groups accumulated more 5-HT than the Fed Sham ($p < 0.01$). This shows that the drug was effective in causing 5-HT to accumulate to levels significantly greater than the Sham state.

Comparison of the results in this study with other studies that have examined the relationship of serotonin and appetite is difficult because no study has examined hypothalamic serotonergic activity before and after food intake by measuring 5-HT accumulation.

Finkelstein et al. ('81) has compared hypothalamic and other brain region 5-HT levels in obese and non-obese Zucker rats. They found approximately 1300 ng 5-HT per gm hypothalamus of both types while in this study the average 0 time 5-HT level 1962 $\pm$ 52 ng per gm in all groups. The differences here may be, in part, because Finkelstein used a

flurometric assay method while this study used the LCEC method. Garthwaite et al. ('79) examined whole brain 5-HT levels in obese (ob/ob) hyperglycemic mice but the results cannot be compared to this study since the whole brain was assayed and a flurometric method for assay was used.

In this study, no treatment differences were found when ANOVA was done on base levels (0-time) of 5-HT while a definite trend for a difference was found in the slope comparison of the S Non-Fed vs. Fed groups. This finding supports the earlier assertion that examination of neurotransmitter activity is more important than neurotransmitter levels for assessing the role of a neuropathway in behavior.

In studies that examined the use of LCEC for assay of 5-HT, Reinhard et al. ('80) found 1080 ± 80 ng/gm hypothalamus and Mefford & Barchas ('80) found 841 ± 59 ng/gm hypothalamus using an LCEC technique very similar to the one used here. These values are almost half the average 0 time amount found in this study, 1962 ± 52 ng/gm hypothalamus, but the S.E.M. is very similar.

Reports by Anderson ('79), Curzon ('81), Boadle-Biber ('82) and Wurtman & Fernstrom ('76) indicate that a high protein (low carbohydrate) as opposed to a low protein (high carbohydrate) meal would decrease the availability of Trp for neuronal uptake since the protein would increase the amounts of (naa) that compete for uptake. In this study it may be possible that the diet had this effect since it was relatively high in protein and low in carbohydrate. Also, the study by Li ('82) indicated that as the protein intake

of a meal increased 5-HT levels in the whole rat brain would be decreased when they were measured 20 minutes after the meal.

However, none of the reports reviewed to date has measured 5-HT production before and after a high fat or any other type of meal. Whether the meal caused a decrease in 5-HT production or whether the observed change was due to a change in TH activity cannot be determined from this study.

Though 5-HIAA declined as expected in response the pargyline, no differences in decline were detected with the Bonferoni T test. The high variability, combined with the lower stability of 5-HIAA as indicated by rapid decay of the 5-HIAA standard, may have caused the variation to be too high to detect differences in 5-HIAA decline.

The greater hypothalamus weight in the OM rat did not appear to contribute extra 5-HT since the OM accumulation rates tended to be lower. The greater weight is consistent with findings of Stone et al. ('81) that OM rats have greater absolute cerebrum weights.

Individual animal variation, the assay technique, and the method for assessing serotonergic activity may have contributed to the large differences in individual sample values at any given time point. Since an individual animal had to be killed for each sample at each time point, the different individual responses to the drug and each animal's variation in enzyme activity would add some variation to the results. The LCEC was very consistent and had a 1-3% error with injection of standards, which is similar to previous

studies (Reinhard '80). Twenty to fifty percent sample decay was seen with duplicate injections in the first set of samples which was due to storage and freezing. Also, 30% overall decay was observed to be caused by freezing. When the samples were assayed immediately after processing and antioxidants were added to the homogenate solution 0 to 3% difference was seen in duplicate injections. The decay in the first half of the samples used to calculate the results could have induced a considerable degree of error.

Omission of Fed Sham

In the preliminary sample collection, the Fed Sham groups in each strain had slopes that were 0 and the Non-Fed Sham groups with 2 values per time point showed that they had a slope of 0. So, to conserve animals and time, the Non-Fed Sham group was omitted from the study.

Choice of Statistical Method

The Bonferoni T test is the method of choice because it incorporates the variance about each test group slope into the test statistic and more degrees of freedom can be used than if ANOVA was done on the average slope (b_1). Both of these aspects increase the power of the Bonferoni test. An ANOVA on individual slopes can only incorporate the variance between slopes derived from one sample at 3 or 4 time points. So, the degrees of freedom are much less than the Bonferoni T and variation is likely to be greater since the slopes are based on one value per time point.

