CARBOHYDRATE AND TRYPTOPHAN INDUCED INCREASE   IN BRAIN SEROTONIN:

BIOCHEMICAL AND BEHAVIORAL CORRELATES

by

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ABSTRACT

Behavioral and biochemical correlates of the carbohydrate and tryptophan induced increase in brain serotonin were investigated in a series of 4 experiments. Experiment 1 was conducted to establish the nadir of brain tryptophan during the dark phase of the light cycle. Following a 16 hour fast, brains were removed at 1600, 1800, 2000, 2400, and 0400, hrs for tryptophan determination. Analyses indicated no differences in brain tryptophan throughout the dark period.

The time course and peak concentrations of the carbohydrate and tryptophan induced increase in brain tryptophan and serotonin were determined in Experiment 2. Rats were fasted from 0030 to 1730 and then offered a control diet and injected with saline or 50 mg/kg tryptophan, or offered a high carbohydrate, protein-free meal and injected with saline. One hour after treatment and hourly for the next 3 hours, brains were obtained for analysis of tryptophan and serotonin. Tryptophan injected rats exhibited a peak in brain tryptophan at 1 hour post injection and a fall in tryptophan to control levels by 2 hours. Carbohydrate fed animals exhibited an increase in brain tryptophan at all times observed. Elevated brain serotonin was found in both tryptophan and carbohydrate treated animals.

Experiment 3 was conducted to establish a behavioral correlate of brain serotonin. Behaviors investigated included: latency to step-down and explore a novel chamber and acquisition and extinction of a passive avoidance response. Animals were fed ad libitum, and 1 hour (1700) prior to behavioral testing, injected with saline or 50 mg/kg tryptophan. Animals did not differ on measures of passive avoidance acquisition or

extinction. However, tryptophan injected animals were found to exhibit a longer latency to step-down and explore a novel chamber than controls.

In Experiment 4, plasma corticosterone, latency to step-down, rearing, urination, and defecation in a novel chamber were assessed. Animals were fasted from 2400 to 1700 and injected and fed as in Experiment 2. One and 2 hours following treatment, behaviors were observed. Thereafter, brains were removed for determination of tryptophan and serotonin and blood obtained for plasma corticosterone analysis. In tryptophan administered rats, brain tryptophan was observed to peak at 1 hour post injection and to remain higher than controls at 2 hours post injection. Carbohydrate fed rats were found to exhibit higher levels of brain tryptophan than control animals at both times assayed. Brain serotonin was found to peak in tryptophan treated rats at 1 hour post injection and to remain elevated at 2 hours. No changes in brain serotonin were revealed in carbohydrate fed animals. No group differences were observed for any of the behavioral measures taken. However, increased plasma corticosterone was found in rats fed the high carbohydrate meal.

These data revealed that injection of tryptophan resulted in an increased latency to step-down and explore a novel chamber when animals were fed ab libitum, whereas carbohydrate ingestion resulted in an increase in plasma corticosterone with no effect on behavior. Confirmation that serotonin mediated these biochemical and behavioral changes awaits further research.

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I. INTRODUCTION

"Does eating influence brain function? To put the
question more specifically, do changes in blood
chemistry that follow the intake of nutrients pro-
duce corresponding changes in the tissues of the
brain? If so, could such diet-induced changes
affect the functional activity of the brain?"
(Fernstrom and Wurtman, 1974).

The traditional answer to these questions has usually been no; when required, the brain extracts oxygen, glucose, and other nutrients from the blood (Fernstrom and Wurtman, 1974). Hence, temporary fluctuations in the concentration of nutrients in plasma have not been thought to affect the brain (Fernstrom and Wurtman, 1974). However, more recent research has revealed some exceptions. Fasted rats fed a high carbohydrate, pro-tein-free meal (Fernstrom and Faller, 1978; Fernstrom, 1975b; Colmenares et al., 1975; Jacoby et al., 1975a; Madras et al., 1974; Fernstrom, 1971) or injected with the amino acid tryptophan (Young et al., 1978; Jacoby et al., 1975b; Fernstrom and Wurtman, 1971) have been shown to exhibit elevations in brain tryptophan and serotonin. That this same phenomenon could occur in humans has also been speculated (Fernstrom and Wurtman, 1974). Thus, the neurochemical response to tryptophan and carbohydrate has been repeatedly demonstrated. However, the functional significance of elevated brain serotonin has not been well established.

Serotonin has been associated with many behaviors. Thus, increases in brain serotonin could have important practical implications. Inhibi-tion of avoidance learning (Essman, 1977), memory (Essman, 1977), food intake (Blundell and Latham, 1979), locomotor activity (Warbritton et al., 1978), and exploration (File and Pope, 1974) have been observed in rats

with elevated levels of brain serotonin. Furthermore, brain serotonin
has been implicated in the regulation of the rat (Yuwiller, 1979), and
human (Modlinger et al., 1979) hypothalamus/pituitary/adrenal axis.

In addition, several metabolic conditions are thought to involve
alterations in serotonin metabolism. An elevated concentration of brain
serotonin has been linked to the etiology of confusion and coma, symptoms
in hepatic encephalopathy (Fischer et al., 1978; Sourkes, 1978). The
traditional form of therapy for this condition has been a high carbo-
hydrate, protein-free diet. Thus, it is possible that this treatment could
potentiate the disease symptoms through further increasing brain sero-
tonin. In addition to hepatic coma, rat neonatal iron deficiency has been
shown to increase brain serotonin (Mackler et al., 1978). Furthermore,
decreased behavioral responsiveness, often linked to elevated serotonin,
has been observed in an open field test of 28 day old rats fed an iron
deficient diet from birth (Weinberg et al., 1979). Thus, ingestion
of a carbohydrate, protein-free meal might be expected to further reduce
responsiveness in iron deficient rats.

Finally, the behavioral and biochemical consequences of carbohydrate
induced increases in brain serotonin are of particular interest because
consumption of refined sugar is high among North American populations.
In the United States sucrose intake has been estimated at 100 pounds
per capita per year (Bogart et al., 1973). Similarly, 68% of children
in a rural British Columbia town were observed to snack, and 28% of the
snacks consumed were high in refined sugar and low in other nutrients
(Onishi, 1980).

From the above, the importance of determining the functional signifi-
cance of the carbohydrate and tryptophan induced increase in brain sero-

tonin is clear. Thus, the present study was conducted to determine if the rise in serotonin following injections of tryptophan or ingestion of a high carbohydrate, protein-free meal, after fasting, has behavioral consequences. In addition, the effect of the carbohydrate and tryptophan induced increase of brain serotonin on plasma corticosterone output was investigated.

The specific objectives of this study were:

(1) To measure brain tryptophan, serotonin, and plasma corticosterone in fasted male rats fed a high carbohydrate meal or injected with tryptophan.

(2) To investigate whether the rise in brain serotonin can be correlated with a change in exploration, a behavior which has been related to brain serotonin.

(3) To determine whether there is a correlation between plasma corticosterone and brain serotonin.

II. REVIEW OF THE LITERATURE

1. Carbohydrate and Tryptophan Induced Changes in Brain Tryptophan and the Indoleamines.

A high carbohydrate protein-free meal fed to fasted rats at the beginning of the light phase, has repeatedly been shown to increase brain tryptophan, serotonin, and 5-hydroxyindoleacetic acid (5-HIAA) (Jacoby et al., 1975; Colmenares et al., 1975; Fernstrom, 1975b, 1974; Fernstrom et al., 1973; Fernstrom, 1971). Within 2 hours after presentation of the carbohydrate, protein-free meal, tryptophan and the indoleamines were observed to rise and remain elevated for 3 hours (Fernstrom, 1971). In addition, brain tryptophan was further elevated when the carbohydrate meal was also fat-free (Madras et al., 1973). Similar findings were obtained when a glucose solution was administered to fasted rats via either a stomach tube or gavage (De Montis et al., 1978; Madras et al., 1974, 1973). However, with these latter methods of administration, the peak in tryptophan occurred 1 hour after glucose presentation.

Tryptophan, serotonin, and 5-HIAA have also been assayed in specific regions within the central nervous system of rats fed a carbohydrate, protein-free meal (Colmenares et al., 1975). While no increase in tryptophan and the 5-hydroxyindoles was found in the hypothalamus or the corpus striatum, examination of the brain stem, spinal cord, and the telencephalon (neocortex, olfactory cortex, and hippocampus) revealed elevations in serotonin, tryptophan, and 5-HIAA (Colmenares et al., 1975). Because the brain stem is believed to contain the perikarya of the serotonergic neurons, while all other regions of the central nervous system are thought to contain the neuronal axons and terminals (Colmenares et al., 1975),

these findings demonstrated that the carbohydrate-induced increase in serotonin occurred in both cell bodies and the pre-synaptic terminals of serotonin-containing neurons. Therefore, upon depolarization, it is possible that more serotonin could be released into the synaptic cleft. This phenomenon is essential for the carbohydrate induced increase in serotonin to be physiologically significant (Colmenares et al., 1975).

Intraperitoneal injection of tryptophan also has been shown to increase brain tryptophan and serotonin (Young et al., 1978; Jacoby et al., 1975b; Fernstrom and Wurtman, 1971). Injections of 12.5 to 125 mg tryptophan/kg body weight resulted in dose related increases in brain tryptophan 1 hour after injection. However, the maximum increase in brain serotonin was obtained with tryptophan doses of 25-50 mg/kg (Young et al., 1978; Jacoby et al., 1975b; Fernstrom and Wurtman, 1971).

Thus, it has been repeatedly demonstrated that brain tryptophan, serotonin, and 5-HIAA are increased following carbohydrate ingestion or tryptophan dosing.

2. Serotonin Distribution and Biosynthesis.

Brain serotonin, or the monoamine 5-hydroxytryptamine, has primarily been found confined to a distinct cluster of neurons, the raphe nuclei (Fernstrom and Wurtman, 1974). The cell bodies of the raphe nuclei are known to be located in the brain stem and have fibres ascending into the remainder of the brain and descending through the spinal cord (Fernstrom and Wurtman, 1974).

Serotonin synthesis has been fully described (Airaksinen and Airaksinen, 1978; Fernstrom, 1978). This metabolic pathway (Fernstrom, 1978) is depicted in Figure 1.

Tryptophan   5-Hydroxytryptophan   Serotonin   5-HIAA
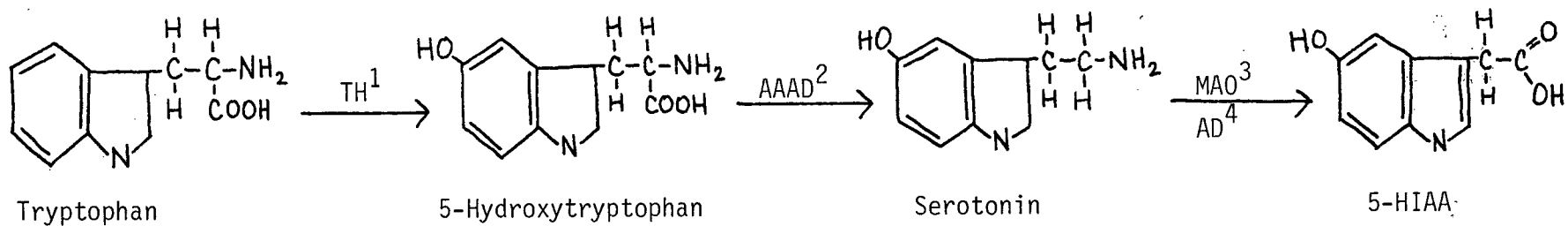
[1] Tryptophan hydroxylase; cofactors: ferrous iron, reduced pteridine cofactor

[2] Aromatic amino acid decarboxylase; cofactor: pyridoxal phosphate

[3] Monoamine oxidase

[4] Aldehyde dehydrogenase

Figure 1. Serotonin biosynthesis

(Fernstrom, 1978)

3. Regulation of Serotonin Synthesis.

Although serotonin synthesis has been extensively investigated, its regulation is still unclear. Tryptophan availability, rate of impulse flow along serotonergic neurons, and product inhibition have all been implicated as factors involved in regulation of serotonin synthesis.

Because the Km of tryptophan hydroxylase for tryptophan is high relative to its normal concentration, i.e. 50 uM versus 30 uM respectively (Fernstrom, 1978), tryptophan availability has been recognized as an important factor in the control of serotonin synthesis. In addition, procedures which alter brain tryptophan concentration have also been found to influence brain serotonin or 5-HIAA. Animals fed a corn based diet, known to be deficient in tryptophan, were observed to exhibit lower levels of brain tryptophan and serotonin (Lytle et al., 1975). Furthermore, lower concentrations of tryptophan and 5-HIAA have been found in rats offered meals of purified amino acids known to block tryptophan entry across the blood brain barrier (Gessa et al., 1974). In addition, presentation of a low protein diet to weanling rats has also been shown to lower brain tryptophan, serotonin, and 5-HIAA. These brain parameters were observed to return to normal after nutritional rehabilitation for 7 days (Dickerson and Pao, 1975).

Similarly, aminophylline, a drug believed to increase brain serotonin, was observed to elevate brain tryptophan (Curzon and Knott, 1974). In addition, brain tryptophan and serotonin turnover were found elevated after 24 hours of food deprivation or immobilization stress (Curzon et al., 1972). Finally, consumption of a high carbohydrate meal (Fernstrom, 1971) or injections of tryptophan (Fernstrom and Wurtman, 1971) were observed to increase brain tryptophan, serotonin, and 5-HIAA. That this

increase in brain serotonin reflected increased synthesis rather than lowered metabolism or release was indicated by the parallel rise in 5-HIAA (Wurtman and Fernstrom, 1976). Moreover, when animals were given a carbohydrate meal along with an aromatic amino acid decarboxylase inhibitor, brain 5-hydroxytryptophan, the immediate precursor of serotonin, was significantly elevated (Jacoby et al., 1975a).

From the above, it is clear that chronic and acute changes in brain tryptophan availability appear to be a major determinant of brain serotonin synthesis. However, tryptophan availability may not be critical in the induction of brain serotonin and 5-HIAA diurnal rhythms. Originally, daily fluctuations of both whole brain (Wurtman and Fernstrom, 1972) and brain stem (Morgan et al., 1975) tryptophan were observed to positively correlate with brain serotonin. However, more recently, circadian modifications in tryptophan and serotonin were shown to be in opposite phase in the fronto-parietal cortex (Hery et al., 1977). Furthermore, tryptophan hydroxylase activity and its rhythm were found to vary depending on the serotonin containing cell groups in the rat brain stem (Kan et al., 1977). Thus, the importance of tryptophan availability in determining serotonin or 5-HIAA circadian rhythmicity is unclear. It is possible that under normal physiological conditions, the relative importance of tryptophan availability is a function of brain area.

