AN INVESTIGATION OF THE EFFECT OF RESTRAINT STRESS,  
CARBOHYDRATE RESTRICTION AND SEROTONIN ON  
MACRONUTRIENT INTAKE IN THE FEMALE RAT. 

by 

INGRID PRICE 

B.Sc., The University of British Columbia, 1991  
M.A., The University of British Columbia, 1994 

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF  

DOCTOR OF PHILOSOPHY  

in  

THE FACULTY OF GRADUATE STUDIES  
(Department of Psychology; Biopsychology) 

We accept this thesis as conforming 
to the required standard 

THE UNIVERSITY OF BRITISH COLUMBIA 

March 2002  
© Ingrid Valerie Price, 2002
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Psychology

The University of British Columbia
Vancouver, Canada

Date April 19, 2002

DE-6 (2/88)
ABSTRACT

Serotonin (5-HT) influences food intake in humans and other species. Low levels of brain 5-HT increase carbohydrate (CHO) intake, whereas elevated levels of 5-HT decrease CHO intake. Stress influences both 5-HT activity and food intake. Two forms of stress were implemented in this dissertation to determine their effect on macronutrient intake (CHO, protein, fat) as well as the possible role of 5-HT in these effects.

Research has revealed that varying the duration of restraint stress differentially influences food intake. These effects may be at least partially mediated through serotonergic mechanisms. Further, the deprivation state of the animal has been shown to alter 5-HT activity, and elevated brain 5-HT levels decrease CHO and to some extent fat intake in the rat. Experiments 1-3 (Chapter 2) investigated the effect of three different durations of restraint stress (20 min, 2 h and 2 h/day chronically for 5 days) on macronutrient intake in the female rat. Results indicated that all restraint durations inhibit fat and CHO intake following 4 h of food deprivation. However, 24 h of food deprivation attenuates the inhibition of CHO intake produced by either 20 min or chronic restraint but not that produced by 2 h of restraint. These data reveal an important interaction between the effect of restraint stress and food deprivation on the intake of specific macronutrients.
Experiment 4 (Chapter 3) attempted to determine if the 5-HT1A agonist, 8-hydroxy-dipropylaminotetralin (DPAT), could counteract the inhibitory effect of 2 h of restraint stress on CHO intake. Rats were tested after 4 or 24 h of food deprivation. Restraint significantly inhibited CHO and fat intake after either 4 or 24 h of food deprivation, and protein intake was inhibited after 4 h but not 24 h of food deprivation. DPAT was able to counteract the effect of restraint stress on CHO intake when rats were food-deprived for 24 h prior to testing. DPAT had no effect on fat or protein intake, nor on CHO intake after 4 h of food deprivation. These results have implications for the effect of restraint stress on macronutrient intake and neurochemical systems.

Experiments 5-8 (Chapter 4) investigated the impact of chronic CHO-restriction on macronutrient preference. Given the relationship between brain 5-HT and CHO, it is reasonable to assume that chronic CHO-restriction would reduce 5-HT activity, which could result in a preferential increase in CHO intake when a choice diet of CHO, protein or fat is offered. Initial experiments showed CHO over-consumption when rats were maintained on a diet restricted to 10% CHO for a 14 day period. CHO-restricted rats were injected with 0, 3 or 6 mg/kg fluoxetine (a 5-HT reuptake inhibitor) to determine whether an increase in 5-HT activity could attenuate the effect of chronic CHO-restriction on subsequent CHO intake. Results indicated that chronic CHO-restriction increased CHO intake while having no effect on protein or fat intake. Furthermore, 6 mg/kg fluoxetine attenuated the effect on CHO intake.
The results of this study extend current research through determining the effect of restraint stress on macronutrient intake. Further, this study extends current findings in the dietary stress literature through employing a CHO-restriction procedure that does not restrict overall caloric intake. In addition, the experiments in this study were conducted with female rats. The vast majority of stress research to date has been conducted in male rats. Results from studies employing female rats may allow for stronger indications of the effect of stress on food intake in women that may have implications for eating disorders. The findings from these experiments are discussed in terms of potential mechanisms and implications for animal models of eating disorders and directions for future research.
TABLE OF CONTENTS

Abstract ...........................................................................................................ii

Table of Contents ...........................................................................................v

List of Figures .................................................................................................vii

Acknowledgements .........................................................................................ix

Dedication .........................................................................................................x

CHAPTER I General Introduction ..................................................................1

1.1 Stress and Food Intake ............................................................................2
  1.1.1 Types of Stressors ...........................................................................2
  1.1.2 Duration of Stressors .......................................................................3

1.2 Stress and Corticosterone ......................................................................5

1.3 Corticosterone and Food Intake .............................................................7

1.4 5-HT and Food Intake ...........................................................................10
  1.4.1 Pharmacological Studies ..................................................................10
  1.4.2 Correlational Studies .....................................................................12
  1.4.3 Macronutrient Intake .....................................................................12
  1.4.4 Brain Mechanisms .........................................................................14

1.5 5-HT, Stress and Food Intake .................................................................15

1.6 Gender and Strain Differences in Stress Response .............................17

1.7 Purpose of this Study ..............................................................................19

CHAPTER II Brief, Chronic & Acute Stress .................................................23

2.1 Methods and Materials ..........................................................................28
  2.1.1 Participants ....................................................................................28
  2.1.2 Restraint Tubes .............................................................................28
  2.1.3 Experimental Diets .......................................................................29
  2.1.4 Acclimatization Procedure .............................................................30
  2.1.5 Testing Procedure ..........................................................................30

2.2 Results ....................................................................................................33
  2.2.1 Experiment 1: 20 min restraint .....................................................33
  2.2.2 Experiment 2: 2 h restraint .............................................................36
  2.2.3 Experiment 3: Chronic restraint ....................................................38
2.3 Discussion ........................................................................................................40

CHAPTER III Acute Stress and DPAT: Experiment 4 .................................................44

3.1 Methods and Materials .................................................................................46
  3.1.1 Participants ...............................................................................................46
  3.1.2 Materials ................................................................................................47
  3.1.3 Procedure .................................................................................................47

3.2 Results ...........................................................................................................49

3.3 Discussion ......................................................................................................54

CHAPTER IV CHO-restriction, Macronutrient Intake and Serotonin .......................59

4.1 General Methods and Materials ..................................................................61
  4.1.1 Experimental Diets .................................................................................61
  4.1.2 Materials ................................................................................................62
  4.1.3 CHO-restriction Procedure ...................................................................63
  4.1.4 Testing Procedure ..................................................................................64

4.2 Experiment 5 ..................................................................................................65
  4.2.1 Methods and Materials ..........................................................................65
  4.2.2 Results and Discussion ...........................................................................66

4.3 Experiment 6 ..................................................................................................69
  4.3.1 Methods and Materials ..........................................................................70
  4.3.2 Results and Discussion ...........................................................................71

4.4 Experiment 7 ..................................................................................................72
  4.4.1 Methods and Materials ..........................................................................73
  4.4.2 Results and Discussion ...........................................................................74

4.5 Experiment 8 ..................................................................................................75
  4.5.1 Methods and Materials ..........................................................................76
  4.5.2 Results and Discussion ...........................................................................77

4.6 Discussion ......................................................................................................79

CHAPTER V General Conclusions .........................................................................83

5.1 Implications ....................................................................................................91
5.2 Directions for Future Research ......................................................................97

REFERENCES .....................................................................................................103

APPENDIX I: Mechanism of 5-HT1A autoreceptor activation in the brainstem to reduce 5-HT release throughout the forebrain .........................................................125
LIST OF FIGURES

Figure 1. The effect of 20 min of restraint stress after 4h (a) and 24 h (b) of food deprivation on macronutrient intake in the female rat .................................................................p. 35

Figure 2. The effect of 2 h of restraint stress after 4 h (a) and 24 h (b) of food deprivation on macronutrient intake in the female rat ........................................................................p. 37

Figure 3. The effect of chronic restraint stress (2h/day for 5 days) after 4 h (a) and 24 h (b) of food deprivation on macronutrient intake in the female rat .................................................................p. 39