SUMMARY & CONCLUSIONS

Pharmacological and feeding studies have linked serotonin with regulation of food intake in laboratory animals. Whether serotonergic activity is different in two rat strains, the Osborne-Mendell (OM) and S 5B/P1 (S), that respond differently to a high fat diet and whether serotonergic activity is increased after feeding are examined here. Hypothalamic 5-HT turnover was measured in OM and S rats by using an MAO inhibitor (pargyline) to assess the 5-HT production rate. 5-HT production can be linked with serotonergic activity since TH (Tryptophan Hydroxylase) can be induced from neuronal activity.

Both strains were divided into 3 groups: Fed Drug, Non-Fed Drug, and Fed Sham. All groups were fed a calorie-dense, high fat diet and were adapted to eat their daily food intake in a 2-hour meal at the start of the dark cycle. The Fed groups were given the food cups for 20 minutes of the usual 2-hour meal before being sacrificed, while the Non-Fed groups were not allowed to feed before sacrifice. At the time of sacrifice, the hypothalamus was removed, weighed, and homogenized in 0.1 N perchloric acid with an internal standard. The homogenate supernatant was assayed for 5-HT using LCEC. After injection of drug or vehicle, one rat from each strain in each treatment group was

sacrificed at 0, 20, 40, and 60 minutes post injection. The rate of 5-HT production, or accumulation, was compared between the groups to determine if there were differences in serotonergic activity between strains and between the Fed and Non-Fed state.

Both strains had greater 5-HT accumulation in the Non-Fed state than in the Fed state. Only the S rats had a significantly greater difference ($P < 0.10$) while the OM difference did not produce a significant trend. Strain comparisons in the treatment groups produced no significant trends through the S slope was greater than the OM and both strains had very similar slopes in the Fed state. The ability of the S rat to show a significant difference in the Non-Fed vs. Fed comparison while the OM could not lends support to the overall hypothesis that the S rat will have a greater serotonergic activity than the OM. The Fed vs. Non-Fed comparisons indicate that serotonergic activity is reduced after feeding and does not support the hypothesis that serotonergic activity would increase. These conclusion are preliminary and indicate that further strain comparisons of 5-HT activity may show some strain differences and further evaluation of the relationship of food intake to serotonergic activity is needed to clarify the role of serotonin pathways in appetite regulation.

POSSIBLE FOLLOW-UP STUDIES

Follow-up studies that extend the design should incorporate the protein:carbohydrate ratio as a factor. This would show whether the high protein diet actually suppressed Trp production here since high carbohydrate intake has been reported to cause more Trp uptake into the neuron (Curzon '81; Wurtman & Fernstrom, '76). So, in the Fed state, 5-HT accumulation from a high carbohydrate diet would be expected to be greater than from a high fat diet and possibly could show differences in 5-HT turnover between the strains. Also, further trials should measure plasma Trp:naa ratio and hypothalamic Trp levels to examine their effects on 5-HT production.

In light of the feeding studies and drug studies that evaluate food choices (Wurtman & Fernstrom, '79; Orthen-Gambill & Kanareck, '82), it would be interesting to examine the choices of fat, carbohydrate and protein between the two strains with and without the administration of a serotonergic drug. Also, the temporal pattern of food intake would be another aspect that could be examined with relative ease.

To really compare serotonergic activity, one would want to measure the amount of 5-HT released into the synapse. Since reliable methods for this are still being developed, we have to rely on more indirect measures. The present

method may not be as sensitive as needed to detect difference in serotonergic activity. This method may be more sensitive if micro punches of specific nuclei regions were sampled instead of the whole hypothalamus since it is possible that different nerve bundles could mediate different aspects of feeding and thus have different responses to a given stimuli which together may offset each other.

APPENDIX

FED DRUG

CH

Post Inj. (min)	Bodywt. (g)	Food Intake ± 2 hr (g)	Food Intake 20 min Meal (g)	Age (Days)	5-HIAA (ng/g)	5-HT (ng/g)	HYPOTHAL. WT. (g)
0							
	144	8.1	5.5	52	1230.0	2011	.0395
	157	8.4	4.0	51	1166.0	2268	.0514
	175	8.2	7.0	53	956.8	1845	.0501
	176	8.3	5.5	56	766.9	1868	.0528
	142	6.8	4.5	53	661.2	--	.0461
	214	11.1	10.0	57	515.6	1645	.0549
	168	9.8	10.0	54	457.2	2163	.0416
20							
	132	8.3	5.0	52	775.0	2766	.0509
	159	7.8	5.0	51	1084.0	2423	.0403
	115	10.6	8.5	53	--	--	.0461
	167	8.8	8.0	56	595.9	2423	.0635
	205	9.7	8.5	54	463.5	2063	.0556
	223	11.3	6.0	57	423.8	1891	.0581
	187	10.0	10.0	54	348.7	2423	.0474
40							
	120	7.4	4.0	52	444.4	1808	.0741
	194	10.4	9.5	51	561.1	2623	.0499
	151	8.4	6.0	53	670.3	2898	.0396
	202	9.6	8.0	56	381.7	2179	0622
	205	10.3	6.0	54	374.7	2470	.0591
	288	11.9	8.0	57	235.6	1958	.0470
	200	10.0	10.0	54	221.1	2508	.0494
60							
	150	7.8	5.0	52	536.9	3288	.0460
	172	9.6	6.5	50	864.2	3171	.0493
	200	11.1	7.0	53	496.7	3262	.0428
	204	11.1	8.0	56	373.5	2468	.0529
	187	10.7	7.0	53	112.0	2560	.0403
	226	11.8	7.0	57	201.4	2279	.0572
	183	9.6	7.0	54	161.4	2797	.0551