In addition to tryptophan availability, serotonin synthesis may be influenced by the rate of neuronal firing (Boadle-Biber, 1979a). Stimulation of serotonergic neurons of the mid brain raphe was shown to increase serotonin synthesis from radiolabelled tryptophan (Shields and Eccleston, 1972). Similarly, in-vitro depolarization of rat brain stem slices in a potassium enriched incubation medium was found to enhance

tryptophan hydroxylase activity as measured by increased tryptophan trans-formation to 5-hydroxytryptophan (Hamon et al., 1979). Both calcium (Boadle-Biber, 1979a,b; Elks et al., 1979) and cyclic adenosine monophos-phate (CAMP) (Boadle-Biber, 1980) have been implicated in the induction of tryptophan hydroxylase activity since manipulations that elevate calcium within nerve tissue were observed to increase tryptophan hydroxylase activity. Similarly, procedures which removed calcium from the incubation medium were found to block enzyme activity following depolarization (Boadle-Biber, 1979b; Elks et al., 1979). In addition, the addition of CAMP has also been shown to increase tryptophan hydroxylase activity in-vitro (Boadle-Biber, 1980).

The role of calcium in the induction of tryptophan hydroxylase activity is not yet understood. Calcium has been shown to chelate trypto-phan which could result in an increase in tryptophan uptake into snyapto-somes (Bruinvels and Moleman, 1979). However, since administration of a calcium blocker to the incubation medium was found to decrease tryptophan activity with no effect on tryptophan uptake (Elks et al., 1979), this mechanism appears unlikely. Calcium induced activation of protein kinase which in turn stimulates tryptophan hydroxylase has also been proposed (Boadle-Biber, 1979a; Elks et al., 1979).

Although it is evident that the rate of neuronal firing may play a role in the regulation of serotonin synthesis through induction of tryptophan hydroxylase activity, activation of this enzyme has not always been followed by increased serotonin synthesis. Administration of pargy-line, a drug which increases serotonin, was reported to markedly reduce the stimulatory effect of neuronal depolarization on tryptophan conver-sion to serotonin (Hamon et al., 1979). In addition, in-vitro brain sero-

tonin synthesis was found inhibited when serotonin was added to the incubation medium (Young et al., 1978). Such results indicate that substrate inhibition may also regulate serotonin synthesis. However, feed back inhibition in the regulation of serotonin synthesis has not been universally supported. When rat brain serotonin levels were elevated pharmacologically with a monoamine oxidase inhibitor and animals then allowed to consume a carbohydrate, protein-free meal, the subsequent increase in brain hydroxyindole levels was comparable to those found in control rats not given the drug (Jacoby et al., 1975a). In addition, the normal diurnal increase in serotonin has been reported to be 25%. However, a 46% increase has been reported when carbohydrate plus a monoamine inhibitor was administered (Jacoby et al., 1975).

Thus, tryptophan availability, rate of neuronal firing, and substrate inhibition may be involved in the regulation of serotonin synthesis. Furthermore, these factors could work together in regulating serotonin production. The relative importance of each of these factors may be contingent upon brain region.

4. Tryptophan Metabolism and Regulation of Tryptophan Entry Across the Blood Brain Barrier.

Of all the essential amino acids, tryptophan has been found to occur in lowest concentration in protein, only 1.5% (Fernstrom, 1978). In contrast to other amino acids, 80%-90% of tryptophan has been observed to circulate bound to albumin while only 10%-20% has been found to circulate freely (Fernstrom, 1978). The metabolic fate of tryptophan is complex with only 1% utilized for serotonin synthesis (Fernstrom and Wurtman, 1976). The main metabolic pathways for tryptophan are depicted in

Figure 2 and include protein synthesis, nicotinic acid production, serotonin synthesis, melatonin formation, and production of other biologically inactive metabolites (Airaksinen and Airaksinen, 1978).

The uptake of tryptophan into the brain is thought to be controlled by three major factors: (1) the blood concentrations of the large neutral amino acids (phenylalanine, tyrosine, leucine, isoleucine, valine, and methionine) which compete for a common transport carrier (Bender, 1978); (2) the concentration of the small fraction of tryptophan not bound to albumin (Curzon and Knott, 1974); and (3) the time of day (Hery et al., 1972).

Competition between plasma tryptophan and the neutral amino acids as a regulatory mechanism was shown when fasted rats offered a protein-containing meal, i.e. lab chow, or a purified amino acid diet, exhibited a significant elevation in plasma tryptophan. But, no change in brain tryptophan was observed. However, if the same purified diet, lacking all the neutral amino acids except tryptophan was fed, elevation of those amino acids consumed was found in the plasma. Furthermore, brain tryptophan, serotonin, and 5-HIAA were also elevated. In contrast, when only aspartate and glutamine, which share a different brain transport carrier from the neutral amino acids, were omitted from the purified diet, all the consumed amino acids were increased in the plasma, while brain tryptophan remained unaffected (Fernstrom et al., 1973). A high correlation (.95) between brain tryptophan and the ratio of serum tryptophan to the sum of the competing neutral amino acids was subsequently demonstrated (Fernstrom and Faller, 1978). A much lower correlation (.66) was shown between brain tryptophan and plasma tryptophan alone (Fernstrom et al., 1973).

Nicotinic acid ⟶ NAD

↑

Quinolinic acid

↑

3-Hydroxyanthranilic acid ⟶ CO_2 + NH_3 + H_2)

B_6 ↑ 5

3-Hydroxykynurenine $\xrightarrow{B_6}$ Xanthurenic acid

↑

Kynurenine $\xrightarrow{B_6}$ Kynurenic acid

5,B_6 ⟶ Anthranilic acid

↑

Indole, skatole indoxylsulphate

Formylkynurenine

↑4

N-methyltryptamine 8 N,N-dimethyltryp-timine (DMT)

Protein ⟷ Tryptophan $\xrightarrow{2}$ Tryptamine ↑8 $\xrightarrow{3}$ Indoleacetic acid

5-Methoxy-DMT

↓1 ⟶ Indolepyruvic acid

8↑

5-Hydroxytryptophan (5-HTP)

5-Methoxytryptamine B_6 ↓2

7

N-methyl-5-HT $\xrightarrow{8}$ Bufotenine

8↗ N-Acetyl-5-HT ⟵ 5-Hydroxytryptamine (5-HT) ⟶ Glucuronides and sulphates

↓7 ↓3

Melatonin 5-Hydroxyindole-acetaldehyde

↓

5-Hydroxyindoleacetic acid (5-HIAA)   5-Hydroxytryptophol

Figure 2.[1] Main metabolic pathways of tryptophan, including certain enzymes and the occurrence of pyridoxal (B_6) as the coenzyme: (1) tryptophan-5-hydroxylase (tryptophan-5-mono-oxygenase, E.C. 1.14.16.1.); (2) aromatic L-amino acid decarboxylase (E.C.4.1.1.28); (3) monoamine oxidase [MAO, Amine oxidase (flavin-containing), E.C. 1.4.3.4]; (4) tryptophan pyrrolase (tryptophan oxidase, tryptophan-2,3-dioxygenase, E.D. 1.1.3.11.11); (5) kynurenmase (E.C. 3.7.1.3); (6) kynurenine trans-minase, (E.C. 2.6.1.7); (7) Hydroxyindole-O-methyltransferase; (8) indole-ethylamine-N-methyltransferase-(+-aromatic-amine-N-methyltrans-ferase?); B_6 pyridoxal phosphate, as coenzyme.

[1] (Airaksinen and Airaksinen, 1978.)

This alteration in plasma neutral amino acid concentration is believed to account for the elevated brain tryptophan concentration when a carbohydrate protein-free meal is consumed (Fernstrom and Faller, 1978; Fernstrom, 1975b; Fernstrom et al., 1973). Carbohydrate has been shown to increase brain tryptophan through insulin secretion. Insulin was shown to increase the ratio of plasma tryptophan to the neutral amino acids which compete for uptake across the blood brain barrier (Fernstrom et al., 1973). Thus, when a high carbohydrate meal is consumed, plasma tryptophan is readily transported into the brain. In contrast, when fasted rats consumed regular lab chow or a carbohydrate meal supplemented with 18% casein, brain tryptophan was not found to rise, despite insulin secretion (Fernstrom et al., 1973). With ingestion of protein, the plasma concentration of competing amino acids was observed to increase considerably more than tryptophan (Fernstrom et al., 1973). Similar findings of an increased ratio of plasma tryptophan to the amino acids which compete with it for brain uptake have been shown in fasted humans fed a glucose solution (Fernstrom and Wurtman, 1974).

Further evidence for the effect of carbohydrate on the plasma neutral amino acid pattern of humans comes from the following. When human subjects consumed high carbohydrate, protein-free meals for 5 days, the diurnal variations in plasma neutral amino acid ratios differed considerably from individuals eating 150 g of protein daily (Fernstrom and Wurtman, 1979). When 0 g of protein were consumed, the ratio of tryptophan to its competitors increased throughout the day until a peak at 1500 hrs and then a gradual decrease to nadir concentrations at 0700 hrs. This rhythm was in opposite phase to that observed in the group fed 150 g protein. Furthermore, the tryptophan to neutral amino acid ratios were consistently

higher at all time points assayed when the high carbohydrate meals alone were consumed (Fernstrom and Wurtman, 1979).

Although the relative concentrations of neutral amino acids play an important role in the regulation of tryptophan transportation across the blood brain barrier, other mechanisms have also been described. Clofibrate administration (Bloxam et al., 1980), starvation, and immobilization (Curzon et al., 1972) were shown to increase brain tryptophan (Bloxam et al., 1980; Curzon et al., 1972) and subsequently serotonin turnover (Curzon et al., 1972). Under these circumstances, plasma free fatty acids were shown to rise and displace tryptophan from albumin binding sites, hence rendering more free tryptophan available to the brain. Thus, the concentration of free tryptophan may play a role in regulating brain tryptophan following treatments that elevate free fatty acids. In addition, time of day has been demonstrated to influence the brain's ability to transport tryptophan (Hery et al., 1972). Hery et al. (1972), observed that radiolabelled tryptophan uptake into brain slices was higher during the light phase than during the dark period.

Thus, three factors appear to regulate tryptophan entry across the blood brain barrier: ratio of plasma tryptophan to the neutral amino acids, concentration of free tryptophan, and time of day.

5. Serotonin and Behavior.

Although considerable research has centered around the regulation of serotonin synthesis, the functional significance of elevated brain serotonin has still not been well established. However, numerous behaviors have been linked to changes in brain serotonin content.

Behaviors motivated by negative reinforcement have been related to

serotonin. Destruction of serotonergic neurons by intracisternal injection of 5,6 dihydroxytryptamine (Lin et al., 1978) or electrolytic lesions (Kohler and Lorens, 1978) was observed to facilitate learning of a 2-way active avoidance shuttle box task. P-chlorophenylalanine, (PCPA), a tryptophan hydroxylase inhibitor, was shown to enhance Y-maze avoidance acquisition, while P-chloroamphetamine was found to improve shuttle box avoidance (Vorhees, 1979). Furthermore, lesions of the raphe nucleus were shown to facilitate a learned taste aversion and 5-hydroxytryptophan administration was found to reverse this effect (Lorden and Oltmens, 1978).

In contrast, 2-way shuttle box avoidance was unaffected and learning of an unsignalled 1-way avoidance response was impaired with PCPA administration (Kohler and Lorens, 1978). Lack of agreement with other PCPA data on shuttle box avoidance may be related to the shorter experimental period employed by Kohler and Lorens (1979) (2 days). Vorhees (1979) observed that PCPA did not exert its effect until the third day of testing.

In addition to avoidance testing, serotonin has been associated with memory (Essman, 1977). Stimulation of the dorsal raphe nuclei impaired the memory of a passive-avoidance response and PCPA blocked this effect (Fibiger et al., 1978). Similarly, PCPA markedly increased extinction in a punished step-down paradigm (Beninger and Phillips, 1979).

Serotonin has also been implicated in several homeostatic behaviors, particularly in the regulation of food intake. Administration of 5-hydroxytryptophan was observed to decrease food consumption, lower meal size, and depress rate of eating per meal (Blundell and Latham, 1979). Such results were in accord with the effects of fenfluramine (Blundell and Leshem, 1975) and Lilly 110140 (Goudie, 1976) drugs known to poten-

tiate serotonin action. Similarly, PCPA treatment was shown to result in hyperphagia and dose dependent increases in food ingestion (Mackenzie et al., 1979; Coscina et al., 1978).

In contrast to the above, tryptophan injections were reported to have no effect on food consumption in deprived rats (Weinberger et al., 1979). Similar findings have been obtained with a methyl ester of PCPA, a serotonin depleter. In addition, intraventricular injection of the amino acid methyl esters of leucine and tryptophan was shown to result in hyperphagia, but no association was observed between over eating and a reduction in brain serotonin (Mackenzie et al., 1979).

In addition to serotonin's possible role in the suppression of food intake, its function in the regulation of either protein or carbohydrate consumption has been discussed (Anderson, 1979; Wurtman and Wurtman, 1979). In the self-selecting rat, an inverse relationship, (r=-.96), was reported between protein intake and the plasma tryptophan concentration relative to its competitors. This plasma amino acid pattern has been directly associated with serotonin concentration (Anderson, 1979). Uncontrolled diabetes, a disease that causes both a marked decrease in the ratio of tryptophan to the neutral amino acids and whole brain tryptophan, was also observed to elevate protein intake (Woodger et al., 1979). Conversely, PCPA administration, mid-brain raphe lesions, and 5,7 dihydroxytryptamine destruction of serotonergic neurons were found to decrease protein intake. Reduced protein consumption was shown to be directly associated with depressed serotonin and 5-HIAA (Ashley et al., 1979). Finally, drugs thought to enhance serotonergic transmission, i.e. fenfluramine, fluoxetine, or MK 212, were observed to selectively decrease consumption of carbohydrate without influencing protein intake (Wurtman and Wurtman,

1979). Thus, serotonergic neurons appear to regulate food intake but, their exact function has not been established.

Inhibition of aggression may be an affective behavior mediated by serotonin. Chlorimipramine, a serotonin re-uptake blocker, was shown to inhibit muricide in natural killer rats but was not found to prevent mouse killing in rats with lesions of the ascending projection of the dorsal and median raphe nuclei (Marks et al., 1978). In addition, raphe lesions were observed to induce aggressive behaviors such as muricide, an increased flight response to tail pinching, a greater struggle response to capturing with a gloved hand, and more squealing upon being caught (Yamaoto and Ueki, 1978). Several of these behaviors were shown to be inhibited by 5-hydroxy-tryptophan, imipramine, and chloroimipramine (Yamaoto and Ueki, 1978). Shock induced fighting was also found to be facilitated in rats offered a tryptophan free diet, although supplementing chow with 0.5% tryptophan had no effect on shock induced fighting or muricide (Kantak et al., 1980).

In contrast, mice offered a purified 12% casein diet supplemented with 0.25 or 0.5% tryptophan were found to exhibit increased territorial aggression (Thurmond et al., 1980). However, a 1% tryptophan supplement had no effect on this behavior. After 6 weeks on the tryptophan supple-mented diets, no diets were observed to alter territorial aggression, despite increased levels of brain tryptophan and serotonin. Interestingly, this same 12% casein diet supplemented with leucine, rather than tryptophan, was shown to lower brain tryptophan, tyrosine, and serotonin, but not to influence aggressive behavior (Thurmond, et al., 1980). Although Thurmond's findings do not support the belief that serotonin inhibits aggressive behavior, his contrasting results could be due to the differ-

ences in experimental design. Both the type of aggression tested and the species differed.