Figure 4. The effect of 2 h restraint stress and DPAT at 4 h (a) and 24 h (b) food deprivation on carbohydrate (CHO) intake ..................p. 51

Figure 5. The effect of 2 h restraint stress and DPAT at 4 h (a) and 24 h (b) food deprivation on protein intake ..............................................p. 52

Figure 6. The effect of 2 h restraint stress and DPAT at 4 h (a) and 24 h (b) food deprivation on fat intake. Values are represented as means ± S.E.M. ..................................................................................p. 53

Figure 7. The effect of chronic exposure to a 10% CHO-restricted diet for 14 days prior to testing on CHO intake for a 30 and 60 min test period .................................................................p. 68

Figure 8. The effect of chronic exposure to a 10% CHO-restricted diet for 14 days prior to testing on protein intake for a 30 and 60 min test period .................................................................p. 68

Figure 9. The effect of chronic exposure to a 10% CHO-restricted diet for 14 days prior to testing on fat intake for a 30 and 60 min test period .................................................................p. 69

Figure 10. The effect of chronic exposure to a 15% or 20% CHO-restricted diet for 14 days prior to testing on macronutrient intake for a 30 min test period compared to a control diet of 45% CHO ..................................................................................p. 72
Figure 11. The effect of chronic exposure to a 10% CHO-restricted and control diet for 14 days prior to testing on macronutrient intake for a 30 min test period after a 24 h food deprivation ..........p. 75

Figure 12. The effect of 14 days of CHO-restriction with and without fluoxetine on subsequent CHO intake ...........................................p. 78

Figure 13. The effect of 14 days of CHO-restriction with and without fluoxetine on subsequent protein intake ...........................................p. 78

Figure 14. The effect of 14 days of CHO-restriction with and without fluoxetine on subsequent fat intake ...........................................p. 79
This dissertation is possible due to the support and encouragement of many individuals. First, to my supervisor, Boris Gorzalka, for taking a risk with this project, I hope that at the end of this journey you feel it has been worth it. I would also like to thank Liisa Galea, Cathy Rankin and Gary Poole for their support, encouragement and mentoring, your belief in me and my work has encouraged me to continue and complete. I would like to thank the many undergraduate students who worked with me in the lab to collect the data for the experiments presented in this dissertation. Their names are really too many to mention, but without their help I would never have been able to complete this work. I would like to thank my parents who have always been there when I needed them. I would like to thank the many friends and colleagues who have encouraged me, listened to me complain and celebrated my wins with me. I have come to realize that while the completion of any sizable achievement may appear on the surface to be due to the discipline and drive of one person, in reality it is due to the support, encouragement and commitment of many individuals, and our wins are always and should always be shared. Thanks to everyone whom I have shared this win with.
DEDICATION

This dissertation is dedicated to my husband, Willard Price and two sons: Evan and Reid Price. First, to Willard, for seeing me through this entire process, from beginning to end, this dissertation is just as much yours as it is mine. There were many times when I wanted to quit and felt that I could not continue, but your faith and belief in me never faltered, much as I would have liked it to at times. I really couldn't have done this without you. To Evan and Reid, who have never known their mother not as a graduate student, with research to do and manuscripts to write up; thank-you for your patience, for your wonderful hugs and for simply being. You have all been my reason for doing this for some time now and completing this major task would have very little meaning without each of you to share it with.
CHAPTER I: GENERAL INTRODUCTION

There is a vast animal literature investigating the neurochemical and behavioural mechanisms of animal models of human mood disorders. For the most part, animal models of depression and anxiety involve exposing animals to some sort of stressor, either acutely or chronically. Exposure to stress may be related to the development of depression (Gold, Goodwin & Chrousos, 1988), anorexia nervosa (AN; Donohoe, 1984), and bulimia nervosa (BN; Powell & Thelen, 1996). Eating disorders, and often depression are characterized by dysfunctional eating behaviours. A considerable amount of research has been devoted to exposing animals to stressful environments and measuring the influence of this exposure on food intake as well as attempting to determine the underlying mechanisms for these effects. Numerous factors can influence the impact of a stressor on the functioning of an animal. For example, gender (Haleem, Kennett & Curzon, 1988), species (Meadows, Phillips & Davey, 1988) and strain (Keim & Sigg, 1976) differences can result in differential responses to stressful stimuli. The direction of this response and the mechanisms through which the stressor influences food intake depends on the type of stressor employed as well as the duration of exposure to the stressor. The experiments conducted in this dissertation will investigate the effect of two forms of stress on food intake: physical restraint stress and diet restriction.
1.1 STRESS AND FOOD INTAKE

Different stress regimes have been shown to increase (e.g., Badiani, Jakob, Rodaros & Stewart, 1996; Espejo & Gil, 1997; Hawkins, Cubic, Baumeister & Barton, 1992), decrease (Grignaschi, Mantelli & Samanin, 1993; Shimizu, Oomura & Kai, 1989; Zurita, Murúa & Molina, 1996) or have no effect (Ely, Dapper, Marasca, Correa, Gamaro, Xavier, et al., 1997; Endo & Shiraki, 2000; Zurita, et al., 1996) on food intake.

1.1.1 Types of stressors

Different kinds of stressors have diverse effects on food intake in the rat. For example, exposure to rapid and frequent changes in daily environmental temperature for several days has been shown to increase food intake and decrease body weight in rats (Kawanishi, Fukuda, Tamura, Nishijo & Ono, 1997). Exposing rats to foot shock for 12 weeks does not influence food intake (Endo & Shiraki, 2000), while chronic exposure to acoustic stress decreases food intake (Alario, Gamallo, Beato & Trancho, 1987; Krebs, Macht, Weyers, Weijers & Janke, 1996) and tail pinch stress increases food intake (Hawkins, et al., 1992; Levine & Morley, 1981; Telegdy, Kadar, Kovacs & Penke, 1984).

There are many examples in the literature of dietary stress influencing feeding behaviour. Rats that have been food-deprived for as little as 2 h at dark onset (Tempel, Shor-Posner, Dwyer & Leibowitz, 1989) and as long as 24 h (Welch, Grace, Billington & Levine, 1994) and 48 h (De Boer, Koopmans, Slangen...
& Van Der Gugten, 1989) increase subsequent fat and CHO intake compared to control animals. Other studies have shown that after a 5 day fast, rats increased their CHO and fat consumption for 23 days to compensate for the energetic deficit from the fast (Piquard, Schaefer & Haberey, 1978). Further, rats that were starved for 3 days decreased their protein intake for the first 2 days of refeeding followed by an increase in CHO consumption relative to controls (Hunsicker, Mullen & Martin, 1992). By contrast, in rats restricted to 5 g of food/day for 5 days, free-feeding tests revealed an immediate increase in food intake, characterized primarily by an increase in CHO intake (Hunsicker, et al., 1992).

Restraint stress (placement in a tube-like restraining device) has generally been shown to decrease food intake (Pitman, Ottenweller & Natelson, 1988; Zurita, et al., 1996). Research has suggested that immobilization stress (taping animals in a prone position to a wire grid) may have more severe influences on behaviour than restraint stress (Grignaschi, et al., 1993). While immobilization stress also decreases food intake in rats it is able to do so after a shorter stress duration than restraint stress (Grignaschi, et al., 1993).

1.1.2 Duration of Stressors

While the nature of the stressor influences the effect of a stressor on food intake, the length of exposure to a stressor can also have an influence on food consumption. For example, a 5 min tail pinch stress has been shown to increase
food intake after one stress session and have no effect on food intake after several daily exposures (Levine & Morley, 1981). In this instance, the animal habituates to the stressor over time and its behaviour is more strongly influenced after one exposure to the stressor than after several exposures. The habituation effect appears to be related to the intensity of the stressor. For example, while food intake has been shown to habituate in rats exposed to immobilization stress (Kennett, Dickinson & Curzon, 1985a; Kennett, Dickinson & Curzon, 1985b), no habituation is evident in studies employing the milder restraint stress (Pitman, et al., 1988). This may be due to the effect of different stressors on stress hormones such as corticosterone. While chronic exposure to immobilization stress results in an habituation of the corticosterone response to stress (Kennett, et al., 1985a), chronic restraint stress does not (Pitman, et al., 1988). Habituation of the corticosterone response to stress has been suggested as an indication of the intensity of a stress (Kennett, et al., 1985a), or where stressors that show an habituation of the corticosterone response are considered to be more intense than stressors that do not reveal an habituation.