S

Post Inj. (min)	Bodywt. (g)	Food Intake ± 2 hr (g)	Food Intake 20 min Meal (g)	Age (Days)	5-HIAA (ng/g)	5-HT (ng/g)	HYPOTHAL. WT. (g)
0							
	134.5	7.1	5.0	56	1265.0	2503	.0441
	122.5	7.7	3.0	57	1030.0	2409	.0394
	137	6.1	7.0	59	1018.0	1979	.0360
	158	7.5	3.0	57	813.3	1789	.0426
	152	8.5	5.0	54	705.9	--	.0413
	134	7.5	5.0	54	694.4	1886	.0451
	175	10.3	6.0	53	478.1	2119	.0502
20							
	167	8.1	5.5	56	763.0	2335	.0545
	130	7.8	5.0	57	--	2475	.0465
	191	9.5	7.0	--	704.9	2207	.0434
	196	10.3	3.0	54	--	--	.0550
	180	9.7	6.0	54	397.7	2373	.0448
	204	10.8	9.0	53	511.2	1961	.0501
	--	--	--	--	--	--	--
40							
	150	6.7	5.0	56	735.0	3087	.0473
	140	7.4	3.0	57	--	--	.0351
	150	7.9	8.0	56	626.6	2509	.0625
	176	9.6	4.0	57	464.9	2287	.0456
	124	6.8	5.0	54	278.8	2446	.0484
	230	12.1	13.0	54	296.2	2247	.0501
	142	9.0	7.0	53	395.5	3304	.0404
60							
	130	7.8	4.0	56	1395.0	2956	.0479
	153	8.3	5.0	57	956.2	3883	.0394
	174	9.5	6.0	56	--	--	.0449
	159	9.3	4.0	57	370.7	2491	.0508
	166	10.3	5.0	54	182.6	2463	.0432
	227	11.3	6.0	54	486.5	2486	.0597
	173	9.4	7.0	53	438.3	2472	.0586

NON-FED RUNS

CM

Post Inj. (min)	Bodywt. (g)	Food Intake ± 2 hr (g)	Age (Days)	5-HIAA (ng/g)	5-HT (ng/g)	HYPOTHAL. WT. (g)
0						
	178	8.9	53	1196.0	2386	.0429
	193	4.9	52	—	2371[a]	.0483
	188	10.1	54	1091.0	2303	.0383
	173	9.8	57	878.6	1959	.0574
	206	11.6	54	679.5	2061	.047
	200	8.6	56	412.8	1240	.0703
	212	11.8	55	321.2	1745	.0477
20						
	135	8.8	53	749.1	2381	.0485
	169	5.5	52	—	3279[a]	.0429
	207	8.3	54	852.8	2673	.0453
	183	9.2	57	558.3	2143	.0384
	213	11.6	54	390.3	2193	.0490
	209	10.3	56	514.9	2046	.0571
	162	8.8	55	544.4	2301	.0642
40						
	199	10.6	53	700.0	3464	.0500
	157	9.2	52	—	3420[a]	.0540
	146	7.1	54	593.1	3211	.0338
	141	8.2	57	—	2428	—
	144[a]	8.9	54	—	1829	.0865
	227	10.9	56	350.4	2324	.0501
	170	9.2	55	284.7	2568	.0439
60						
	159	8.4	53	—	—	.0467
	116	10.1	52	—	—	.0540
	136	7.2	54	436.7	3780	.0307
	178	11.1	57	452.5	2716	.0530
	206	10.7	54	282.1	2745	.0415
	199	11.0	56	345.6	2186	.0472
	188	9.5	55	304.8	2569	.0533