In addition to the above affective behavior, serotonin has been associated with sleep (Essman, 1978). Tryptophan administration to humans (Hartman, 1978) was reported to reduce sleep latency and to reduce both sleep latency and REM (rapid eye movement) latency in rats (Hill and Reyes, 1978). Recently, 4 g of tryptophan was shown to increase the duration of stage 3 sleep in humans, but, only trends were found for decreased awake activity, increased REM, and decreased drowsy sleep (Nicholson and Stone, 1979). Also, tryptophan was observed to decrease slow wave sleep latency in rats. This reduced latency correlated with elevated serotonin and 5-HIAA, and depressed dopamine and homovanillic acid (Fornal, et al.,1979). However, when a tryptophan analogue was administered, the effect on sleep latency and the catecholamines, including norepinephrine, was repeated. Serotonin was only slightly increased and no differences in 5-HIAA were obtained (Fornal et al., 1979). Therefore, the tryptophan effect was thought to be due to a weakening of catecholamine function (Fornal et al., 1979).

Locomotor activity has also been related to serotonin (Warbritton et al., 1978). Direct infusion of serotonin into the right lateral cerebral ventricle of rats was reported to produce dose dependent decreases in spontaneous locomotor behavior as measured by a 6-channel electronic activity monitor (Warbritton et al., 1978). In addition, when rats (Fibiger and Campbell, 1971) or mice (Modigh, 1972) were injected with 5-hydroxytryptophan and placed in a motility meter (Modigh, 1972) or a stabilimeter (Fibiger and Campbell, 1971), decreased locomotor activity was observed. Rats injected with 5-hydroxytryptophan, similarly

exhibited decreased bar pressing for food which correlated with increased brain serotonin (Aprison and Hingten, 1962).

In accord with the above, procedures that deplete serotonin have been observed to increase locomotor activity. Destruction of the serotonergic neurons by selective lesions of the raphe nuclei, were reported to induce hyperactivity when rats were tested in a stabilimeter (Geyer et al., 1976; Jacobs, 1974). This effect was potentiated following amphetamine administration (Geyer et al., 1976). In addition, when measured in either a stabilimeter or a running wheel, dose dependent increases in locomotor activity were produced when rats were given PCPA (Mabry and Campbell, 1973; Fibiger and Campbell, 1971). PCPA has also been reported to potentiate the effect of amphetamine on locomotor activity (Breese et al., 1974). In addition, injections of 5-hydroxytryptophan were shown to reverse the hyperactivity induced in rats by amphetamine or apomorphine, dopamine stimulants (Baldessarini et al., 1975; Breese et al., 1974; Mabry and Campbell, 1973). Moreover, a carbohydrate induced increase in serotonin also reversed the hyperactivity induced in rats injected with cocaine, and weakened the effect of amphetamine (Taylor and Ho, 1979).

Brain serotonin has also been linked to exploration. Rats injected with PCPA were reported to make more head dips in a hole board apparatus, and both between and within session habituation were found prolonged (File, 1977). In addition, chlorpromazine, a drug which increases brain tryptophan and serotonin, was shown to decrease the number of head dips and holes explored in the same apparatus (File and Pope, 1974). Furthermore, mice intraventricularly injected with serotonin were observed to walk and rear less than mice injected with vehicle (Herman, 1975). More-

over, very low doses of tryptophan have been reported to reduce locomotion of rats in an open field (Tricklebank et al., 1978; Taylor, 1976). However, when methionine, an amino acid which competes with tryptophan for uptake across the blood brain barrier, was administered with tryptophan, no decrease in locomotion was observed (Taylor, 1976).

Thus, serotonin has been associated with many behaviors. Although results are controversial, in general, serotonin appears to function as an inhibitor of most behaviors discussed.

6. Serotonin and Corticosterone.

The role of serotonin in the regulation of the hypothalamus/pituitary/adrenal axis has been widely researched.

Complete or frontal deafferentation of the rat hypothalamus, the probable locus of the cortical releasing hormone (CRH) neurons (Yuwiller, 1979), was reported to deplete serotonin and elevate corticosterone (Vermes et al., 1973). In addition, the adrenal response to ether and surgical stress was found blocked due to implantation of serotonin into the medial hypothalamus (Vermes and Telegdy, 1972). Reserpine, a serotonin blocker, was observed to increase ACTH in dogs (Egdahl et al., 1956). Elevated plasma corticosterone was also found in response to ether stress when rats were administered PCPA (Vermes and Telegdy, 1973).

Thus, the above studies seem to suggest that serotonin inhibits the release of hormones of the hypothalamus/pituitary/adrenal system. However, results to the contrary have also been reported. Intraperitoneal injection of 5-hydroxytryptophan was found to elevate plasma corticosterone (Popova et al., 1972). Furthermore, increased corticosterone was also observed

in rats injected with 5-hydroxytryptophan (Fuller and Snoddy, 1979), fluoxetine, an inhibitor of serotonin uptake (Fuller and Snoddy, 1979), and quipazine (Fuller and Snoddy, 1979) and fenfluramine (Schettini et al., 1979), serotonin agonists. Similarly, electrolytic lesions of the nucleus raphe median or intraventricular injection of 5,7 dihydroxy-tryptamine, a serotonin neurotoxin, was shown to block the fenfluramine induced increase in plasma corticosterone (Schettini et al., 1979). In addition, metergaline, a serotonin antagonist, was found to reverse the increased corticosterone response produced by quipazine (Fuller and Snoddy, 1979). Finally, Modlinger et al. (1979, 1980) observed that an oral dose of 10 g tryptophan in humans increased plasma cortisol.

From the above, it would appear that serotonin plays a role in the regulation of corticosterone output. The heterogeneity of results may indicate that serotonin is involved in both inhibition and stimulation of corticosterone release. In this regard, it is possible that more than one system of CRF neurons could exist and that they could respond to serotonin in different ways (Yuwiller, 1979). In addition, other systems have also been reported to mediate the output of CRF (Yuwiller, 1979). Thus, the investigation of serotonin's role in the regulation of the hypothalamus/pituitary/adrenal axis is very complicated. A more complete understanding may not be achieved until the locus of the CRF neurons has been established and serotonin pathways innervating the CRF neurons have been identified.

7. Summary.

Fasted rats fed a high carbohydrate meal or injected with tryptophan have been shown to exhibit an elevation in brain serotonin. However, the behavioral consequences of this neurochemical increase have not been well established. In addition, the role of serotonin in the regulation of corticosterone output is still poorly understood. Thus, the present study was conducted to: (1) investigate the significance of the brain serotonin elevation relative to exploration of a novel environment, a behavior related to brain serotonin content; and (2) determine plasma corticosterone concentrations following tryptophan and carbohydrate induced increases in brain serotonin.

8. Rationale.

Elevated brain serotonin has been shown to occur in rats injected with tryptophan or fed a high carbohydrate meal. In addition, reduced exploratory behavior has been observed when serotonin has been increased. Thus, it is hypothesized that fasted rats fed a high carbohydrate meal or injected with tryptophan will display decreased exploratory behavior relative to controls and that the reduction in exploration will be positively correlated with increased serotonin. Serotonin has been implicated as both an inhibitor and stimulator of the hypothalamus/pituitary/adrenal axis. However, it is expected that the tryptophan and carbohydrate induced increase in serotonin will increase plasma corticosterone levels since elevated cortisol output has been observed in humans administered an oral dose of tryptophan. Similarly, rats given a sucrose solution have also been shown to exhibit an increase in plasma corticosterone (Hart et al., 1980).

III. GENERAL METHODS

1. Animals and Rations.

Male Wistar rats weighing 220-280 g were used in all studies. Eight
to 10 days prior to each experiment, rats were offered water and a puri-
fied control diet (Appendix Table I) ad libitum. According to the National
Research Council, this diet meets the nutrient requirements of growing
rats (N.R.C., 1978). Animals were housed individually in suspended wire
mesh cages and exposed to light for 12 hrs. daily, 0600 to 1800 hrs.
Room temperature was maintained at 21 ± 2°C. Stress has been shown to
increase brain serotonin turnover in the rat (Curzon et al., 1972). Thus, all
animals were handled twice daily in order to acclimatize them to the
handling that would occur on the day of testing.

2. Biochemical Determinations.

Whole brain tryptophan was measured using the methods of Denkla and
Dewey (1967), as modified by Bloxam and Warren (1974), and whole brain
serotonin was assayed by the method of Curzon and Green (1970). Plasma
corticosterone was measured by the method of Glick et al., (1964).

Double distilled water was used to make up all reagents and solu-
tions. All water was deionized through a purification cartridge
(Barnstead High Capacity, Barnstead Company, Boston, Mass.) and was sub-
sequently distilled. High purity HCl (HCl Aristar, British Drug House,
Vancouver, B.C.) was used in the preparation of all HCl containing solu-
tions.

Tryptophan, corticosterone, and serotonin stock solutions and the
H_2SO_4/ETOH mixture were stored at 4°C. Ortho-phthaldehyde (OPT) (Sigma

Chemical Company, Saint Louis, Missouri) was stored in a dark bottle at -20°C. Serotonin creatinine sulphate (Sigma Chemical Company) and corticosterone (Sigma Chemical Company) were kept in a dessicator at 4°C and all other chemicals were maintained at room temperature. The OPT and cysteine (British Drug House) solutions were prepared immediately before use.

Prior to analyses, frozen tissues were homogenized in ice cold butanol acidified with 0.01 N HCl and centrifuged (1000 x G) at 4°C for 10 min. Four ml of the butanol supernatant were then added to 5 ml cold heptane plus 0.5 ml of 1% L-cysteine in 0.1 N HCl. After vortexing for 2.5 min, samples were again centrifuged as above. The butanol/heptane phase was subsequently aspirated and aliquots of the HCl extract were used for brain tryptophan and serotonin analysis. Samples were kept ice cold throughout processing.

Brain Tryptophan.   An aliquot of 0.1 ml of the HCl extract was added to 2 ml cold 10% TCA with 0.2 ml of 2% formaldehyde. Next, 0.1 ml of $FeCl_3$ (6.0×10^{-3} in cold 10% TCA) was added and the tubes immediately placed in a boiling water bath. The fluorophore norharmen was produced during this heating procedure. Tubes were then cooled, replenished to 2.4 ml with 10% TCA, and read fluorometrically at wavelengths of 370/452 mu (Farrand Manual Spectrofluorometer, Farrand Optical Inc.). Brain tryptophan was expressed as ug/g brain.

The working tryptophan (J.T. Baker, Chemical Co., Phillipsburg, N.J.) stock solution was prepared by diluting a 40.8 ug/ml tryptophan solution (4.08 mg/100 ml 0.1 N NH_4OH) to 4.08 ug/ml. A standard curve was read daily at concentrations of 0.17 ug, 0.34 ug and 0.51 ug of tryptophan/tube.

Brain Serotonin.   To an 0.3 aliquot of the HCl extract was added 0.2 ml of OPT (0.5% in methanol) and 1.5 ml of concentrated HCl.   After vortexing briefly, samples were placed in a boiling water bath for 10 min, cooled, and replenished to 2 ml with concentrated HCl.   Brain serotonin was read fluorometrically at wave lengths of 360/470 mu and expressed as ug/g brain.

The serotonin stock solution, prepared from 40 mg of serotonin creatinine sulphate in 100 ml 0.01 N HCl, was serially diluted to 40 ug/ml. A standard curve was read daily from duplicates of 4 ug/ml, 2 ug/ml, and 1 ug/ml of serotonin.

Plasma Corticosterone.   To 0.05 ml plasma were added 0.50 ml distilled water and 0.03 ml iso-octane.   Samples were then vortexed 15 sec and centrifuged 4 min at (1000 x G).   Next, the iso-octane supernatant was aspirated and 0.7 ml of chloroform were added.   Samples were vortexed and centrifuged as above.   The supernatant was again removed and 0.05 ml of 0.1 N NaOH were added to the remaining chloroform layer.   Tubes were subsequently vortexed, and centrifuged as previously described and the NaOH supernatant aspirated.   Next, 0.35 ml of a H_2SO_4/ETOH (13:7) mixture were added, vortexed and again centrifuged.   Finally, the chloroform supernatant was removed, the remaining H_2SO_4/ETOH phase was transferred to 1 ml glass cuvettes, and read fluorometrically using a Turner 110 fluorometer, with 47-B and 2A-12 primary and secondary filters.   Corticosterone was expressed as ug/100 ml plasma.

A working corticosterone stock solution was prepared from 1000 ug/ 100 ml in ETOH.   Concentrations of 10 ug/100 ml, 20 ug/100 ml, 30 ug/ 100 ml, and 40 ug/100 ml were read daily to produce a standard curve.

IV.   EXPERIMENT 1

BRAIN TRYPTOPHAN DURING THE DARK PHASE AFTER FASTING

1.   Introduction.

During the dark phase of the lighting cycle, a gradual increase in whole brain tryptophan has been demonstrated in rats fed ad libitum (Wurtman and Fernstrom, 1972). However, the effect of fasting on this daily elevation has not been studied. Thus, the objectives of the first experiment were:

1)   to determine if a 16 hour fast, terminated at various times during the dark cycle, would alter brain tryptophan rhythmicity;

2)   to establish the nadir in brain tryptophan during the dark period.

These results would establish the starting time of the second experiment.

2.   Experimental Procedure.

Thirty rats were randomly assigned to 5 groups, offered water ad libitum, but deprived of food at either 2400, 0200, 0400, 0800, or 1200 hrs. Sixteen hours later, rats were decapitated at either 1600, 1800, 2000, 2400, or 0400 hrs respectively. Brains were removed, immediately frozen in liquid nitrogen, and stored at -70° for determination of brain tryptophan.

The data were analyzed by a 1-way analysis of variance (ANOVA).

3.   Results and Discussion.

Table I shows the mean concentrations of whole brain tryptophan in rats fasted 16 hrs. The ANOVA indicated that during the dark phase, brain tryptophan did not differ when rats were deprived of food for 16 hrs.

TABLE I

BRAIN TRYPTOPHAN FOLLOWING A 16 HOUR FAST

Time	Brain Tryptophan (ug/g $\pm$ SEM)
1600 (5)[*]	3.36 $\pm$ 0.14
1800 (6)	3.30 $\pm$ 0.12
2000 (6)	3.46 $\pm$ 0.16
2400 (6)	3.24 $\pm$ 0.08
0400 (5)	3.55 $\pm$ 0.11

* Number of animals/group

During the dark phase, rats fed ad libitum have been observed to exhibit a gradual increase in whole brain (Wurtman and Fernstrom, 1972), brain stem (Morgan et al., 1975), cerebral cortex (Hery et al., 1977), and fronto-parietal cerebral cortex (Hery et al., 1977) tryptophan. Daily fluctuations in the ratio of plasma tryptophan to the neutral amino acids are believed to generate the brain tryptophan rhythm (Fernstrom and Wurtman, 1979). Moreover, considerable evidence has indicated that the cyclical consumption of protein contributes to the daily change in plasma neutral amino acids (Fernstrom et al., 1979b). Thus, Fernstrom (1979) has proposed that the daily intake of protein generates the rhythm in brain tryptophan.

Results from the present study support the possibility that food intake may contribute to the daily increase in tryptophan during the dark phase. However, this interpretation must be viewed with caution because an ad libitum control was not included in this experiment. These data further indicated that brain tryptophan did not fluctuate during the dark period after a 16 hour fast.