There are several factors that influence the impact of restraint stress on food intake. For example, acute and chronic stress can often differentially influence food intake in the rat. The direction of this effect has varied with chronic (e.g., Marti, Marti & Armario, 1994) and longer acute stress sessions (1-2 hours; e.g., Grignaschi, et al., 1993; Shimizu, et al., 1989) often resulting in reduced feeding, while brief acute sessions (5-20 min) can increase feeding
Stress, serotonin and macronutrient intake

Badiani, et al., 1996; Espejo & Gil, 1997). The method through which stressors are administered is important in the resulting effects on food intake. For example, tube restraint stress for 1 h has no effect on feeding (Ely, et al., 1997; Zurita, et al., 1996), while at 2 h food intake is reduced (Zurita, et al., 1996) and at 20 min it is increased (Badiani, et al., 1996). In contrast, immobilization stress through taping to a wire grid is effective in reducing feeding after 1 h (Grignaschi, et al., 1993). There does not appear to be any research which varies the duration of food deprivation or restriction to determine any differences in subsequent food intake; however longer dietary stress paradigms modify corticosterone levels (Garcia-Belenguer, Oliver & Mormede, 1993; Gursoy, Cardounel, Hu & Kalimi, 2001; Heiderstadt, McLaughlin, Wright, Walker & Gomez-Sanchez, 2000) which influence food intake (Cooper & Liebman, 1992; Leibowitz, 1988; Tempel, Yamamoto, Kim & Leibowitz, 1991).

1.2 STRESS AND CORTICOSTERONE

The effect of stress on an organism is often mediated by an increase in the adrenal hormone cortisol (in humans) or corticosterone (in rats and some other species).

Chronic restraint stress has differing effects on corticosterone release at different periods of the light cycle (Ottenweller, Servatius & Natelson, 1994). It may be that both chronic and acute restraint stress regimes influence 5-HT
activity through an increase in corticosterone release from the adrenal gland (Saphier, Farrar & Welch, 1995).

Dietary restriction increases plasma corticosterone levels in rats (Garcia-Belenguer, et al., 1993; Gursoy, et al., 2001; Heiderstadt, et al., 2000). However, these increases in corticosterone are only evident when the dietary stress paradigm is sufficiently severe. For example, rats restricted to 50% of their ad libitum food intake for 4 weeks had increased plasma corticosterone levels, while rats exposed to the less severe caloric restriction of 80% of their ad libitum food intake did not have elevated corticosterone levels (Garcia-Belenguer, et al., 1993).

Corticosterone increases 5-HT release and modifies 5-HT receptor function in the brain (Saphier, et al., 1995; Tejani-Butt & Labow, 1994). Specifically, corticosterone decreases 5-HT1A receptor density in the dorsal raphe nucleus of the brainstem and the hippocampus (Tejani-Butt & Labow, 1994) and increases 5-HT2A receptor density in the prefrontal cortex and hippocampus (Yamada, Watanabe, Nankai & Toru, 1995). Moreover, stress has been shown to increase 5-HT2A receptor density in the cerebral cortex (McKittrick, Blanchard, Blanchard, McEwen & Sakai, 1995). In a study conducted by Berendsen and colleagues (Berendsen, Kester, Peeters & Broekkamp, 1996), chronic (2 times/day for 4 days) but not acute administration of a high dose of corticosterone (50 mg/kg) decreased behaviours associated with 5-HT2C receptor activation, increased behaviours associated with 5-HT2A receptor activation, and had no effect on 5-
HT1A receptor-mediated behaviours. It should be noted that the dose of 50 mg/kg corticosterone was chosen because it produces plasma corticosterone levels comparable to those seen in animals stressed by immobilization (Dickinson, Kennett & Curzon, 1985; Hodges & Jones, 1963).

Therefore, while acute stress may cause an increase in corticosterone release from the adrenal gland that increases brain 5-HT, it has no effect on post-synaptic 5-HT receptors. However, chronic corticosterone release from the adrenal in response to chronic stress may elevate 5-HT levels for a sufficient period of time to allow an increase in 5-HT2A receptors and a decrease in 5-HT2C receptors and may or may not decrease 5-HT1A receptors.

1.3 CORTICOSTERONE AND FOOD INTAKE

The major function of corticosterone in rats and cortisol in humans is to enhance CHO stores in the body (Bray, York & Fisler, 1989; Hers, 1986). Corticosterone and cortisol play an important role in regulating CHO intake particularly at the beginning of the natural feeding cycle, when blood levels of the hormone peak and glycogen stores are low (Tempel & Leibowitz, 1989). At this time in the circadian cycle, corticosterone acts in concert with γ-aminobutyric acid (GABA), norepinephrine (NE), and neuropeptide Y (NPY) to increase CHO intake (Cooper & Liebman, 1992; Leibowitz, 1988). A primary site of action for these substances in the brain is a medial region of the hypothalamus, in particular, the paraventricular nucleus (PVN), where these
neurotransmitters and glucocorticoid receptors are co-localized (see Leibowitz, 1992). In both rats and humans, CHO is most strongly preferred during the early hours of the feeding cycle, perhaps because it is more efficient in rapidly replenishing glucose stores in the hungry animal (De Castro, 1987; Tempel, et al., 1989). Thus, in association with the CHO-rich meals that naturally occur at this time, there is a peak in the levels or release of the hypothalamic neurochemicals and in the density or responsiveness of their receptors (Dumont, Martel, Fournier, St-Pierre & Quirion, 1992; Leibowitz, 1988; Leibowitz, 1991).

Additional research supports this relationship between corticosterone and CHO intake. Corticosterone has been shown to restore CHO intake at dark onset in adrenalectomized rats (Tempel, et al., 1991). And, in food restriction experiments, increasing CHO in diets of animals with elevated plasma corticosterone levels results in a normalization of corticosterone levels (Garcia-Belenguer, et al., 1993).

However, both chronic (e.g., Marti, et al., 1994) and acute (Zurita, et al., 1996) stress often decrease food intake. If stress acts to decrease food intake and increase corticosterone release, one would expect that corticosterone treatment would decrease feeding and CHO intake. Some studies employing chronic mild stress models in rats have shown that while liquid sucrose consumption is reduced, food intake is either unaffected or increased by chronic stress (see Willner, 1997). Further, some human research has revealed that depressed individuals choose a higher proportion of carbohydrates in their diet
relative to protein and fat than nondepressed people (Christensen & Somers, 1996; Kazes, Danion, Grange, Pradignac, Simon, Burrus-Mehl, et al., 1994). Whether this occurs in animal models of depression (chronic stress models) has yet to be determined. Animal research on chronic stress has found the opposite, that chronic mild stress decreases sucrose consumption. Willner (1997) has suggested this indicates that the animal is in an anhedonic state that reduces the palatability of the diet. It may be that chronic stress influences drinking a high CHO beverage differently than eating a high CHO meal. For example, results from one study indicate that chronic restraint stress acts to increase the consumption of a high CHO cereal (Ely, et al., 1997).

Because elevated corticosterone increases brain 5-HT (Berendsen, et al., 1996; Saphier, et al., 1995; Tejani-Butt & Labow, 1994) one could speculate that corticosterone's effect on CHO intake is independent of its effect on 5-HT activity. The correlational research mentioned earlier suggests that the effect of corticosterone on CHO intake is more likely due to increased activity of GABA, NPY and NE whose levels are increased at the beginning of the active cycle and whose receptors are co-localized in the PVN (Cooper & Liebman, 1992; Leibowitz, 1988; see Leibowitz, 1992). It may be that the effect of corticosterone to increase brain 5-HT occurs later in the circadian cycle, resulting in a satiety signal for CHO.
1.4 5-HT AND FOOD INTAKE

In recent years links have been made between the intake of food and the serotonergic system. Overall, there is consensus that 5-HT serves an inhibitory role in feeding, however the specific mechanisms through which this occurs have yet to be resolved (see Simansky, 1996). In general, increases in 5-HT within the hypothalamus have been shown to decrease food intake in rats (Liebowitz, Weiss, Walsh & Viswanath, 1989). Further, at the beginning of the dark cycle, when hypothalamic 5-HT levels are low (Martin & Marsden, 1985) food intake is increased (Tempel, et al., 1989). Experiments employing both pharmacological and correlational methods have investigated the overall effect of 5-HT on food intake as well as the specific receptor subtypes that may be involved.