S

Post Inj. (min)	Bodywt. (g)	Food Intake ± 2 hr (g)	Age (Days)	5-HIAA (ng/g)	5-HT (ng/g)	HYPOTHAL. WT. (g)
0						
	136	8.6	56	1354.0	2528	.0454
	188	6.2	58	—	2397[a]	.0460
	142	7.7	57	877.4	1989	.0350
	150	6.9	58	832.0	1829	.0545
	152	7.9	55	604.3	4277	.0522
	170	8.3	56	390.1	1417	.0543
	244	13.2	56	395.8	1931	.0585
20						
	136	8.1	56	927.6	2449	.0389
	174	6.6	58	—	3814[a]	.0574
	126	9.1	57	830.8	2492	.0443
	129	7.3	58	1033.0	2682	.0396
	163	9.3	56	437.3	2248	.0441
	163	8.9	53	497.6	2186	.0486
	235	11.5	56	318.4	2190	.0391
40						
	152	8.4	56	561.1	3183	.0434
	126	9.1	58	—	4060[a]	.0455
	130	6.7	54	584.4	3201	.0305
	155	8.2	58	—	2386	.0419
	151	8.6	55	383.4	2358	.0465
	184	10.6	53	346.7	2424	.0592
	190	9.8	56	533.1	2625	.0600
60						
	169	7.3	57	435.4	3256	.0538
	111	9.6	58	—	—	.0496
	183	10.4	57	715.7	3644	.0539
	141	8.1	58	613.8	4360	.0315
	151	9.3	56	318.9	3455	.0492
	156	9.1	53	357.9	2882	.0539
	188	10.2	56	224.2	2724	.0454

[a] Estimated loss.

FED SHAM

CM

Post Inj. (min)	Bodywt. (g)	Food Intake ± 2 hr (g)	Food Intake 20 min Meal (g)	Age (Days)	5-HIAA (ng/g)	5-HT (ng/g)	HYPOTHAL. WT. (g)
0							
	192	8.2	7.5	54	1034.0	1766	.0543
	185	9.1	5.5	56	781.1	1879	.0432
	191	12.3	6.0	54	495.3	2139	.0542
	193	10.0	9.0	55	341.4	1404	.0288
	189	10.6	9.0	55	647.5	1750	.0524
	182	11.1	8.0	54	952.4	1903	.0600
20							
	148	8.1	5.5	54	1054.0	2243	.0402
	185	8.7	5.5	56	808.3	1896	.0594
	179	9.0	10.0	55	580.4	1885	.0531
	182	8.3	8.0	55	694.8	1907	.0591
	212	11.6	11.0	59	669.3	1788	.0537
40							
	169	7.1	5.5	55	829.3	3470	.0387
	191	9.8	7.0	55	930.5	2135	.0678
	162	10.0	4.0	54	412.3	1684	.0591
	189	11.0	5.0	55	339.3	1427	0490
	220	11.8	10.0	54	532.1	1762	.0464
	234	10.6	7.0	59	554.7	1509	.0699
60							
	157	10.4	8.0	54	—	—	.0374
	164	7.4	7.0	55	878.9	2147	.0415
	179	10.5	5.0	54	332.1	1240	.0617
	180	9.4	7.0	53	450.7	1570	.0457
	187	11.0	8.0	54	630.8	1612	.0517
	230	10.1	7.0	59	710.9	2028	.0498

S

Post Inj. (min)	Bodywt. (g)	Food Intake ± 2 hr (g)	Food Intake 20 min Meal (g)	Age (Days)	5-HIAA (ng/g)	5-HT (ng/g)	HYPOTHAL. WT. (g)
0							
	141	10.1	6.0	58	1035.0	2183	.0339
	195	9.4	6.5	57	835.7	1715	.0513
	181	10.5	10.0	54	422.6	1547	.0274
	144	8.4	6.0	55	635.5	1756	.0526
	157	9.1	6.0	56	762.1	—	.0491
	212	10.2	5.0	55	779.9	1965	.0534
20							
	140	8.1	5.0	55	—	2424	.0411
	161	9.1	5.0	56	994.2	1949	.0428
	—	—	—	—	—	—	—
	159	9.3	8.0	54	693.4	1725	.0460
	143	12.2	9.0	55	900.1	2033	.0573
40							
	141	8.1	4.0	55	1212.0	2473	.0397
	169	9.0	5.0	56	850.8	2011	.0550
	138	8.75	9.0	54	590.6	2209	.0445
	138	8.1	5.5	55	598.5	1927	.0573
	141	9.8	5.0	54	567.3	1583	.0565
	240	8.6	4.0	55	790.8	2061	.0443
60							
	176	7.4	4.0	55	1197.0	2257	.0467
	154	8.1	5.0	56	1012.0	2135	.0383
	145	8.75	7.0	54	453.4	2215	.0568
	161	9.6	6.0	53	568.3	1940	.0461
	159	8.4	7.0	54	588.2	1638	.0470
	208	11.0	11.0	55	844.7	2110	.0525

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