V. EXPERIMENT 2

EFFECT OF TRYPTOPHAN ADMINISTRATION AND CARBOHYDRATE INGESTION
ON BRAIN TRYPTOPHAN AND SEROTONIN

1. Introduction.

Brain tryptophan and serotonin have repeatedly been shown to increase in rats fasted over night and injected with tryptophan, or offered a high carbohydrate, protein-free meal at the beginning of the light phase. Whether this same phenomenon occurs when rats are fasted primarily during the light cycle and then fed carbohydrate at the beginning of the dark period has not been determined. Thus, the purpose of the second experiment was to determine the time course and peak levels of brain tryptophan and serotonin in fasted rats fed a control meal, a high carbohydrate, protein-free meal, or injected with tryptophan early in the dark phase.

2. Experimental Procedure.

The experimental design is summarized in Figure 3. At 0030 hrs rats were randomly assigned to 4 groups and deprived of food but permitted free access to water. At 1730 hrs the fast was terminated and rats were offered water ad libitum and treated as follows:

Group 1 - decapitated, brains removed, frozen in liquid
nitrogen, and stored at -70°C;

Group 2 - injected with 0.9% saline, (pH 9.5) and offered the
purified control diet ad libitum;

Group 3 - injected with 0.9% saline, (pH 9.5) and offered the
protein-free purified diet, isocaloric to the con-
trol diet with protein derived calories replaced
with carbohydrate (Table 1 - appendix);

Group 4 - injected with L-tryptophan, (50mg/kg) dissolved in
saline (pH 9.5) and offered the control diet ad
libitum.

ANIMALS FASTED
0030

1730

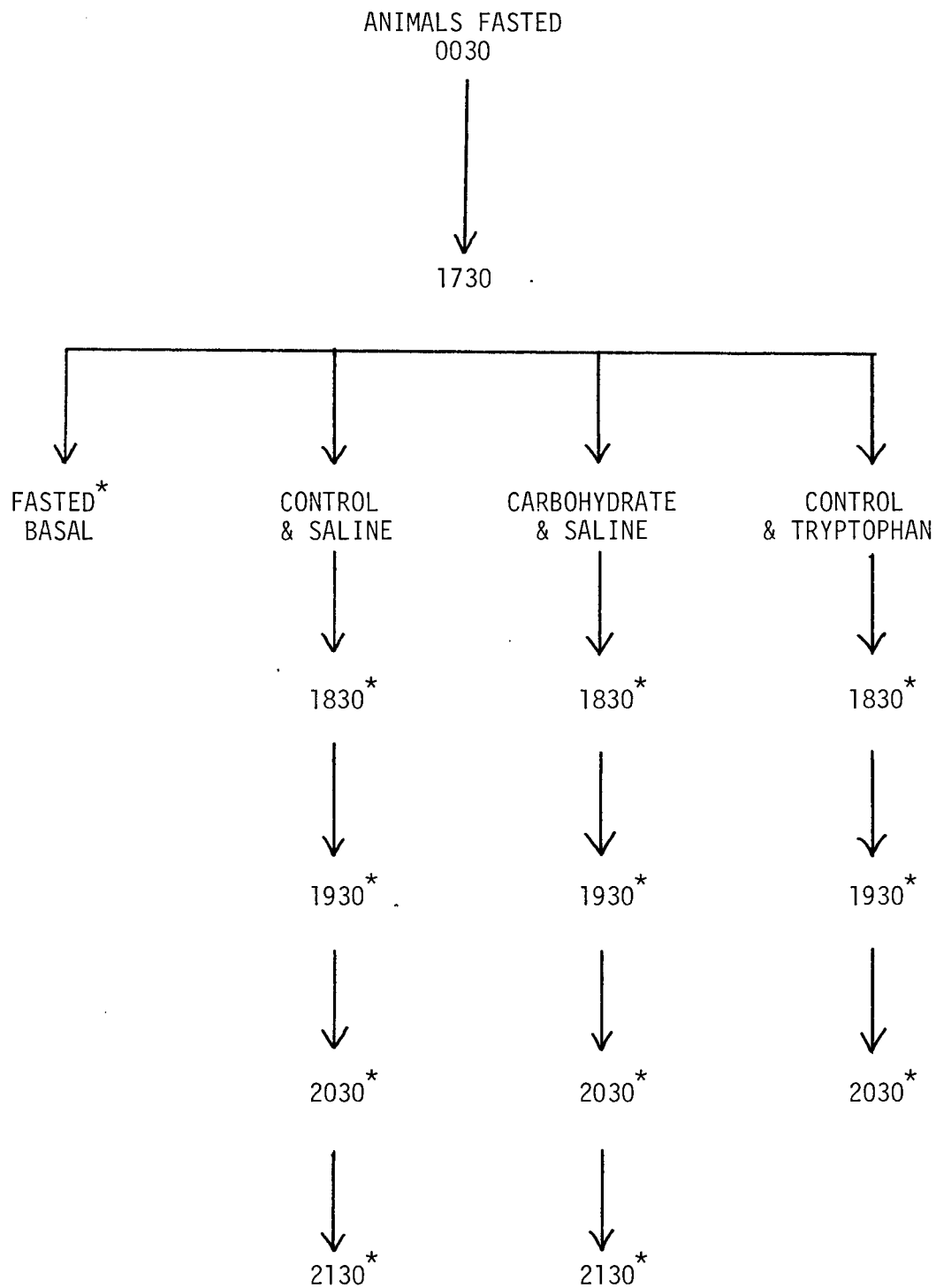| FASTED[*] BASAL	CONTROL & SALINE	CARBOHYDRATE & SALINE	CONTROL & TRYPTOPHAN
	1830[*]	1830[*]	1830[*]
	1930[*]	1930[*]	1930[*]
	2030[*]	2030[*]	2030[*]
	2130[*]	2130[*]	

Figure 3.   Effect of tryptophan administration and carbo-
hydrate ingestion on brain tryptophan and
serotonin: Experimental design.

[*]Brains removed for analysis of brain tryptophan and
serotonin.

One hour post treatment and hourly for the next 3 hours, animals from groups 2, 3, and 4 were decapitated and brains removed, frozen in liquid nitrogen, and stored at -70°C. Brain tryptophan and serotonin were analyzed by a Group (3) x Time (4) ANOVA.

3. Results.

Brain Tryptophan. The time course and peak levels of brain tryptophan in fasted rats offered a control meal, a high carbohydrate meal, or injected with tryptophan are illustrated in Figure 4 and Appendix Table II. The ANOVA revealed a Group x Time interaction $F(5,51)=29.27$, $p < .001$. Tests for simple main effects indicated a main effect of group at 1 hour, $F(2,51)=101.75$, $p < .01$, 2 hours $F(2,51)=4.29$, $p < .05$, 3 hours $F(2,51)=4.44$, $p < .05$, and 4 hours $F(2,51)=3.31$, $p < .05$, post treatment. Neuman Keuls post hoc analyses showed that animals injected with tryptophan had higher brain tryptophan levels than controls, $p < .01$, and carbohydrate treated rats, $p < .01$, at 1 hour post injection. Brain tryptophan of tryptophan injected animals fell to control levels by 2 hours. When compared to controls, brain tryptophan was also elevated, $p < .05$, in rats offered the high carbohydrate meal. Although the difference continued for 4 hours post treatment, $p < .05$, the greatest difference occurred 2 hours after food presentation.

Brain Serotonin. Figure 5 and Appendix Table III illustrate the time course of brain serotonin in fasted rats fed either a control diet, a high carbohydrate meal, or injected with tryptophan. The ANOVA indicated a main effect of group $F(2,51)=6.50$, $p < .001$, and Neumann Keuls post hoc analyses revealed that brain serotonin in rats injected with tryptophan or offered the high carbohydrate, protein-free meal was elevated over that of controls, $p < .05$.
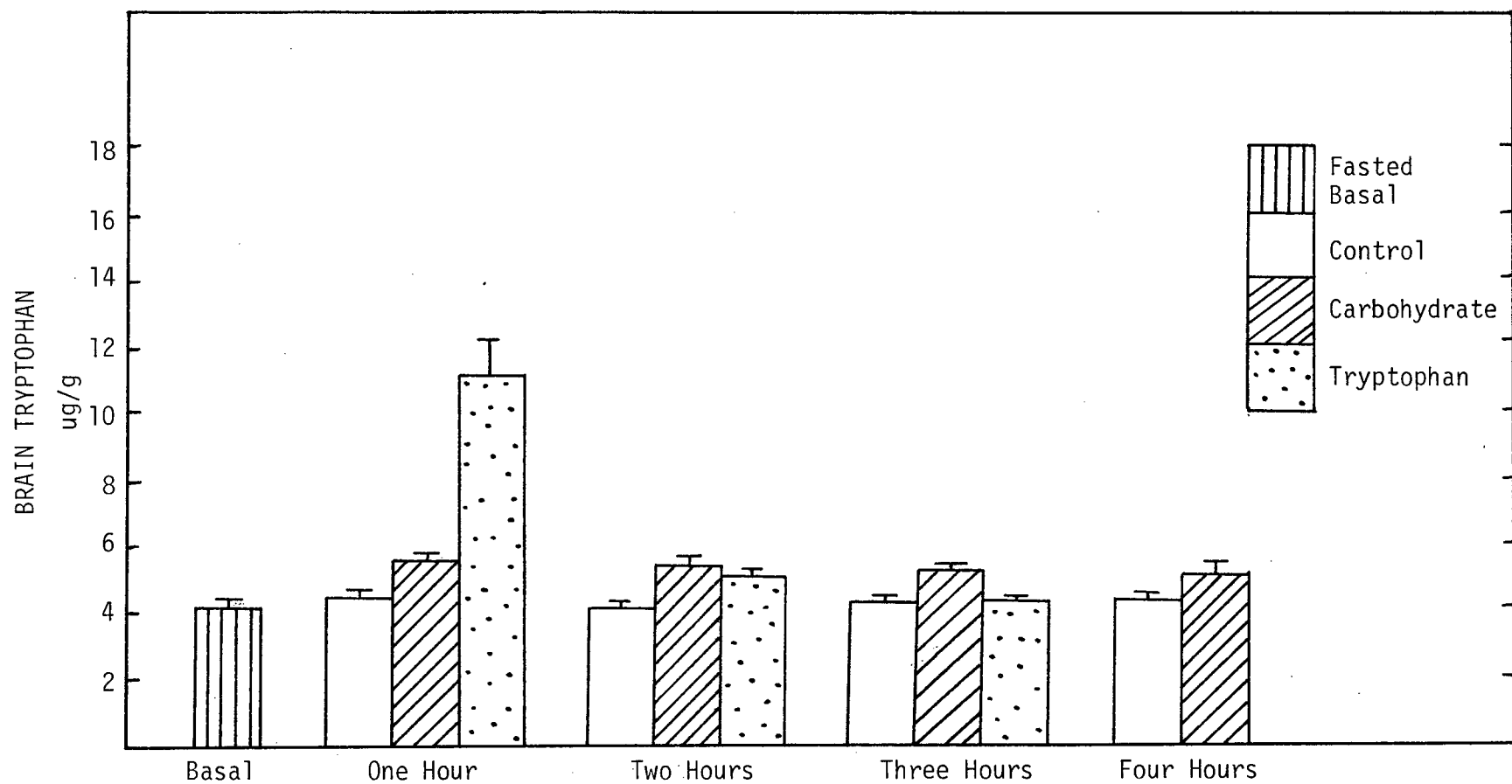
Figure 4. Brain tryptophan following tryptophan administration or ingestion of carbohydrate (mean + SEM)
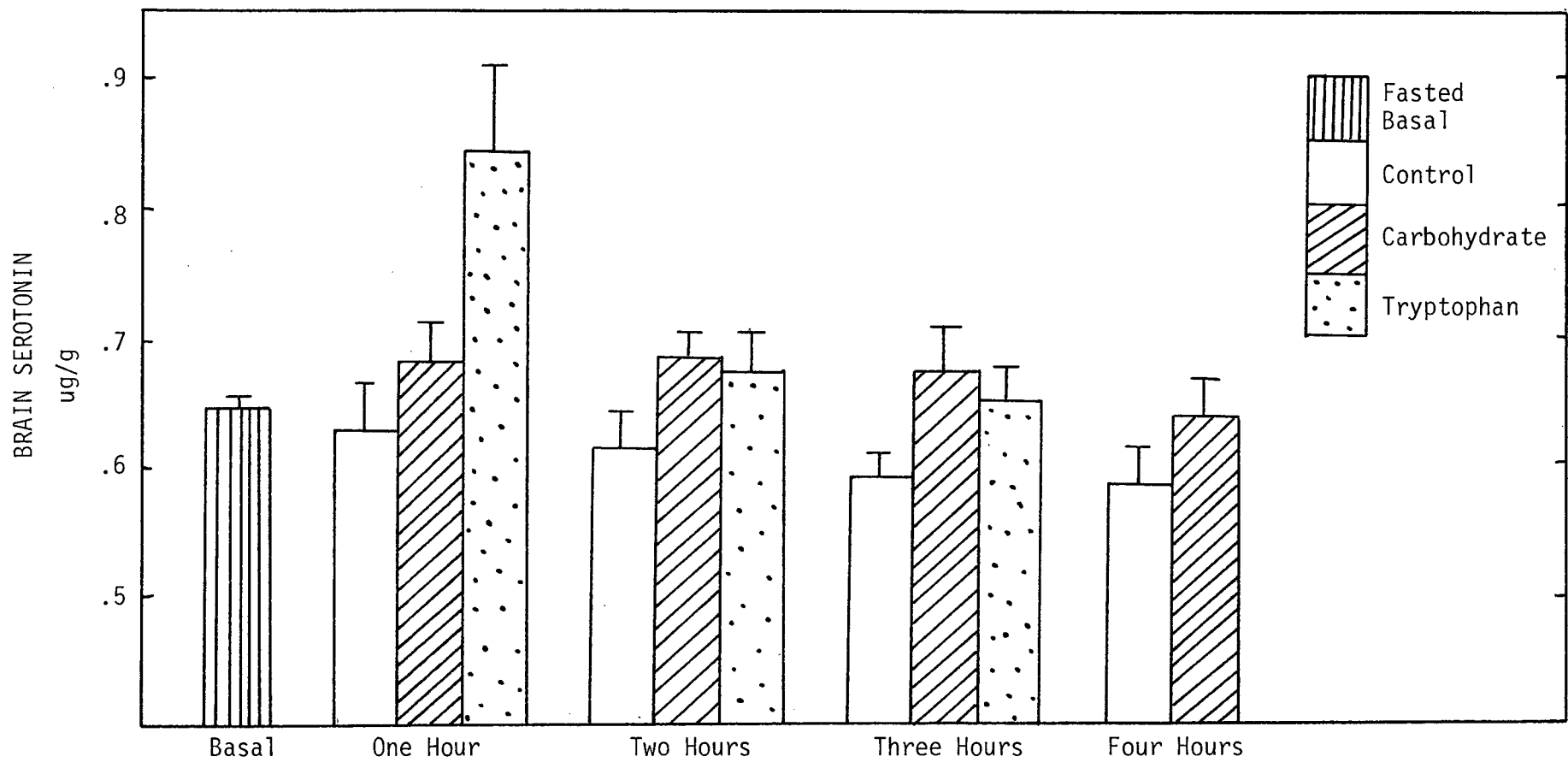
Figure 5: Brain serotonin after tryptophan administration or carbohydrate ingestion (mean + SEM)

4. Discussion.

These data are in accordance with the findings of Fernstrom (1971, 1975b). Furthermore, the results demonstrate that the carbohydrate induced increase in brain tryptophan and serotonin content occurs during the early stage of the dark cycle. As previously observed (Fernstrom, 1971), brain tryptophan in this study peaked 1 hour after tryptophan injection. Feeding carbohydrate generated the greatest change in brain serotonin at 2 and 3 hours after food intake.