1.4.1 Pharmacological Studies

Food-deprived rats treated peripherally with 5-HT show the full behavioural sequence of satiety (grooming and/or locomotor activity followed by a period of resting) suggesting that 5-HT is a likely candidate for a satiety signal (Edwards & Stevens, 1991). As well, selective serotonin reuptake inhibitors such as fluoxetine, paroxetine and sertraline accelerate the onset of satiety without altering the behavioural sequence of satiation (Clifton, Barnfield & Philcox, 1989; McGuirk, Muscat & Willner, 1992; Simansky & Vaidya, 1990). Further, peripheral administration of general 5-HT postsynaptic receptor agonists such as quipazine and m-chlorophenylpiperazine (mCPP) reduce food intake in animals (Kennedy,
Gibson, O'Connell & Curzon, 1993; Samanin, Bendotti, Candelaresi & Garattini, 1977; Samanin, Caccia, Bendotti, Borsini, Borroni, Invernizzi, et al., 1980), and treatment with a low dose (0.5 mg/kg) of methysergide (a general 5-HT antagonist) increases sucrose intake in food-deprived rats (Islam, Dougherty, Koch & Bodnar, 1994). Research investigating the specific receptor subtypes that may be involved in 5-HT-induced satiation has revealed that while the 5-HT1A agonist 8-hydroxy-dipropylaminotetralin (DPAT) increases feeding (see Dourish, 1992), activation of 5-HT2A/2C receptors by (±)-2,5-dimethoxy-4-iodoamphetamine (DOI; Price, Gorzalka, White & Arkinstall, 1998; see Simansky, 1996) or 5-HT1A/1B receptors by RU 24969 (5-methoxy-3-[1,2,3,6-tetrahydropyridin-4-yl]-1H-indole; Kennett & Curzon, 1988) inhibits feeding. The effect of DPAT to increase food intake is due to its binding to presynaptic 5-HT1A receptors in the brainstem which, when activated, reduce 5-HT release throughout most of the brain (see Cooper, 1992). Investigations into the specific aspects of food intake affected by different 5-HT receptor subtypes suggest that stimulation of postsynaptic 5-HT2A receptors disrupts the continuity of feeding, while stimulation of postsynaptic 5-HT1B receptors reduces the size of the meal ingested and stimulation of postsynaptic 5-HT2C receptors inhibits the rate of eating (see Simansky, 1996). Normal satiation occurs after co-activation of 5-HT1B and 5-HT2C receptors (see Simansky, 1996).

Studies performed in humans have also revealed that serotonergic drugs exert an influence on food intake (Goodall, Feeney, McGuirk & Silverstone,
Stress, serotonin and macronutrient intake

1992). For example, serotonin reuptake inhibitors, fenfluramine (e.g., Curzon, Gibson & Oluyomi, 1997; Goodall, et al., 1992) and fluoxetine (e.g., Curzon, et al., 1997) decrease food intake in humans.

1.4.2 Correlational Studies

Correlational studies have largely focused on investigating the circadian rhythm of brain 5-HT and how this is related to food intake. During the dark period of the light cycle, rats consume 80-90% of their daily calories (Armstrong, 1980; Siegal, 1961). Studies in rats have revealed that 5-HT levels are lowest in the period just prior to dark onset (Leibowitz, et al., 1989; Wesemann & Weiner, 1990). At dark onset, rats will choose a meal that has a high proportion of CHO relative to protein (Leibowitz, et al., 1989). This increase in CHO consumption relative to protein causes brain 5-HT levels to rise (Leibowitz, et al., 1989). Finally, the increased brain 5-HT induces a satiety signal for CHO resulting in a preferential shift to protein and fat intake at later phases of the dark cycle (Tempel, et al., 1989).

1.4.3 Macronutrient Intake

Increased brain 5-HT decreases CHO intake while sparing protein intake (Leibowitz, et al., 1989). When CHO is ingested, the insulin release caused by food ingestion increases the cellular uptake of all large neutral amino acids (LNAA) except tryptophan, 5-HT's metabolic precursor (Wurtman & Fernstrom,
Further, more tryptophan is released from the cells and enters the bloodstream which produces an increase in the plasma tryptophan/LNAA ratio (Wurtman & Fernstrom, 1976). This allows more tryptophan to cross the blood-brain barrier, due to reduced competition for transport as a result of lower levels of all other LNAA’s in the bloodstream (Wurtman & Fernstrom, 1976). In the brain, tryptophan, the rate-limiting step of 5-HT synthesis, is readily converted to 5-HT (Wurtman & Fernstrom, 1976). The newly synthesized 5-HT binds to 5-HT receptors in the brain resulting in a satiety signal for CHO consumption (Leibowitz et al., 1989). This increase in tryptophan relative to other LNAA’s is dependent on the ratio of CHO to protein contained in the meal, and not simply the absolute amount of CHO ingested. When protein is consumed, the level of all LNAA’s, including tryptophan, rise (Fernstrom & Wurtman, 1972). These LNAA’s compete for entry into the brain, resulting in a reduction in the amount of tryptophan entering the brain (Fernstrom & Wurtman, 1972). Increased brain 5-HT does not produce a satiety signal for protein (Leibowitz et al., 1989), however there may be a relationship between fat intake and 5-HT (Blundell & Lawton, 1995; Mullen & Martin, 1992). While the exact mechanism for fat intake and satiety has not been established, increased brain 5-HT reduces fat intake (Blundell & Lawton, 1995; Mullen & Martin, 1992) and fat ingestion increases tryptophan levels in the brain (Hutson, Knott & Curzon, 1976).
1.4.4 Brain mechanisms

Food intake is increased after local injection of 5-HT1A agonists into the dorsal or median raphe nuclei of the brainstem (Bendotti & Samanin, 1986; Fletcher & Davies, 1990). The raphe nuclei house the cell bodies of serotonergic neurons that project throughout most of the brain (see Cooper, 1992). Further, there is a high density of presynaptic 5-HT1A receptors localized within these nuclei (see Cooper, 1992). Therefore, 5-HT1A agonists stimulate the presynaptic receptors that decrease release of 5-HT within the raphe nuclei and ultimately inhibit the firing of the neurons that project from there to most of the forebrain (see Cooper, 1992; Dourish, 1992; see Appendix I). This causes an overall reduction of 5-HT throughout the brain that presumably causes an increase food intake (see Cooper, 1992; Dourish, 1992).

The hypothalamus is an area of major importance in food intake. Shortly after dark onset 5-HT activity in the medial hypothalamus reaches a peak (Martin & Marsden, 1985). This neurochemical change at dark onset has been revealed in the PVN, ventromedial hypothalamus (VMH) and suprachiasmatic nucleus (SCN) of the hypothalamus (Faradji, Cesuglio & Jouvet, 1983; Hery, Faudon, Dusticier & Hery, 1982; Martin & Marsden, 1985; Mason, 1986; Meyer & Quay, 1976; Stanley, Schwartz, Hernandez, Leibowitz & Hoebel, 1989). These three nuclei respond to serotonergic stimulation influencing suppression of CHO intake at dark onset (see Leibowitz, et al., 1989). Serotonin may act in the PVN, VMH and SCN to coordinate temporal patterns of macronutrient choice (Leibowitz, et
al., 1989). There is extensive evidence indicating that the SCN has a primary role as a circadian master oscillator (Moore, 1983; Rosenwasser & Adler, 1986). Moreover, reports suggest that the VMH and PVN play a role in a variety of physiological and behavioural responses to the light-dark cycle including feeding behaviour (Rosenwasser & Adler, 1986; Stoynev & Ikonomov, 1987; Szafarczyk, Ixart, Alonso, Malaval, Nougier-Soule & Assenmacher, 1983). These three hypothalamic nuclei receive a moderate to dense innervation from serotonergic projections of the raphe nuclei (Steinbusch, 1981) and have a relatively dense concentration of 5-HT1B receptor sites (Pazos & Palacios, 1985) that play a role in meal size (see Simansky, 1996). Further, the serotonergic activity in the raphe nucleus (Agren, Koulu, Saaverda, Potter & Linnoila, 1986; Rosenwasser, Trubowitsch & Adler, 1985), as well as 5-HT’s inhibitory effect on neural firing in the SCN (Mason, 1986), is strongest around the time of dark onset. Because anatomical and functional links exist between the SCN and the PVN or VMH (Rosenwasser & Adler, 1986), it is likely that these three nuclei interact closely in coordinating temporal patterns of macronutrient intake (Leibowitz, et al., 1989).