However, in contrast to earlier research, the percent increases in brain tryptophan and serotonin were small in the present study. Such results are not surprising. Reduced radiolabelled tryptophan uptake in both hypothalamus and brain stem slices has been observed during the dark phase (Hery et al., 1972). Also, reduced tryptophan availability could explain the smaller percent increase in serotonin obtained in this study. However, a greater turnover of serotonin to 5-HIAA may have occurred as well, since increased serotonin turnover has been demonstated during the dark phase (Hery et al., 1972).

Thus, the results of this experiment indicated that tryptophan dosing and carbohydrate ingestion resulted in elevated brain tryptophan and serotonin during the dark phase. The greatest change was observed at 1 and 2 hours post treatment.

VI. EXPERIMENT 3

BEHAVIORAL EFFECTS OF TRYPTOPHAN ADMINISTRATION

This experiment was conducted to establish a behavioral correlate of increased brain serotonin concentration. Three behaviors believed to be associated with serotonin were assessed: exploratory behavior, acquisition of a passive-avoidance response, and extinction.

1. Experimental Procedure.

The experimental procedure is summarized in Figure 6. Rats were offered purified control diet and water ad libitum. From 1700 to 2000 hrs on the training night, 48 rats were randomly selected and injected with either 0.9% saline (pH 9.5) or 50 mg/kg tryptophan in 0.9% saline (pH 9.5). On the nights of extinction, the 2 groups were further divided so that half the original saline and tryptophan animals were administered saline and half were injected with tryptophan. Thus, 4 groups of 12 rats were established and injected from 1800 to 1900 hrs. Behavioral testing occurred 1 hour post injection, the time when brain serotonin was shown to peak.

Animals were tested in a dark, sound controlled room (white noise, 56db) adjacent to the animal quarters. The behavioral apparatus was a wooden box, 25 x 29 x 40 cm, having one plexiglass wall for observation and a copper grid floor which could be electrified with a scrambled 1.25 mamp current. A platform, 7.5 x 25 x 7 cm, extended across 1 wall of the apparatus and a 20 watt incandescent light evenly illuminated the box.

One hour after injection, each rat was individually placed on the narrow platform and the latency to step-down and explore the experimental

NIGHT 1 - TRAINING

SALINE   TRYPTOPHAN

NIGHTS 2-5 - EXTINCTION

SALINE   TRYPTOPHAN   SALINE   TRYPTOPHAN
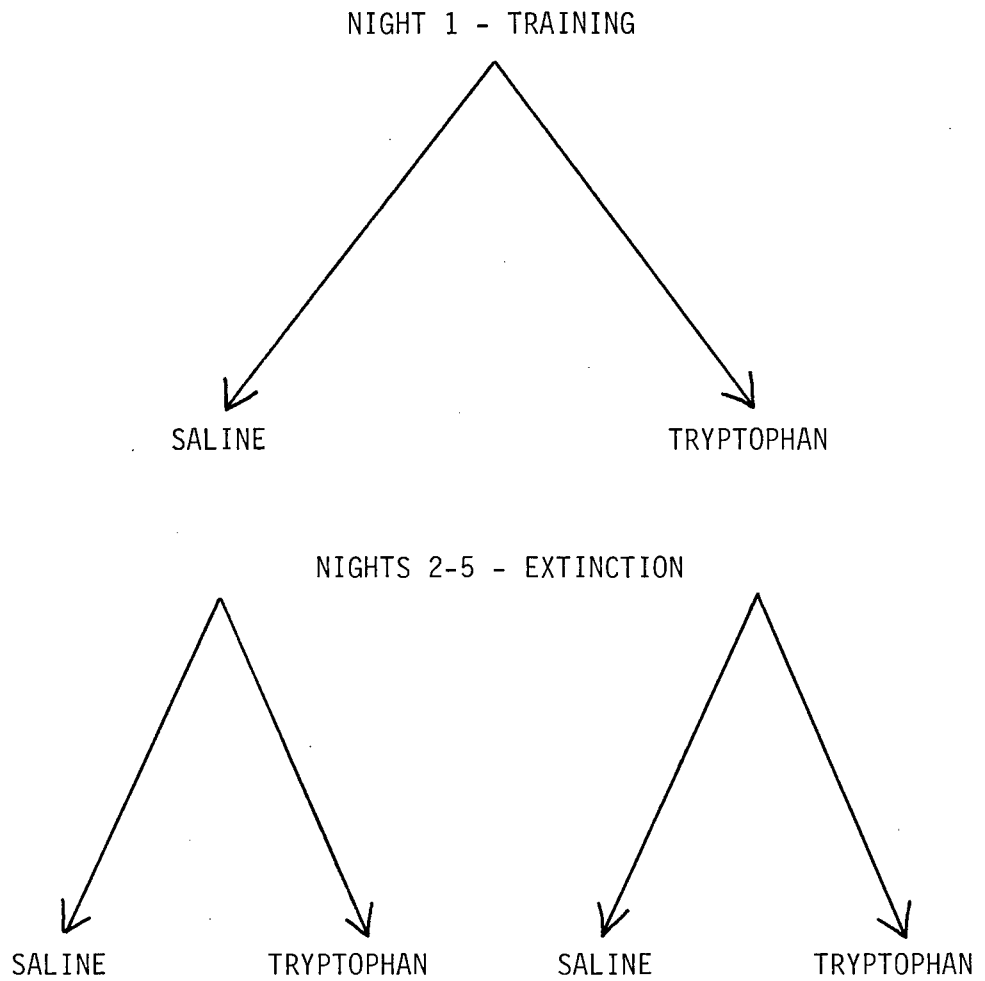
Figure 6. Behavioral effects of tryptophan
           administration: Experimental design.

box on 2 - 1 min trials was measured by 2 trained observers. Between

trials, rats were returned to a small plastic holding cage for 1 minute.

On the third trial, the grid floor was electrified and the following

recorded:

(1) latency to escape shock;

(2) total number of step-downs (4 feet on floor);

(3) total number of step-down. attempts (1-3 feet on floor);

(4) total time to reach criterion (remain on the platform for 2
     consecutive minutes).

During extinction trials (3 min duration), run on the 5 subse-

quent nights, the following behaviors were recorded:

(1) extinction (latency to step-down and remain on the floor a
     criterion of 30 seconds);

(2) number of step-down attempts (1-3 feet on floor or 4 feet on the
     floor but failure to meet the 30 second criterion);

(3) number of step-down attempts and total time to meet criterion on
     the first night of extinction;

(4) total nights to reach criterion;

(5) total seconds to extinguish;

(6) total step-down attempts prior to extinction.

Rats were eliminated from the experiment once extinction occurred.

Measures recorded during learning and the extinction trials were

analyzed by the Student's t-test and 1-way ANOVA respectively. A Group (2)

x Time (2) ANOVA with time as a repeated measure was used to test the mean

latencies to step-down on 2 trials.

2. Results.

Acquisition/Extinction Testing.   The group means for each measure

taken during acquisition and extinction are shown in Table II and Table III.

No differences were observed for any of the parameters tested.

TABLE II

ACQUISITION OF AN AVOIDANCE RESPONSE FOLLOWING INJECTION OF SALINE OR
TRYPTOPHAN

Treatment	Escape latency	Step-down attempts	Step-downs	Total time
Control (23)* (Saline)	$28.91 \pm 4.43^{\dagger}$	(24) $1.33 \pm 0.36^{\ddagger}$	(24) 0.33 ± 0.13	(24) $177.17 \pm 10.71^{\dagger}$
Tryptophan (24)	34.89 ± 10.57	(24) 0.96 ± 0.27	(24) 0.25 ± 0.11	(24) 189.33 ± 13.54

* Number of animals/group

† Mean seconds $\pm$ SEM

‡ Mean number of step-down attempts/trial

TABLE III
EFFECT OF TRYPTOPHAN OR SALINE ON EXTINCTION

Treatment Training	Extinction	Total Seconds	Total Step-down Attempts	Total Seconds Night 1	Total SDA Night 1	Total Nights
Saline	Saline[*]	$249.17 \pm 91.00^{\Pi}$	$2.08 \pm 0.57^{\S}$	$115.92 \pm 19.14^{\dagger}$	$1.50 \pm 0.56^{\ddagger}$	$1.83 \pm 0.44^{\#}$
Saline	Trypto-phan	196.83 ± 66.06	1.85 ± 0.60	119.00 ± 20.47	1.33 ± 0.48	1.75 ± 0.35
Trypto-phan	Saline	220.08 ± 65.14	2.75 ± 0.72	123.42 ± 15.89	2.00 ± 0.48	1.67 ± 0.38
Trypto-phan	Trypto-phan	142.58 ± 35.73	2.00 ± 0.63	104.50 ± 20.83	1.25 ± 0.39	1.42 ± 0.39

[*] N = 12 animals/group

[Π] Total seconds $\pm$ SEM to extinguish

[$\S$] Total step-down attempts $\pm$ SEM over 5 nights of extinction

[$\dagger$] Mean seconds $\pm$ SEM first night of extinction

[$\ddagger$] Mean number of step-down attempts $\pm$ SEM first night of extinction

[$\#$] Total nights $\pm$ SEM to extinguish

Latency to Step-Down.   The ANOVA revealed a main effect of group $F(1,45) = 4.64$, $p < .05$, and time $F(1,45) = 12.41$, $p < .001$, for the latency to step-down and explore the novel chamber.   Figure 7 and Appendix Table IV illustrate that tryptophan injected animals took longer than controls to step-down on both trials, $p < .05$.   In addition, both the control and tryptophan groups stepped down faster on the second trial than on the first trial, $p < .05$.

3. Discussion.

Acquisition of an Avoidance Response.   Depletion of brain serotonin has repeatedly been shown to facilitate acquisition of an avoidance task (Vorhees, 1979; Lin et al., 1978; Koher and Lorens, 1978).   Thus, it was hypothesized that an elevation of serotonin, via tryptophan administration, would impair learning of a punished step-down response (passive-avoidance). Although the results of this experiment do not support the above hypothesis, they are in accord with the findings of Engel and Modigh (1974) who observed that 600-800 mg/kg tryptophan impaired acquisition of shuttle box active-avoidance and tryptophan doses of less than 600 mg/kg were ineffective in affecting avoidance response learning.   Thus, the tryptophan dose (50 mg/kg) used in the present study, may have been too low to affect learning of the punished step-down response.   Engle and Modigh's (1974) results also suggest that the tryptophan impairment of shuttle box avoidance might not be related to serotonin, because much lower doses of tryptophan (50 mg/kg) have been shown to produce maximum increases in brain serotonin (Fernstrom and Wurtman, 1971; Young et al., 1978).

In addition to the above, these findings may be due to the behavior studied, i.e. passive-avoidance.   Although serotonin has been related to active-avoidance, a task which involves initiation of a response, serotonin
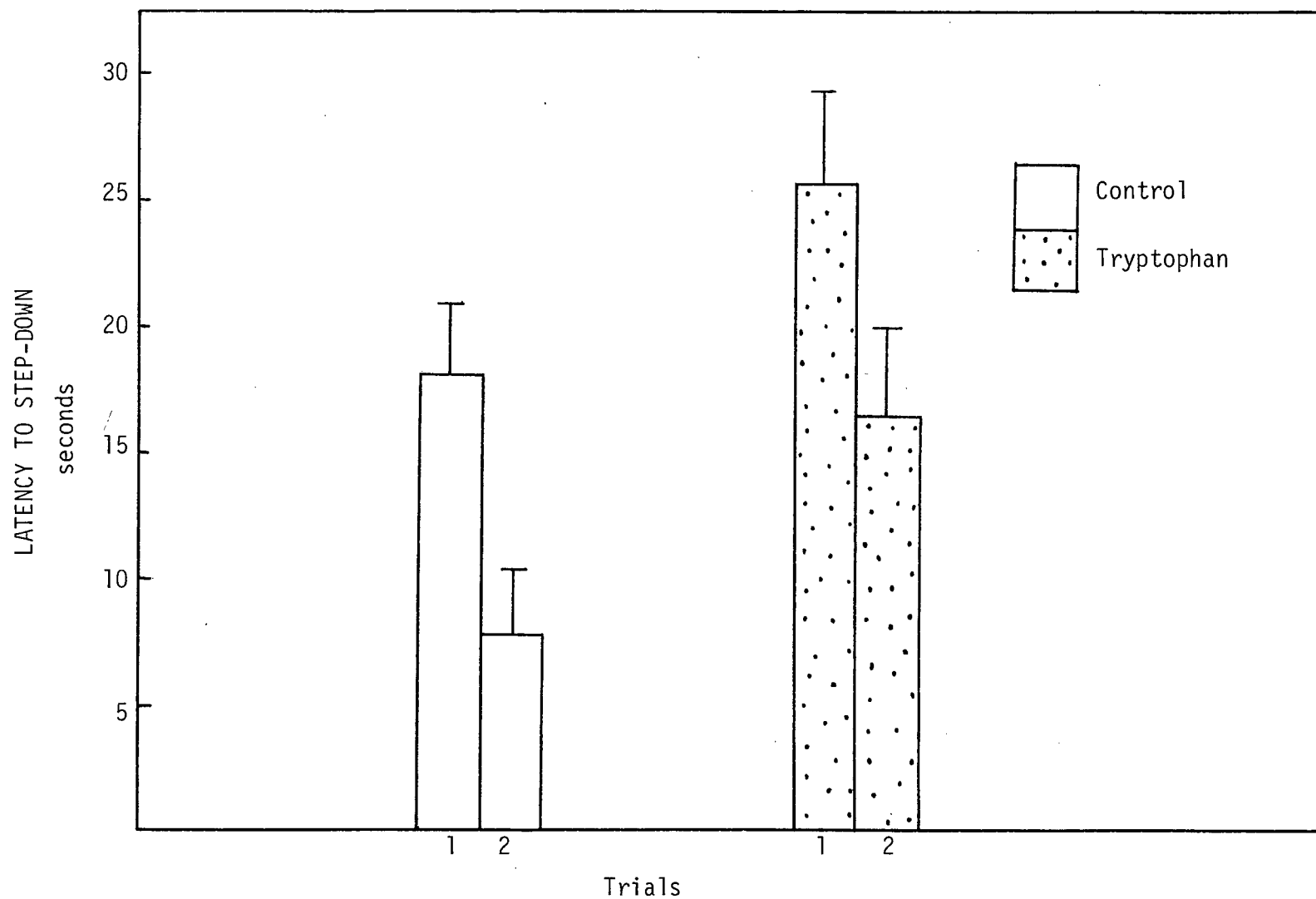
Figure 7. Latency to step-down following tryptophan administration (mean + SEM).

may not function in the same manner in passive-avoidance, a task which requires response inhibition. In this regard, these data confirm those of Fibiger et al. (1978) who observed that electrical stimulation of the dorsal raphe nucleus, a brain region rich in serotonergic perikarya, did not affect acquisition of a simple passive-avoidance task.