1.5 5-HT, STRESS AND FOOD INTAKE

Many studies have established that 5-HT plays a role in the effect of restraint stress on food intake in the rat. For example, the feeding inhibition induced by acute restraint stress is blocked by centrally administered 5-HT antagonists as well as peripherally administered antagonists which cross the
blood-brain barrier. However, xylamidine, a 5-HT antagonist that does not act centrally, fails to elicit this effect (Grignaschi, et al., 1993). These results suggest that the role 5-HT plays in acute stress-inhibited feeding involves central and not peripheral mechanisms.

Research on dietary restriction has revealed similar results. For example, when female rats are restricted to 5 g/day of food and then presented with a self-selection diet of CHO, protein or fat, they increase their total food intake specifically by increasing their intake of CHO (Hunsicker, et al., 1992). Further, in normal and overweight men and women, three weeks of dieting (caloric restriction through decreasing CHO and fat intake) decreased plasma tryptophan levels (Walsh, Oldman, Franklin, Fairburn & Cowen, 1995) and the tryptophan/LNAA ratio (Anderson, Parry-Billings, Newsholme, Fairburn & Cowen, 1990; Goodwin, Cowen, Fairburn, Parry-Billings, Calder & Newsholme, 1990). By testing women who were on a four-week calorie-reduced diet (decreased in CHO and fat) during the follicular phase of their menstrual cycle, when both estrogen and progesterone levels are low, Wolfe and colleagues (Wolfe, Metzger & Stollar, 1997) confirmed that fluctuation in circulating ovarian hormones did not account for the previous results in women. Researchers have suggested that caloric restriction in women (which may or may not be due to a reduction in the proportion of CHO in the diet) may increase post-synaptic 5-HT receptor responsiveness, as revealed through increased levels of prolactin in blood plasma in response to a tryptophan (Anderson, et al., 1990; Delgado, Charney, Price,
Landis & Heninger, 1989; Goodwin, Fairburn & Cowen, 1987), fenfluramine (Walsh, et al., 1995) and mCPP (Cowen, Clifford, Williams, Walsh & Fairburn, 1995) challenge.

1.6 GENDER AND STRAIN DIFFERENCES IN STRESS RESPONSE

When one considers the nature of the stressor, it is reasonable to imagine that what is stressful for one member of a species may not be for another. Research has shown that some women appear to be influenced more negatively by a stressor than men (Weinstein, Shide & Rolls, 1997). Specifically, in women, trait anxiety, disinhibited eating and concerns with body weight and shape are positively correlated with increased food intake in response to a stressor, while no such correlations are found with men (Weinstein, et al., 1997). Female rats have been shown to respond to 20 min (Zylan & Brown, 1996), 2 h (Mendelson & McEwen, 1991) and chronic stress (Haleem, et al., 1988) with significantly higher corticosterone levels than males. While one study found that females do not develop the enhanced postsynaptic 5-HT receptor sensitivity after chronic immobilization stress that occurs in male rats (Kennett, Chaouloff, Marcou & Curzon, 1986), another study found that females showed a reduction in plasma corticosterone compared to males on day 5 of a 2 h/day restraint stress regime (Mendelson & McEwen, 1991). These data suggest that female rats respond more severely to acute restraint sessions (20 min and 2 h), and may differ from males in whether they show an habituation to chronic restraint. It has also been revealed that the 5-HT systems of female rats are more labile than those of male
Researchers have suggested that these differences between males and females may partially explain why depression is more common in women than men (Kennett, et al., 1986).

The vast majority of experiments investigating the relationship between stress and food intake have been conducted in males. Given sex differences in response to stress, it is important that the relationship between stress and macronutrient intake is investigated in the female rat. In all of the following experiments, female rats were used as subjects.

Gonadal hormones organize brain structures during development. There are sex differences in the anatomy and physiology of the hypothalamus and cerebral cortex (see Matsumoto, 2000). These differences are due to the presence of gonadal hormones during development (see Matsumoto, 2000). The turnover and levels of brain 5-HT change during the estrous cycle of the rat (Fludder & Tonge, 1975). Further, estradiol and progesterone treatment modify 5-HT uptake in the hypothalamus (Endersby & Wilson, 1974) and other brain regions (Wirz-Justice, Hackmann & Lichsteiner, 1974). Female rats are more sensitive than males to an elevation of brain 5-HT, responding to lower doses of pargyline plus tryptophan (Biegon, Bercovitz & Samuel, 1980). Finally, 5-HT receptor density fluctuates in the basal forebrain during the estrous cycle of the rat (Biegon, et al., 1980), resulting in a 50% lower density on proestrus (high estrogen levels) than on diestrus (low estrogen levels). In order to control for the potential confound of fluctuating hormone levels and differences in hormone
levels between rats and their subsequent impact on the 5-HT system, all subjects in this study were ovariectomized in adulthood.

In addition to gender, other genetic factors are important in the stress response. For example, in the rat there are differences between some strains in animals’ stress responses. Rats of the Wistar strain have been shown to be most affected by stress, those of the Sprague Dawley strain are moderately affected and those of the Long Evans strain are the least affected by exposure to stressors (Keim & Sigg, 1976). All subjects in this study were of the Wistar strain as this strain has been shown to be most responsive to stress.

1.7 PURPOSE OF THIS STUDY

The results of study will extend current research through discovering the effect of restraint stress on macronutrient intake. The effect of restraint stress on macronutrient intake has never been investigated. Further, this study will extend current findings in the dietary stress literature through employing a CHO-restriction procedure that does not restrict overall caloric intake. In addition, the experiments in this study will be conducted with female rats. The vast majority of stress research to date has been conducted in male rats. Results from studies employing female rats may allow for stronger indications of the effect of stress on food intake in women which may have implications for eating disorders.
This study will develop two stress paradigms that have opposing effects on CHO intake in the female rat. One stress paradigm will be developed that inhibits CHO intake, which may have implications for AN, while a second stress paradigm will be developed that results in an increase in CHO intake, which may have implications for BN. While several forms of stress have been shown to influence food intake in humans and other species, this dissertation will focus on two relatively distinct forms of stress: physical restraint stress and dietary stress (CHO-restriction). This study will also determine whether these stressors influence macronutrient intake in a similar fashion or have a differential effect on specific macronutrients and whether these effects can be attenuated through pharmacological manipulation of 5-HT.

Although considerable research has investigated the effect of total caloric restriction on 5-HT functioning and subsequent food intake, there have not been any studies investigating the effect of CHO-restriction, without overall caloric restriction, on macronutrient intake and 5-HT functioning. It is unknown whether simply restricting rats in their CHO intake without decreasing overall caloric intake is sufficient to increase CHO preference through influencing the 5-HT system. Given the close relationship between CHO intake and 5-HT, it seems logical that this form of dietary stress would have an effect on the 5-HT system and possibly the preference to consume CHO.