Extinction. Serotonin depletion with PCPA has also been shown to increase extinction time of a punished step-down response (Beninger and Phillips, 1980). Thus, it was predicted that in this experiment extinction of the step-down behavior would be reduced if serotonin was elevated via a tryptophan injection. However, these results were not in agreement with those of Beninger and Phillips (1980). Again, the dose utilized (50 mg/kg) in this study may have been too low to influence extinction, even though 50 mg/kg tryptophan is known to produce a maximum elevation in brain serotonin (Fernstrom and Wurtman, 1971; Young et al., 1978). In addition, manipulations that alter serotonin content, within normal physiological range, may not be sufficient to affect extinction. Indeed, PCPA has been shown to greatly deplete serotonin through inhibition of tryptophan hydroxylase activity (Beninger and Phillips, 1980).

Exploratory Behavior. Results from the present study suggest that tryptophan injected rats display reduced exploratory behavior when exposed to a novel chamber. These findings are in agreement with those of File and Pope (1974) who also demonstrated that hole board exploratory behavior was reduced when rats were administered chlorpromazine, a drug which elevates brain serotonin. Similarly, decreased walking behavior in an open field was observed when rats were injected with tryptophan (Tricklebank et al., 1978) whereas, enhanced exploration in a hole board apparatus was shown when serotonin was depleted with PCPA (File, 1977).

However, in addition to exploratory behavior, the latency to step-down may simply reflect the animal's activity level. Reduced locomotor behavior has been observed in rats with elevated concentrations of brain serotonin (Taylor, 1976). Although it was difficult to separate these two behaviors in this experiment, the latency scores did appear to be correlated with serotonin levels.

VII. EXPERIMENT FOUR

TRYPTOPHAN AND CARBOHYDRATE INDUCED INCREASES IN BRAIN SEROTONIN: BIO-
CHEMICAL AND BEHAVIORAL CORRELATES

1. Introduction.

The purpose of the final study was to determine if the carbohydrate
induced increase in brain serotonin would yield behavioral results com-
parable to the tryptophan induced elevation. To help clarify whether
the latency measure reflects exploratory behavior or locomotor activity,
rearing behavior on both trials was tabulated as another component of
exploration. Defecation and urination, behaviors often negatively associ-
ated with serotonin (Kameyama et al., 1980), were also recorded. Finally,
plasma corticosterone was assayed as a biochemical indicator of increased
brain serotonin since this neurotransmitter has been shown to regulate
the hypothalamus/pituitary/adrenal axis (Yuwiller, 1979).

2. Experimental Procedure.

The experimental procedure is outlined in Figure 8. Seventeen hours
prior to drug injections or diet presentation, rats were randomly assigned
to 5 groups and 80 of 88 animals were deprived of food but not water.
The remaining 8 rats were given free access to both food and water. Fast-
ing was terminated between 1700 and 1800 and animals were treated as
follows:

    Group 1 - (non-fasted group) - decapitated, blood and brains
              removed;

    Group 2 - decapitated, blood and brains removed;

    Group 3 - injected with 0.9% saline and offered the high carbo-
              hydrate, protein-free meal ad libitum;

ANIMALS FASTED
2400   NON-FASTED

1700   1700

FASTED*   CONTROL   CARBOHYDRATE   CONTROL   NON-FASTED*
BASAL   SALINE   SALINE   TRYPTOPHAN   BASAL

1800*   1800*   1800*

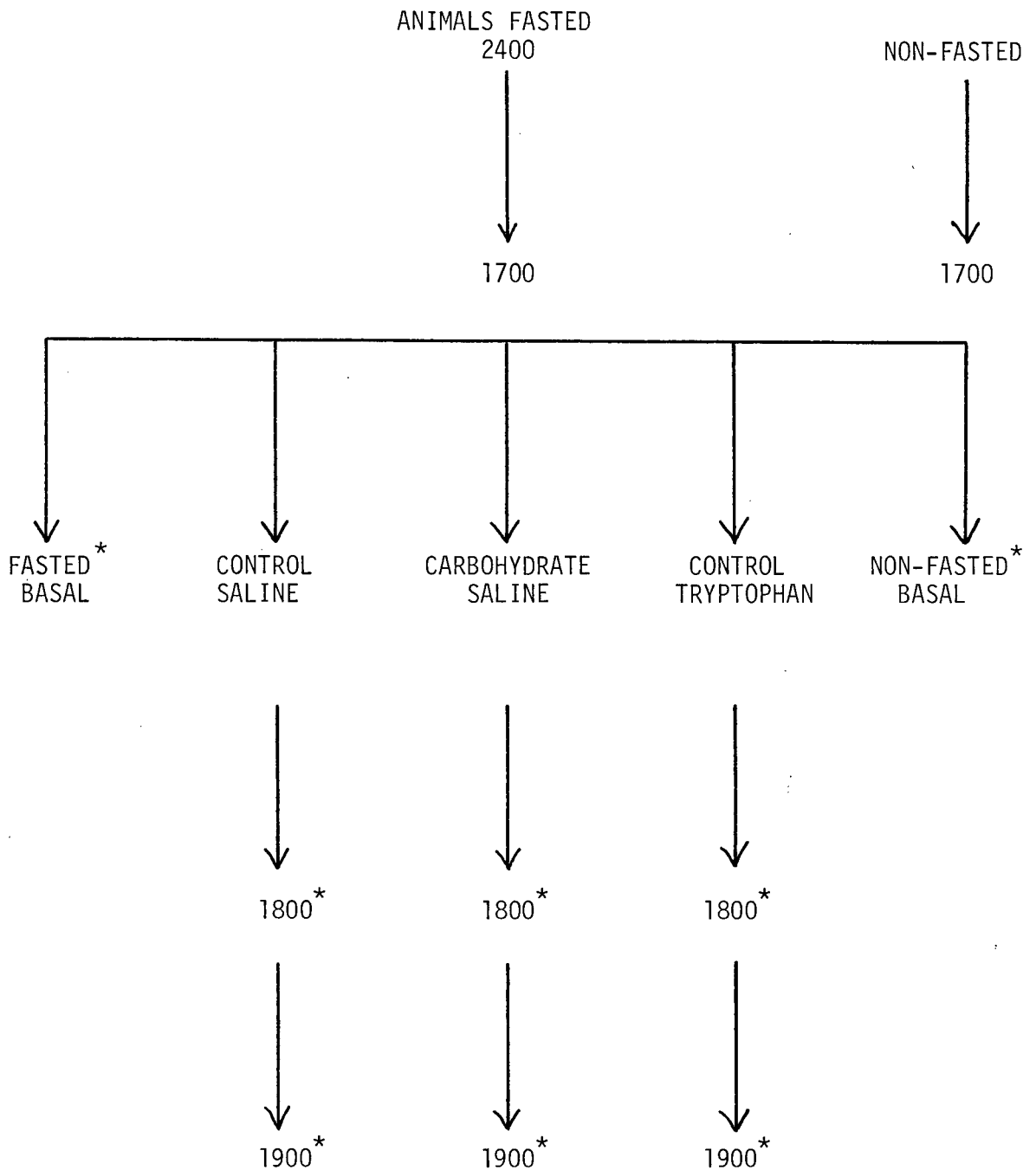1900*   1900*   1900*

Figure 8. Tryptophan and carbohydrate induced increases in
           brain serotonin: biochemical and behavioral
           correlates: Experimental design.

*Brains removed for analysis of brain tryptophan and
 serotonin and blood collected for plasma corticosterone.

Group 4 - injected with 0.9% saline and offered the control
    diet ad libitum;

Group 5 - injected with 50 mg/kg tryptophan and offered the
    control diet ad libitum.

At 1 or 2 hours post treatment, rats from groups 3, 4 and 5 were individually

placed in the novel chamber. Behaviors recorded included:

(1) latency to step-down on 2 trials;

(2) number of rears per trial once on the floor;

(3) total number of boluses and urination pools after 2 trials.

Immediately following behavioral testing each rat was decapitated. Trunk

blood was collected in heparinized tubes. After centrifugation at (1000

x G) for 20 min, plasma was collected and stored at -20°C. Brains were

removed, rapidly frozen in liquid nitrogen, and stored at -70°C.

Statistics. The Student's t-test was used to analyze fasted versus

non-fasted basal brain tryptophan, serotonin, and plasma corticosterone.

Brain tryptophan, serotonin, plasma corticosterone, latency to step-down,

rearing, urination, defecation, and food intake, measures taken 1 and 2

hrs post treatment, were analyzed by a Group (3) x Time (2) ANOVA.

3. Results.

Brain Tryptophan. Brain tryptophan from fasted basal or non-fasted

basal rats and the time course and peak concentrations of brain tryptophan

in animals fed a high carbohydrate, protein-free meal, injected with trypto-

phan, or fed the control diet are presented in Figure 9 and Appendix Table

V. The Student's t-test indicated that brain tryptophan was higher in

fasted versus non-fasted basal animals $T(13)=4.12$, $p < .01$. In addition,

the ANOVA revealed a Group x Time interaction $F(2,64)=119,26$, $p < .001$.

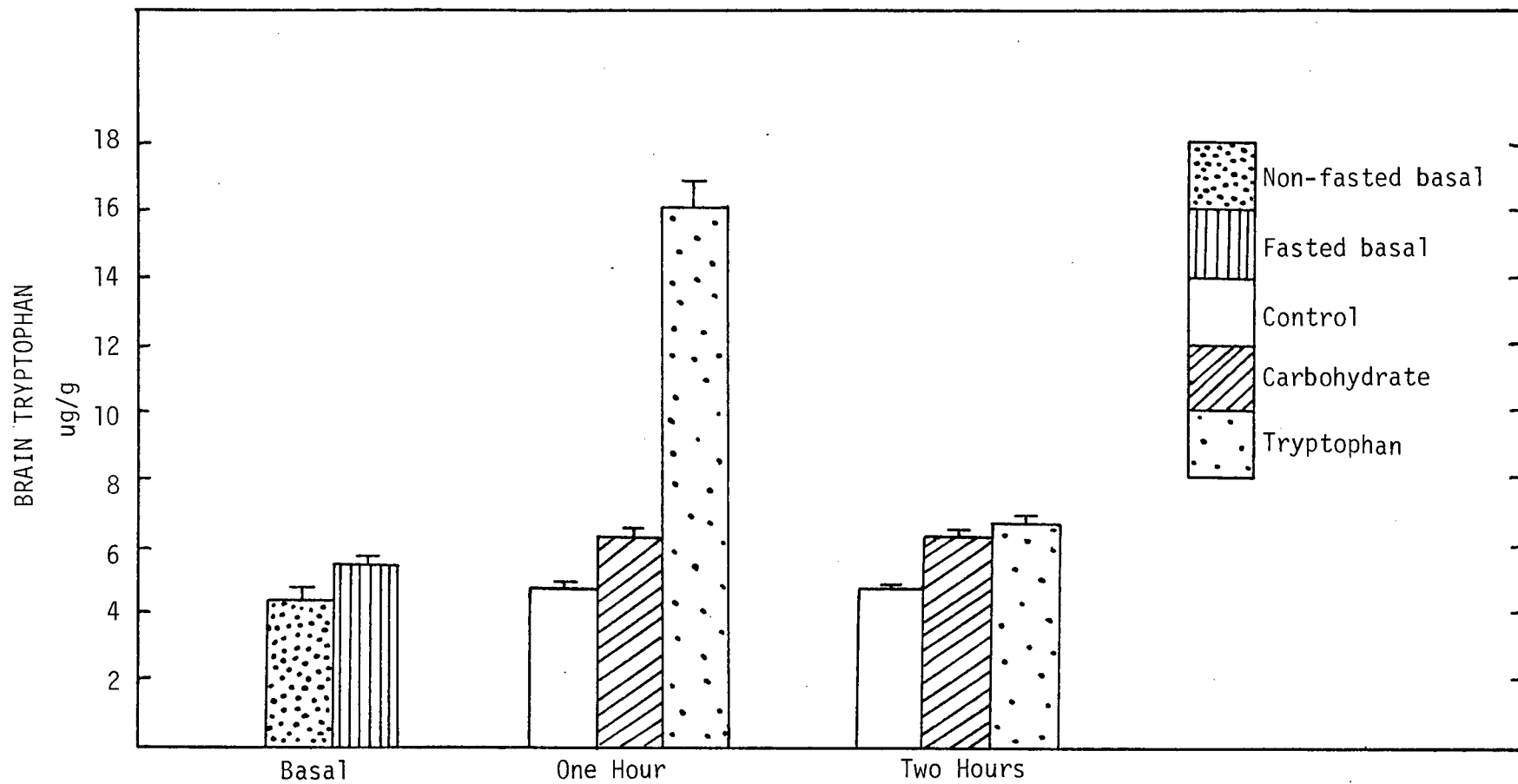Tests for simple main effects indicated a main effect of group at 1 hour

Figure 9. Brain tryptophan following ingestion of carbohydrate and tryptophan
administration (mean + SEM)

$F(2,63)=319,2$, $p < .01$, and 2 hours $F(2,65)=9.55$, $p < .01$, after food and drug administration. A main effect of time $F(2,65)=175.5$, $p < .01$, was also found. Neuman-Keuls post hoc analyses showed that animal injected with trypotophan exhibited higher brain tryptophan concentrations than both control and carbohydrate treated rats at 1 hour post injection, $p < .01$. By 2 hours, brain tryptophan fell in the tryptophan injected animals, but concentrations were still significantly more elevated than controls, $p < .01$. Post hoc analyses also revealed that the carbohydrate fed animals displayed an increase in brain tryptophan over controls at 1 and 2 hours after food presentation, $p < .01$.

Brain Serotonin. Figure 10 and Appendix Table VI show fasted and non-fasted basal concentrations of brain serotonin and the time course and peak levels of serotonin in animals fed the control or experimental diets or injected with tryptophan. Brain serotonin in fasted and non-fasted rats was not different when analyzed by the Student's t-test. However, the ANOVA revealed a Group x Time $F(2,65)=5.30$, $p < .01$, interaction for rats assayed 1 and 2 hours after tryptophan injection or food presentation. A main effect of group was shown at both $1, F(2,65)=50.1$, $p < .01$, and $2, F(2,65)=13.91$, $p < .01$, hours after treatment by the test of simple main effects. Neuman-Keuls post hoc analyses revealed that animals injected with tryptophan had a higher concentration of brain serotonin, $p < .01$, than carbohydrate and control fed rats at 1 hour post injection. By 2 hours brain serotonin fell, but concentrations were still higher than controls, $p < .01$. Carbohydrate fed animals were not shown to differ from controls at either times assayed.