Restraint stress has been shown to increase 5-HT activity and modify 5-HT receptors in the brain (Hunsicker, et al., 1992; Saphier, et al., 1995).
Restraint stress has also been shown to modify food intake in the rat (Badiani, et al., 1996; Grignaschi, et al., 1993; Zurita, et al., 1996). Research has also revealed that restraint stress increases corticosterone release (Ottenweller, et al., 1994; Saphier, et al., 1995), and corticosterone has been shown to increase CHO intake in the rat (Cooper & Leibman, 1992; Leibowitz, 1992). What is unclear from the literature at this stage is whether restraint stress will influence self-selection of macronutrients (CHO, protein and fat) to reveal a preferential inhibition of CHO and possibly fat intake and whether 5-HT is involved in this effect. Further, it is unknown whether differences exist between brief, acute and chronic restraint stress in terms of effect on macronutrient intake.

It is predicted that restraint stress will decrease CHO and likely fat intake. Further, neither CHO-restriction nor restraint stress will influence protein intake. Experiments 1 to 3 investigate how different lengths of restraint stress (20 min, 2 h or 2 h/day for 5 day) influence macronutrient intake. In addition, a reduction in 5-HT activity, through the application of DPAT, will attenuate the effect of restraint stress on CHO and possibly fat intake. Experiment 4 investigates this prediction by determining whether 5-HT is involved in the effect of a 2 h restraint session on CHO intake through the application of DPAT. Finally, Experiments 5 to 8 answer the question: Can dietary stress that limits the intake of CHO without limiting overall caloric intake have an impact on subsequent food intake and if so, does 5-HT play a role in this effect? Experiments 5-7 will determine whether chronic CHO-restriction can lead to an
increase in CHO intake, and Experiment 8 will determine whether fluoxetine administration can attenuate this effect.
CHAPTER II: BRIEF, ACUTE & CHRONIC STRESS

Modifying the length of exposure to a stressor can have differential effects on feeding behaviour. For example, chronic stress (2 h/day for 5-7 days) has been shown to decrease food intake in some experiments (Kennett, et al., 1986), and have no effect in others (Haleem & Parveen, 1996; Kennett, et al., 1985a; Kennett, et al., 1985b). Further, while acute stress sessions (1-2 h; e.g. Kennett, et al., 1986; Haleem & Parveen, 1996; Grignaschi, et al., 1993; Shimizu, et al., 1989; Kennedy, et al., 1993) typically result in a reduction of food intake, brief acute sessions (20 min or less) have been shown to decrease (Zylan & Brown, 1996) or increase food intake (Badiani, et al., 1996; Espejo & Gil, 1997).

Corticosterone release in response to stress may play a role in some of these results. Corticosterone increases 5-HT release and modifies 5-HT receptor function in the brain (Saphier, et al., 1995; Tejani-Butt & Labow, 1994). Further, 2 h of immobilization or restraint stress increases plasma corticosterone (Kennett, et al., 1985b; Mendelson & McEwen, 1991; Pitman, et al., 1988) and 5-HT levels (Haleem & Parveen, 1996; Kennett, et al., 1985b). In addition, while 2 h of immobilization or restraint stress (Kennett, et al., 1986; Haleem & Parveen, 1996; Kennett, et al., 1985a; Grignaschi, et al., 1993; Shimizu, et al., 1989; Kennedy, et al., 1993) decreases food intake, 5-HT postsynaptic receptors do not appear to play a role in this effect (Kennett, et al., 1985a; Kennedy, et al., 1993). Finally, compounds that decrease brain 5-HT activity attenuate the effect

While brief restraint of 5, 15 and 20 min increases corticosterone release (Zylan & Brown, 1996; Kant, Lenox, Bunnell, Mougey, Pennington & Meyeroff, 1983), it is unclear whether this accounts for the effect on food intake (Zylan & Brown, 1996; Badiani, et al., 1996). Dietary factors appear to be important in determining the nature of the response on food intake to 20 min of restraint. For example, brief restraint of 20 min will increase food intake when rats are fed a standard laboratory chow diet (Badiani, et al., 1996; Espejo & Gil, 1997) and inhibit food intake when animals are fed more palatable foods (Zylan & Brown, 1996).

Chronic immobilization stress (2 h/day for 5-7 days) does not increase corticosterone (Kennett, et al., 1985a; Pitman, et al., 1988) or 5-HT (Haleem & Parveen, 1996) levels, although an increase in postsynaptic 5-HT receptor sensitivity has been revealed (Kennett, et al., 1985a; Kennett, et al., 1985b). There has been speculation that it is the modification in 5-HT receptors that allows animals exposed to chronic immobilization to show habituated responses to the stressor, including a lack of inhibition of food intake (Kennett, et al., 1985a; Kennett, et al., 1985b). It appears that early in the chronic immobilization regime, corticosterone levels are elevated; however there is subsequent adaptation and animals fail to respond with increased corticosterone (Kennett, et al., 1985a). Further, elevated corticosterone levels may play a role
in opposing this adaptation process. When animals are given a compound that blocks corticosterone release, the adaptation to chronic immobilization stress is revealed earlier in the course of the stress treatment (Tejani-Butt & Labow, 1994). Much less is known about the effect of restraint stress on corticosterone release. One study found that 2 h of restraint for 5 days inhibited food intake and failed to reveal habituation of the corticosterone response (Pitman, et al., 1988). These data are supported by previous studies that employed the more severe immobilization stress regimes resulting in habituated corticosterone response and habituation of the stress-induced inhibition of food intake. Therefore, it may be that chronic restraint stress (2 h/day for 5 days) is relatively mild and does not alter 5-HT postsynaptic receptor sensitivity as chronic immobilization stress does and, therefore, does not reveal the same habituated attenuation of stress-inhibited food intake.

When considering the relationship between corticosterone, 5-HT and food intake it is important to remember that endogenous corticosterone increases CHO intake in the rat (Leibowitz, 1992) as well as increasing 5-HT release and modifying 5-HT post-synaptic receptor function (Saphier, et al., 1995; Tejani-Butt & Labow, 1994). Correlational data investigating the circadian rhythms of corticosterone release, CHO intake and 5-HT levels (Leibowitz, 1992) allow for the speculation that brief stress (20 min) may increase corticosterone release and reveal in increase in CHO intake, while longer acute sessions (2 h) would increase corticosterone release and 5-HT levels but have no effect on 5-HT.
postsynaptic receptors, resulting in an inhibition of CHO intake. Finally, chronic stress (2 h/day for 5 days) may initially increase corticosterone release, which would increase 5-HT levels and eventually, result in a modification of 5-HT postsynaptic receptors, producing an attenuation of the stress-induced inhibition of CHO intake. Alternately, chronic restraint stress might not result in an habituation of the corticosterone response nor an habituation of stress-induced inhibition of food intake (Pitman, et al., 1988).

Given the relationship between 5-HT and macronutrient intake outlined previously, the following series of experiments will investigate the effects of 20 min, 2 h and chronic (2 h/day for 5 days) restraint stress on macronutrient intake. While several studies have investigated the effects of restraint on overall food intake, this is the first study to look at the impact of different lengths of restraint stress on specific macronutrient intake.

With regards to food intake, female and male rats have both been shown to inhibit food intake after 20 min of restraint (Zylan & Brown, 1996) and 2 h of immobilization stress (Kennett, et al., 1986). Also, a chronic immobilization stress regime of 2 h/day for 5 days does not elicit an habituated rise in food intake to control levels in female rats (Kennett, et al., 1986).