Plasma Corticosterone. The time course of plasma corticosterone concentrations is illustrated in Figure 11 and Appendix Table VII. The ANOVA revealed a main effect of group $F(2,64)=5.18$, $p < .01$. Neuman-Keuls post
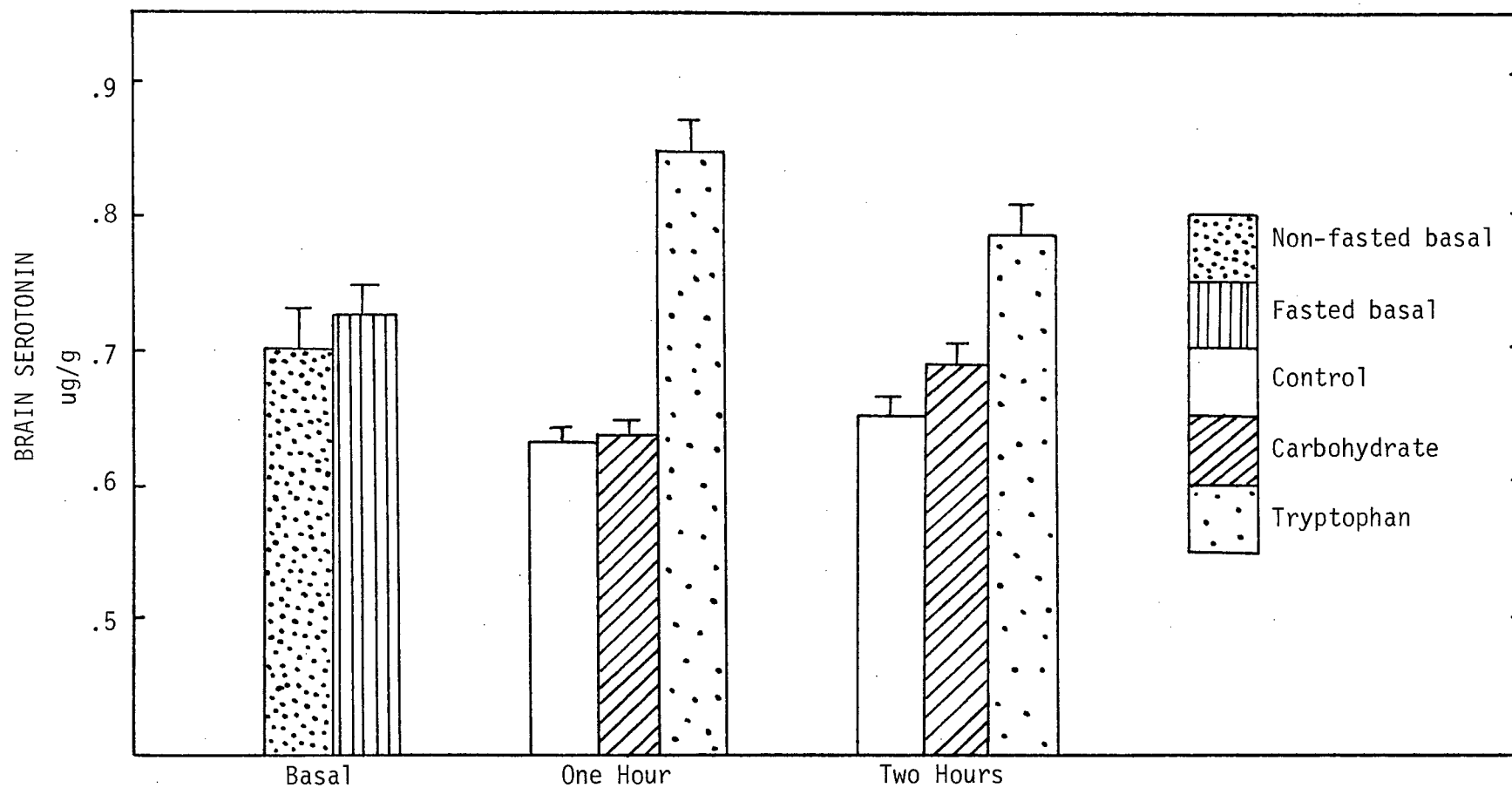
Figure 10. Brain serotonin following ingestion of carbohydrate or tryptophan
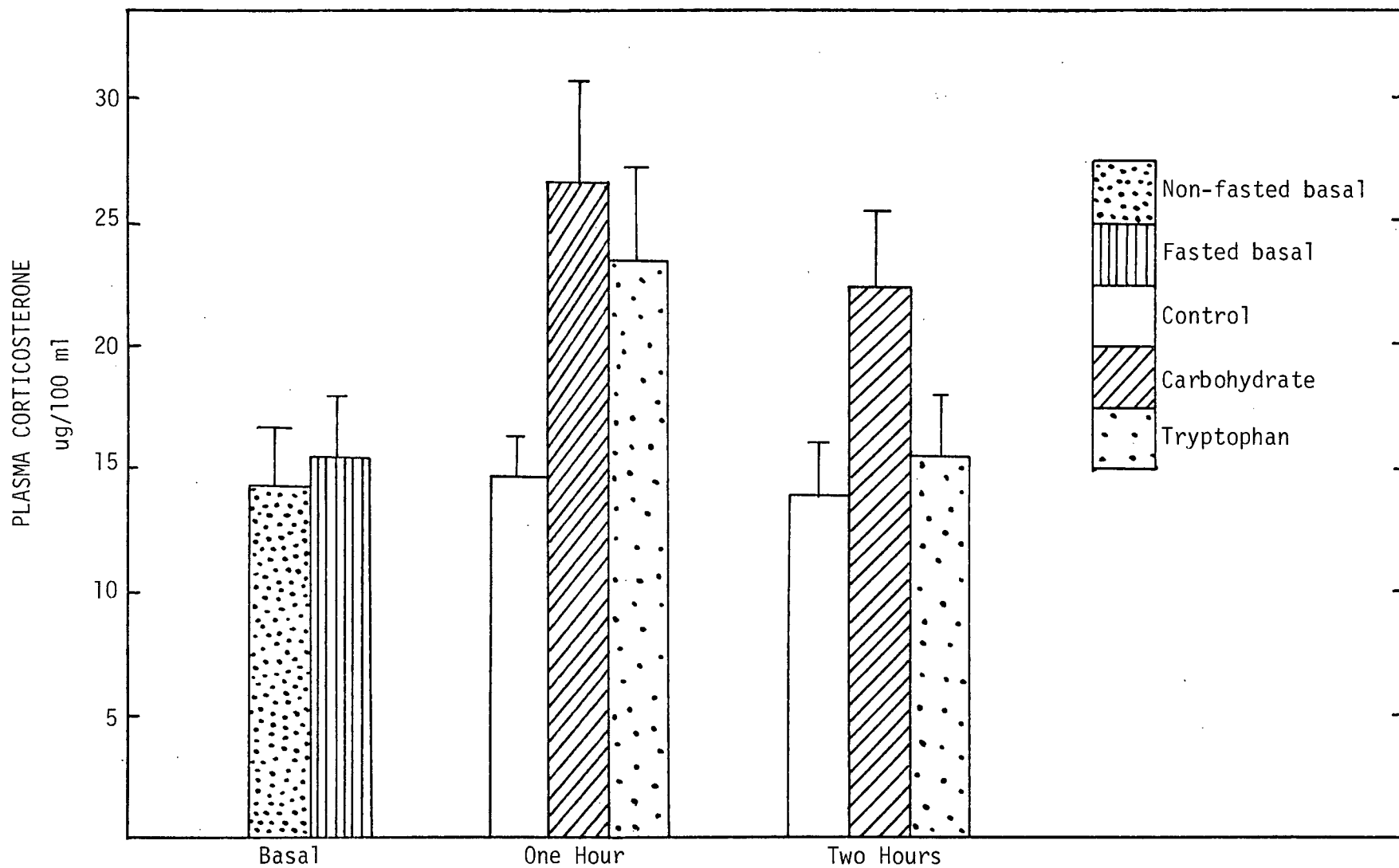administration (mean + SEM).

Figure 11.   Plasma corticosterone following carbohydrate ingestion or tryptophan administration (mean + SEM).

hoc analyses indicated that carbohydrate fed rats exhibited a significantly higher level of corticosterone than controls, $p < .01$.

Food Intake. Table IV includes the amount of food and carbohydrate consumed by animals either injected with tryptophan, offered the high carbohydrate meal, or presented the control diet. ANOVA revealed that the animals offered the high carbohydrate protein-free meal ate less food than those given the control diet and injected with either trypto-phan or saline $F(2,62)=14.57$, $p < .01$. However, carbohydrate consumption did not differ among groups.

Latency To Step-Down/Rearing. Mean rearing and latency to step-down for animals injected with tryptophan, offered the high carbohydrate meal, or given control diet is given in Table V and Figure 12 and Appendix, Table VIII, respectively. The ANOVA revealed that injection of tryptophan or administration of a high carbohydrate meal did not affect rearing or the latency to step-down on either trial, at 1 or 2 hours post treatment.

Urination and Boluses. Urination and bolus measures are presented in Table VI. No significant effect of group was obtained when the urina-tion and bolus data were analyzed by ANOVA. However, a trend, $p < .09$, for time was suggested for urination. Both the tryptophan and control animals tended to urinate less 2 hours after injection or food consumption.

4. Discussion.

Brain Tryptophan. The elevations in brain tryptophan observed in fasted basal rats over that of non-fasted basal rats in this study are in accord with the findings of Curzon et al. (1972). These results were anticipated because fasting has been observed to increase the level of both plasma free tryptophan and subsequently brain tryptophan (Curzon et al., 1972). As previously discussed, fasting has been shown to increase

TABLE IV

TOTAL FOOD AND CARBOHYDRATE INTAKE OF ANIMALS FED CONTROL DIET, CARBO-
HYDRATE MEAL, OR INJECTED WITH TRYPTOPHAN

Treatment	Food Intake (g $\pm$ SEM)	Carbohydrate Intake (g $\pm$ SEM)
Control	(21)* 4.74 $\pm$ 0.23	2.56 $\pm$ 0.12
Carbohydrate	(24) 3.06 $\pm$ 0.21	2.39 $\pm$ 0.16
Tryptophan	(23) 4.71 $\pm$ 0.31	2.54 $\pm$ 0.17

* number of animals/group
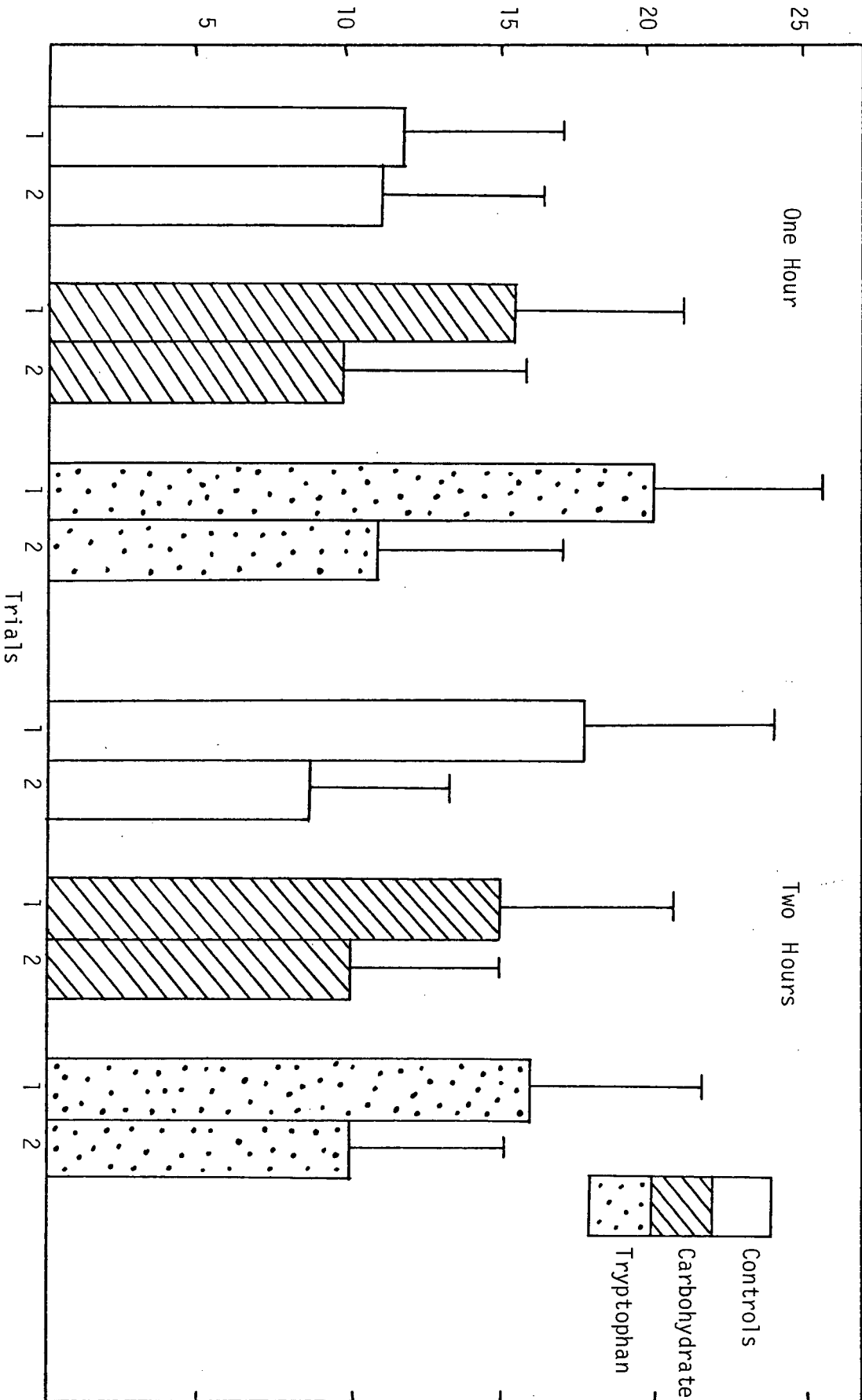
Figure 12. Latency to step-down following carbohydrate ingestion or tryptophan administration (mean + SEM).

TABLE V

EFFECT OF CARBOHYDRATE INGESTION OR A TRYPTOPHAN INJECTION ON REARING

| Treatment | | One Hour | | Two Hours | |
		Rear 1	Rear 2	Rear 1	Rear 2
Control	(12)*	4.42 ± 0.60[†]	3.27 ± 0.72	(12) 4.83 ± 0.96	3.42 ± 1.00
Carbohydrate	(12)	4.50 ± 0.86	2.75 ± 0.59	(12) 3.17 ± 0.58	3.50 ± 0.73
Tryptophan	(11)	3.64 ± 0.65	3.09 ± 0.63	(12) 2.67 ± 0.56	2.83 ± 0.76

* number of animals/group

† Mean number of rears/trial $\pm$ SEM

TABLE VI

URINATION OR DEFECATION FOLLOWING INTAKE OF CARBOHYDRATE, CONTROL DIET OR A TRYPTOPHAN INJECTION

Treatment	Urination		Bolus	
	One Hour	Two Hours	One Hour	Two Hours
Control*	1.75 ± 0.50	0.25 ± 0.25	0.75 ± 0.41	0.00 ± 0.00
Carbohydrate	0.42 ± 0.23	0.67 ± 0.31	0.08 ± 0.83	0.50 ± 0.29
Tryptophan	1.67 ± 0.79	1.08 ± 0.31	0.50 ± 0.50	0.58 ± 0.40

* 12 animals/group

fatty acid output which in turn elevates plasma free tryptophan by displac-
ing tryptophan from albumin binding sites.

The effect of carbohydrate ingestion and tryptophan dosing on
brain tryptophan and its time course replicated the findings of the
second experiment.