While some restraint experiments do not employ food deprivation (Kennett, et al., 1986; Shimizu, et al., 1989; Zurita, et al., 1996; Zylan & Brown, 1996), others employ 23-24 h of food deprivation (Grignaschi, et al., 1993; Kennedy, et al., 1993). This duration of food deprivation has significant effects
on 5-HT levels (Chaouloff, Berton, Aquerre, Hay & Mormede, 1997; Hunsicker, et al., 1992; Knott & Curzon, 1974; Loullis, Felton & Shea, 1979; Nishimura, Nishihara, Torii & Takahashi, 1996). 24 h of food deprivation increases 5-HT levels in the raphe nucleus of the brainstem without affecting 5-HT1A autoreceptors (Chaouloff, et al., 1997) and 5-HT release in the raphe nucleus decreases 5-HT release throughout the brain (Zifa & Fillion, 1992). Given the importance of 5-HT in the effect of stress on food intake, it seems appropriate to determine whether the length of food deprivation prior to testing interacts with stress on macronutrient intake. The following series of experiments will investigate the effect of 4 and 24 h food deprivation on the impact of 20 min, 2 h and chronic restraint stress on macronutrient intake in the female rat. Research investigating neurochemical responses to food deprivation and restraint stress suggests that 24 h of food deprivation will attenuate the effect of restraint stress on subsequent macronutrient intake through reducing brain 5-HT levels. It may be that the effect 24 h of food deprivation has on stress-induced inhibition of macronutrient intake will vary depending on the length of the stress session. It is conceivable for example, that animals exposed to 24 h of food deprivation prior to 20 min of restraint stress will consume macronutrients at levels similar to that of a control group, while after 2 h of restraint stress macronutrient intake will remain inhibited.
2.1 METHODS AND MATERIALS

2.1.1 Participants

Female Wistar rats (Animal Care Unit, University of British Columbia, Vancouver, Canada) between the ages of 4 and 5 months of age (350-450 g body weight) were used as subjects. Rats were singly housed in standard hanging wire mesh cages during the experiment and group-housed in standard triple hanging wire mesh cages at all other times. Temperature conditions in the colony were maintained at 21 +/- 1°C. Colonies were maintained on a 12/12 h light-dark cycle with lights off at 1230 h. Except during food deprivation and testing, rats had free access to Purina rat chow and tap water. All animals were bilaterally ovariectomized at 3 months of age to control for hormone fluctuation during the estrous cycle. Animals were ovariectomized using a combination of 75 mg/kg ketamine and 7 mg/kg xylazine anesthesia. Surgical procedures were performed 1 month prior to commencement of experiments in order to allow for post-ovariectomy weight gain. Therefore, females were tested for the effect of restraint stress on macronutrient intake 5 (Experiments 1 and 2) and 6 (Experiment 3) weeks after ovariectomy.

2.1.2 Restraint Tubes

Placement in white plastic cylinder tubes was used to induce restraint stress. These tubes were 8 inches (20.3 cm) long and 2.5 inches (6.35 cm) in diameter. Each tube was covered on one end with a Plexiglas covering that had
Stress, serotonin and macronutrient intake

a small air hole 0.25 inches (.64 cm) in diameter in the centre. These tubes fit snugly to the body size of the rats and inhibited movement. After placement in the tubes, rats were placed horizontally in plastic carrying cages so that the open end of the tubes were close to the wall of the carrying cage in order to prevent the escape of rats from the tubes.

2.1.3 Experimental Diets

Three separate diets of protein, CHO and fat were used in this study. Previous studies have revealed that free access to these diets allow for normal body weight gain and consistent day-to-day intake scores (Leibowitz, et al., 1989). All recipes for the macronutrient diets were obtained from Leibowitz and colleagues (1989). The fat diet (7.7 kcal/g) consisted of 86% lard (No Name Brand, Safeway, Canada) combined with 8% minerals (Rogers & Harper Salt Mixture, ICN Pharmaceuticals, Costa Mesa, CA) and 6% vitamins (AIN-76 Vitamin Mixture, ICN Pharmaceuticals, Costa Mesa, CA). The CHO diet (3.7 kcal/g) was composed of 37% sucrose (BC Sugar, Safeway, Canada), 28% dextrin (Dextrin type II, ICN Pharmaceuticals, Costa Mesa, CA), 28% cornstarch (Canada Brand, Safeway, Canada), 4% minerals and 3% vitamins. The protein diet (3.7 kcal/g) consisted of 93% casein (Granulated, enzymatic casein, ICN Pharmaceuticals, Costa Mesa, CA), 4% minerals, 2.97% vitamin and 0.03% L-cysteine (ICN Pharmaceuticals, Costa Mesa, CA). Each macronutrient diet was placed in one of
three small petri dishes (5 cm in diameter) tightly set in metal trays to minimize spillage.

2.1.4 Acclimatization Procedure

Prior to any experimental procedure, rats were given a 6-day period to acclimatize to single housing and to the macronutrient diets. During this period of adaptation, rats were exposed to the macronutrient diets at dark onset (1230 h) and allowed access to the diets for 40 min. The metal trays with the diets in them were placed in the front right hand corner of the rats' cages, away from their water source. The location of the food dishes within the rats cages which contained macronutrient diets was changed each day in order to control for place preference consumption. The order of the macronutrients was the same for all rats on any given day. During the acclimatization period, all rats were allowed free access to laboratory chow and water.

2.1.5 Testing Procedure

20 min (Experiment 1) and 2 h (Experiment 2) Restraint Experiments

Experiment 1 employed 110 rats as subjects, rats were randomly assigned to 4 treatment groups: restraint/4 h food-deprivation (n=28), restraint/24 h food-deprivation (n=28), control/4 h food-deprivation (n=27), control/24 h food-deprivation (n=27).

In Experiment 2, 114 rats were employed as subjects. Animals in this experiment were randomly assigned to four treatment groups: restraint/4 h...
food-deprivation (n=29), restraint/24 h food deprivation (n=29), control/4 h food deprivation (n=28), control/24 h food deprivation (n=28).

On the day of testing for both experiments, all rats were food-deprived for 4 h or 24 h prior to restraint. During this time, rats were allowed free access to water. In order to ensure similarity between groups, a matched-pairs design by body weight was used to define treatment groups. At dark onset (1230 h), rats assigned to the restraint group were placed in plastic cylinders and moved to another dark room for the entire restraint period. Rats in the control group (no restraint) were left in their home cages for this period. At the end of the restraint period (20 min for Experiment 1; 2 h for Experiment 2), restrained rats were returned to their home cages and all animals received the three macronutrients for a period of 40 min. The order of the macronutrients was the same for all rats in each experiment. Each diet dish was weighed on an analytical balance before and after the 40 min exposure period. Differences in weights were then calculated for each macronutrient. Spillage was relatively negligible and similar across treatment groups. The amount of food consumed was calculated as g per 100 g body weight. A score was determined for protein, CHO and fat intake for each animal.

Experiment 3: Chronic Restraint Experiment

This experiment employed 112 rats as subjects, which were randomly assigned to four treatment groups: restraint/4 h food-deprivation (n=30),
restraint/24 h food-deprivation (n=30), control/4 h food-deprivation (n=26),
control/24 h food-deprivation (n=26). After the acclimatization period, rats in
the restraint group were restrained for 2 h/day for 4 days prior to the day of
testing. In order to ensure similarity between groups, a matched-pairs design by
body weight was used to define treatment groups. On each of these days, the
restraint period began at 1230 (dark onset) and continued for a 2 h period.
During this time, control rats were left in their home cages with free access to
laboratory chow and water. On the day of testing, all rats (control and
restrained) were food-deprived for 4 h or 24 h prior to restraint. During this time,
rats were allowed free access to water. The rats assigned to the restraint group
were placed in plastic cylinders and moved to another dark room for the entire
restraint period. Rats in the control group (no restraint) were left in their home
cages for this period. At the end of the 2 h restraint period, rats were returned
to their home cages and all subjects received the three macronutrient diets for a
period of 40 min. The order of the macronutrients was the same for all rats in
each experiment. Intake of each macronutrient was determined as outlined in
the procedure for the 20 min and 2 h restraint experiments.

In all experiments, data were analyzed using a repeated-measures
Multivariate Analysis of Variance (MANOVA) with CHO, protein and fat as the
three dependent variables. The independent variables were restraint (restrained
or control group) and length on food deprivation (4 and 24 h). A separate
Analysis of Variance (ANOVA) for each macronutrient was conducted when a significant interaction with one or both of the independent variables was revealed with the MANOVA. Results were considered to be significant when \( p < .05 \). All procedures were in strict accordance with the ethical standards of the American Psychological Association and the Canadian Council on Animal Care, and were approved by the University of British Columbia Animal Care Ethics Committee.

### 2.2 RESULTS

#### 2.2.1 Experiment 1: 20 min restraint

As revealed in Figure 1, CHO and fat intake were inhibited by 20 min of restraint, and CHO intake was differentially influenced by length of food deprivation while fat intake is not. Further, protein intake was not influenced by restraint stress or length of food deprivation.