Brain Serotonin.   Injection of tryptophan induced an
increase in brain serotonin similar to the effect observed in Experiment 2.
However, in contrast to the previous study, brain serotonin did not rise
in rats fed the high carbohydrate protein-free meal.   The elevated plasma
corticosterone concentrations also observed in the carbohydrate fed rats,
could offer an explanation for these contradictory results, i.e. the
carbohydrate fed group may have been more stressed than the control rats.
Certain stressful procedures have been shown to increase the turnover of
serotonin to 5-HIAA (Yuwiller, 1979).   Thus, although increased serotonin
synthesis may have occurred, the stress of a protein-free meal after fast-
ing, plus exposure to the novel chamber could have been powerful enough
to cause an even greater turnover of serotonin.   Confirmation could be
obtained by measuring labelled 5-hydroxytryptophan, an indicator of in-
creased serotonin synthesis.   In addition, 5-HIAA could be assayed as a
measure of serotonin turnover.

Plasma Corticosterone.   Although rats injected with tryptophan
exhibited an increase in brain serotonin, plasma corticosterone levels
did not differ from those of control or carbohydrate fed animals.
These results were surprising since they are not in agreement with
those of Modlinger et al. (1979, 1980) who showed that cortisol was in-
creased in humans administered an oral dose of tryptophan.   In addition,
our data are not in accord with the rapidly growing body of literature

which suggests serotonin plays a role in the regulation of the hypothalamus/ pituitary/adrenal axis. However, it is noteworthy that when the corticosterone concentrations of the tryptophan versus control rats (1 hour post injection) were analyzed with a t-test, the differences were significant. Unfortunately, the design of the present experiment invalidates the use of this statistic. However, it is possible that a change in experimental design, to include only the tryptophan and control group at 1 hour post injection, would yield findings in agreement with those in the literature.

In contrast to the tryptophan effect, rats fed the high carbohydrate, protein-free meal had significantly elevated concentrations of plasma corticosterone. From this study, serotonin's role in mediating this response is not clear, especially since brain serotonin concentrations were not increased. However, since brain tryptophan was elevated, it is possible that serotonin turnover and release were increased. Confirmation of the serotonin mechanism must await further research when 5-HIAA can be assayed or the corticosterone response to carbohydrate can be analyzed following administration of a serotonin blocker.

Possible evidence that serotonin has no role in the carbohydrate induced increase in plasma corticosterone comes from a study in which a sucrose solution was administered to rats (Hart et al., 1980). Although elevated corticoids were shown, the time course appeared to differ from that normally found for serotonin following carbohydrate ingestion. Rats given a high carbohydrate, protein-free meal have been reported to exhibit increases in serotonin and 5-HIAA for 2-3 hours after food presentation. However, Hart et al. (1980) observed that the corticoid levels peaked at 50 min and fell to control concentrations by 100 min. Thus,

some non-serotonergic mechanism could have mediated the corticosterone response found in Hart et al.'s (1980) experiment and in the present study following carbohydrate ingestion.

In addition, acetylcholine has been implicated in the regulation of the hypothalamus/pituitary/adrenal system (Jones et al., 1977). Thus, it may have mediated the corticosterone response after sugar consumption. However, to date, the carbohydrate effect on brain acetylcholine has not been studied.

Although the mechanism of the carbohydrate induced increase in corticosterone is unclear, it appears that these animals may have been stressed more than controls. Corticosterone release has been shown to occur as part of the stress response (Yuwiller, 1979). However, because of the design of this study, the specific stressor is not clear. Carbohydrate per se could have generated the corticoid response. But, this seems unlikely since the amount of carbohydrate eaten was the same among groups. The more probable stressor may have been the lack of dietary protein.

Food Intake. The data from the present study reveal that fasted rats offered a high carbohydrate, protein-free meal consume less food than rats given the control diet or injected with tryptophan. Thus, the protein-free meal may have greater short-term satiety value, perhaps through inducing a more pronounced elevation in plasma glucose. Crapo et al. (1976) observed that in humans, consumption of a sucrose solution produced a greater rise in plasma glucose than ingestion of the same sugar with added protein and fat. The significance of elevated plasma glucose in satiety has also been demonstrated. Administration of 2-deoxyglucose, an inhibitor of glucose metabolism, was observed to stimu-

late food intake (Houpt and Hance, 1971), whereas, duodenal and portal infusions of glucose were found to suppress feeding in fasted animals (Russek, et al., 1980).

In addition, intake of carbohydrate may have been regulated by serotonin. Several researchers have suggested that serotonin may influence carbohydrate intake. Nance and Kilbey (1973) observed that sucrose preference was increased when PCPA treated rats were depleted of serotonin and that this behavior was reversed by 5-hydroxytryptophan. Similarly, Wurtman and Wurtman (1979) showed that drugs which elevate serotonin concentration lowered carbohydrate intake but had no effect on protein ingestion. Both researchers suggested serotonin may regulate carbohydrate intake.

Although the results of the present study do not prove the above proposal, elevated serotonin turnover in the carbohydrate fed rats may have mediated the reduction in food intake so that total carbohydrate consumed would not exceed amounts ingested by control rats.

Latency To Step-Down. Neither tryptophan or carbohydrate administered rats exhibited differences in latency to step-down from control fed animals. That serotonin also was not elevated in the carbohydrate fed rats could explain the lack of behavioral results in this group. However, the inability to repeat the tryptophan effect on the latency scores is more difficult to explain. One possibility could be the unavoidable changes in experimental design. Rats were offered food ad libitum in the previous experiment. But, in this study, animals were fasted 17 hours and then presented food immediately after injection.

Bolles (1965) observed increased home cage activity in rats fasted 24 hours and then limited to 10-12 g food daily for 15 days, even on the first day of testing. In addition, the rats of this study were observed

to be highly excitable when handled and exposed to the novel chamber (when compared to animals of the preliminary experiment).

Thus, the possible effects of food deprivation could explain the non-significant results obtained in this study.

VIII. SUMMARY

In this series of studies, behavioral and biochemical correlates of the tryptophan and carbohydrate induced increase in brain serotonin were investigated.

1. Brain Tryptophan and Serotonin.

Brain tryptophan and serotonin were consistently found to peak in tryptophan administered rats at 1 hour post injection. Both tryptophan and serotonin dropped to control levels by 2 hours after injection. This time course was replicated.

Carbohydrate was found to induce an increase in brain serotonin; however, when animals were also exposed to a novel chamber, the carbohydrate induced serotonin elevation was no longer present.

2. Latency to Step-Down.

Aminals fed ad libitum and injected with tryptophan exhibited an increased latency to step-down and explore a novel chamber. However, these data were not replicated in the final study when animals were fasted prior to injection and food presentation.

3. Plasma Corticosterone.

Since brain serotonin has been implicated in the regulation of the hypothalamus/pituitary/adrenal axis, plasma corticosterone was analyzed as a biochemical indicator of increased serotonin activity. Tryptophan administration did not increase plasma corticosterone above control concentrations, but ingestion of a high carbohydrate, protein-free meal did produce an elevation in plasma corticosterone.

IX. CONCLUSIONS

The data from the present studies indicate that tryptophan injections result in an increased latency to step-down and explore a novel chamber, providing the animals are fed ad libitum. However, a tryptophan induced corticoid response was not clearly demonstrated. In contrast, carbohydrate ingestion after fasting did not affect step-down latency under the conditions of this experiment, i.e. food presentation after a 17 hour fast and behavioral testing during the dark phase, but did elevate plasma corticosterone. Confirmation of the role of serotonin in mediating the behavioral and biochemical responses observed in this study, must await future investigation when analyses are conducted following administration of a serotonin blocker.

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APPENDIX TABLE 1

Purified control and carbohydrate diet[1]

Ingredients	Control Kg/1 Kg	Carbohydrate Kg/1 Kg
Casein, vitamin free[2]	.240	-
Dextrose[3]	.185	.265
Dextrin[4]	.185	.265
Sucrose[5]	.165	.247
Corn oil[6]	.150	.150
Agar[7]	.035	.035
Mineral mix[8]	.027	.027
Choline Chloride Premix[9]	.007	.007
Vitamin mix[10]	.004	.004
Methionine[11]	.002	-
	1.000 Kg	1.000 Kg

[1] Modified from Fernstrom and Faller, (1978).

[2] Supplied by Teklad Test Diets, ARS/Sprague-Dawley Division of the Mogul Corporation, Madison, Wisconsin.

[3] Supplied by Grand Island Biological Co., Grand Island, New York.

[4] Supplied by ICN Nutritional Biochemicals, Cleveland, Ohio.

[5] Supplied by B.C. Sugar Refinery, Vancouver, British Columbia.

[6] Supplied by Best Foods Div., The Canadian Starch Co. Ltd., Montreal, Quebec.

[7] Supplied by ICN Nutritional Biochemicals, Cleveland, Ohio.

Appendix Table 1 (cont'd)

[8] Contains 16.10 g $CaHPO_4$; 6.82 g KCl; 1.98 g $MgSO_4$; 1.54 g Na_2HPO_4; 0.64 g $CaCO_3$; 0.22 g $FeC_6H_5O_7xH_2O$ (16% Fe); 0.15 $MnSO_4$; 0.02302 g $ZnCO_3$; 0.01256 g $CuSO_4$; 0.00221 g NaFl; 0.001537 g $CrCl_36H_2O$; 0.0002188 g NaSe; 0.0001962 g Kl; 13 g sucrose; Minerals supplied by J.T. Baker Chemical Co., Phillipsburg, New Jersey.

[9] Contains 2 g choline chloride; 5 g sucrose. Choline chloride supplied by ICN Pharmaceutials Inc., Life Sciences Group, Cleveland, Ohio.

[10] Contains 200 mg inositol; 120 mg vit. E (50% dl-a-tocopheral acetate): 40 mg nicotinic acid; 20 mg P-aminobenzoic acid; 16 mg dl calcium pantothenate; 16 mg vit. A acetate; 12 mg pyridoxine HCl; 8 mg thiamine HCl; 6 mg riboflavin; 2 mg folic acid; 1 mg d-biotin; 1 mg menadione (vit. K); 0.1 mg vit. B_{12}; 0.05 mg vit. D (calciferol); 13 g sucrose; Vitamins supplied by ICN Pharmaceuticals Inc., Life Sciences Group, Cleveland, Ohio.

[11] Supplied by ICN Pharmaceutals Inc., Life Sciences Group, Cleveland, Ohio.

BRAIN TRYPTOPHAN FOLLOWING INGESTION OF A CONTROL OR CARBOHYDRATE MEAL OR INJECTION OF TRYPTOPHAN

Treatment	One Hour	Two Hours	Three Hours	Four Hours
Control	(5)* 4.48 ± 0.18[†]	(6) 4.12 ± 0.15	(6) 4.22 ± 0.18	(6) 4.37 ± 0.22
Carbohydrate	(5) 5.60 ± 0.17	(6) 5.36 ± 0.31	(6) 5.33 ± 0.13	(6) 5.16 ± 0.20
Tryptophan	(4) 11.06 ± 1.20	(6) 4.98 ± 0.24	(6) 4.21 ± 0.13	

* Number of animals/group

† Mean tryptophan (ug/g ± SEM)

BRAIN SEROTONIN FOLLOWING CARBOHYDRATE INGESTION OR TRYPTOPHAN ADMINISTRATION.

Treatment		One Hour		Two Hours		Three Hours		Four Hours
Control	(5)[*]	$.629 \pm .038$[†]	(6)	$.616 \pm .030$	(6)	$.589 \pm .022$	(6)	$.585 \pm .030$
Carbohydrate	(5)	$.683 \pm .028$	(6)	$.684 \pm .020$	(6)	$.672 \pm .033$	(6)	$.631 \pm .033$
Tryptophan	(4)	$.845 \pm .061$	(6)	$.675 \pm .031$	(6)	$.657 \pm .021$		

* Number of animals/group
† Mean serotonin (ug/g $\pm$ SEM)

APPENDIX TABLE IV

LATENCY TO STEP-DOWN FOLLOWING TRYPTOPHAN OR SALINE INJECTIONS

Treatment	Mean Latency to Step-Down $\pm$ SEM[†]	
	Trial 1	Trial 2
Saline (24)[*]	18.29 $\pm$ 2.8	7.88 $\pm$ 2.4
Tryptophan (23)	25.57 $\pm$ 3.8	16.25 $\pm$ 3.6

* number of animals/group
† Mean seconds

APPENDIX TABLE V

BRAIN TRYPTOPHAN FOLLOWING CARBOHYDRATE INTAKE OR TRYPTOPHAN ADMINISTRATION

Treatment	Brain Tryptophan
Non-fasted (8)[*] basal	4.38 ± 0.15[†]
Fasted basal (7)	5.42 ± 0.37

Treatment		One Hour		Two Hours
Control	(12)	4.76 ± 0.09	(12)	4.69 ± 0.10
Carbohydrate	(12)	6.29 ± 0.21	(12)	6.29 ± 0.17
Tryptophan	(12)	16.18 ± 0.80	(11)	6.73 ± 0.25

[*] Number of animals/group

[†] Mean tryptophan (ug/g $\pm$ SEM)

APPENDIX TABLE VI

BRAIN SEROTONIN FOLLOWING INGESTION OF A HIGH CARBOHYDRATE MEAL, OR IN-
JECTION OF TRYPTOPHAN

Treatment	Brain Serotonin ug/g
Non-fasted basal (7)*	.702 ± .030[†]
Fasted-basal (8)	.731 ± .019

Treatment		One Hour		Two Hours
Control	(12)	.632 ± .011	(12)	.653 ± .014
Carbohydrate	(12)	.635 ± .011	(12)	.688 ± 0.15
Tryptophan	(12)	.849 ± .023	(11)	.786 ± .030

* Number of animals/group
† Mean serotonin (ug/g ± SEM)

APPENDIX TABLE VII

EFFECT OF CARBOHYDRATE INTAKE OR TRYPTOPHAN ADMINISTRATION ON PLASMA CORTICOSTERONE

Treatment	Plasma Corticosterone
Non-fasted basal (8)[*]	14.3 ± 2.59[†]
Fasted basal (8)	15.4 ± 2.72

Treatment	One Hour		Two Hours
Control (11)	14.60 ± 1.86	(11)	13.87 ± 2.24
Carbohydrate (12)	26.67 ± 4.12	(12)	22.44 ± 3.24
Tryptophan (12)	23.48 ± 3.95	(12)	15.45 ± 2.66

[*] Number of animals/group

[†] Mean corticosterone (ug/100 ml $\pm$ SEM)

APPENDIX TABLE VIII

EFFECT OF A TRYPOTOPHAN INJECTION OR INGESTION OF A CARBOHYDRATE OR CONTROL DIET ON LATENCY TO STEP-DOWN

Treatment		Latency to Step-Down One Hour			Latency to Step-Down Two Hours	
		1	2		1	2
Control	(12)*	11.88 ± 5.21[†]	11.08 ± 5.40	(12)	17.96 ± 6.20	8.67 ± 4.75
Carbohydrate	(12)	15.42 ± 5.60	9.92 ± 6.00	(12)	15.08 ± 5.71	10.17 ± 4.95
Tryptophan	(12)	20.05 ± 5.71	11.25 ± 5.95	(11)	16.00 ± 5.67	10.00 ± 5.06

* number of animals/group

† Mean seconds ± SEM