A MANOVA revealed significant interactions between restraint and macronutrient intake \([F(2,180)=4.57, p=.012]\) and between length of deprivation and macronutrient intake \([F(2,180)=3.56, p=.030]\). There was no interaction between restraint, length of food deprivation and macronutrient intake \([F(2,180)=0.59, p=.554]\). Simple effects analyses revealed that CHO intake was inhibited by restraint \([F(1,98)=7.44, p=.008]\) and was significantly enhanced by food deprivation \([F(1,98)=5.68, p=.019]\). Observation of Figure 1
suggests that these effects could be due to 4 h food deprivation inhibiting CHO intake in the restrained group. As Figure 1 reveals, protein intake was not affected by restraint [F(1,98)=1.26, p=.264] nor by length of food deprivation [F(1,98)=1.41, p=.242], however this may be due to a low level of overall protein intake which would decrease the opportunity to reveal an inhibitory effect of restraint stress on protein intake. Fat intake was significantly inhibited by 20 min of restraint [F(1,104)=14.98, p<.001] regardless of length of food deprivation [F(1,104)=1.69, p=.201].
Figure 1. The effect of 20 min of restraint stress after 4 h and 24 h of food deprivation on macronutrient intake in the female rat. Values are represented as mean ± S.E.M. (* = significant effect of restraint on macronutrient intake, a = significant effect of length of food deprivation on macronutrient intake)
2.2.2 Experiment 2: 2 h restraint

Two h of restraint stress inhibited CHO and fat intake, and length of food deprivation influenced fat intake while having no effect on CHO or protein intake (see Figure 2). As in Experiment 1, protein intake was not influenced by restraint stress or length of food deprivation.

A MANOVA revealed a significant interaction between restraint and macronutrient intake \([F(2,188)=4.20, p=.016]\) as well as an interaction between length of food deprivation and macronutrient intake \([F(2, 188)=8.09, p<.001]\). There was no interaction between restraint, length of food deprivation and macronutrient intake \([F(2,188)=0.19, p=.83]\). Simple effects analyses revealed that CHO \([F(1,107)=19.13, p<.001]\) and fat \([F(1,105)=8.25, p=.005]\) intake were significantly inhibited by restraint stress, while protein intake was less affected \([F(1,107)=2.97, p=.09]\). Relative to 4 h of food deprivation, 24 h of food deprivation increased fat intake \([F(1,105)=9.58, p=.003]\), while having no effect on CHO \([F(1,107)=.002, p=.97]\) or protein \([F(1,107)=2.72, p=.10]\) intake (see Figure 2).
Figure 2. The effect of 2 h of restraint stress after 4 h and 24 h of food deprivation on macronutrient intake in the female rat. Values are represented as mean ± S.E.M. (* = significant effect of restraint on macronutrient intake, a = significant effect of length of food deprivation on macronutrient intake)
2.2.3 Experiment 3: Chronic restraint

As indicated in Figure 3, CHO and fat intake were inhibited by chronic restraint, and that CHO intake was differentially influenced by length of food deprivation while fat intake was not (an effect similar to Experiment 1). Protein intake was not influenced by chronic restraint or length of food deprivation (an effect also established with 20 min and 2 h of restraint in Experiments 1 and 2). Further, there is some indication that when rats are food-deprived for 24 h prior to testing, the control rats show a macronutrient intake pattern similar to chronically restrained rats, however this appears to be largely due to a similarity in CHO and protein intake, as there is still some difference in fat intake (Figure 3).

A MANOVA revealed a significant interaction between restraint and macronutrient intake \[F(2,170)=6.92, p=.001\]. There was no interaction between length of food deprivation and macronutrient intake \[F(2,170)=1.24, p=.29\] or restraint, length of food deprivation and macronutrient intake \[F(2,170)=2.33, p=.10\]. Simple effects analyses revealed that restraint stress inhibited CHO intake \[F(1,88)=4.63, p=.034\] which appears to be due to an inhibition in animals food-deprived for 4 h. Further, restraint stress inhibited fat intake \[F(1,108)=21.96, p<.001\] while having less of an effect on protein intake \[F(1,104)=3.51, p=.06\]. As Figure 3 reveals, 24 h of food deprivation inhibited
overall macronutrient intake when compared to animals food-deprived for 4 h prior to testing \[F(1,85)=11.02, \ p=.001\].

![4 h](image)

![24 h](image)

**Figure 3.** The effect of chronic restraint stress (2h/day for 5 days) after 4 h and 24 h of food deprivation on macronutrient intake in the female rat. Values are represented as mean ± S.E.M. (* = significant effect of restraint on macronutrient intake)
2.3 DISCUSSION

This study revealed that all three lengths of restraint stress employed (20 min, 2 h or chronic) decreased CHO intake and fat intake while having no effect on protein intake. Further, in the 20 min and chronic restraint paradigms, 24 h of food deprivation attenuated the difference between restrained and control animals on CHO intake while maintaining the difference in fat intake between these groups. However, 24 h of food deprivation did not prevent the inhibitory effect of 2 h restraint on CHO intake, but increased overall fat intake while still maintaining a difference between restrained and control animals.

The influence of different lengths of restraint stress and food deprivation on 5-HT may help explain these findings. 24 h of food deprivation decreases central 5-HT release (Chaouloff, et al., 1997; Hunsicker, et al., 1992; Knott & Curzon, 1974; Loullis, et al., 1979; Nishimura, et al., 1996) and decreased 5-HT activity increases CHO intake (Leibowitz, et al., 1989) and has some impact on fat intake (Blundell & Lawton, 1995; Mullen & Martin, 1992). Therefore, 24 h of food deprivation may increase motivation to consume CHO and fat through a reduction in overall brain 5-HT. It may be that 2 h of restraint increases 5-HT levels more than 20 min or following chronic stress, preventing the effect of 24 h of food deprivation to override the effect of stress. In the case of 20 min of restraint, it is possible that this length of stress is too brief to have a powerful effect on CHO intake when animals are highly motivated to eat after a long food deprivation period (e.g., 24 h) resulting in an increase in CHO intake in
restrained animals to control levels. However, in the chronic restraint experiment, CHO intake in control animals decreased to the level of restrained animals. Further, overall food intake was inhibited in all animals. It may be that the inhibition of food intake following 24 h of food deprivation reflects an increase in \( \beta \)-endorphin in the hypothalamus (Mitev, Almeida & Patchev, 1993) because an increase in opiate activity through restraint stress inhibits food intake (Zurita, et al., 1996). A reduction in food intake may also be due to the effect of 24 h of food deprivation to decrease plasma norepinephrine (NE; De Boer, et al., 1989). Recall that NE in the brain is involved in increasing food intake at the beginning of the active cycle (Leibowitz, 1992) and increases CHO intake when applied directly to the PVN (Leibowitz, Weiss, Yee & Tretter, 1985). However, the fact that an overall reduction in food intake was not seen after 24 h of food deprivation in the 20 min or 2 h restraint paradigms suggests that there may be some differences in the effects of an acute restraint session and chronic restraint on the functioning of the animals.

It is important to point out that the effect of all lengths of stress to inhibit fat intake remains after 24 h of food deprivation. In the 2 h restraint stress experiment, 24 h food deprivation increased fat intake while maintaining a difference between restrained and control animals. Therefore, it may be that the effect of restraint stress on fat intake is due to the impact of stress on a nonserotonergic neurotransmitter system, one that has been linked to fat intake. Researchers have investigated the effect of a variety of neurotransmitters such
as NE and opiates on macronutrient intake (e.g., Loullis, et al., 1979; Marks-Kaufman, 1982; Shor-Posner, Azar, Jhanwar-Uniya, Filart & Leibowitz, 1986b), however there are no data that can account for the effect of restraint stress to inhibit fat intake through these systems. In addition to the effect of 5-HT, it is likely that other neurotransmitters contribute to the inhibition of fat intake by restraint stress.

Overall, these data suggest that 20 min, 2 h and chronic restraint can inhibit CHO and fat intake. This confirms and potentially reconciles differences in previous research on the effects of restraint stress on total food intake (Kennett, et al., 1986; Haleem & Parveen, 1996; Kennett, et al., 1985a; Grignaschi, et al, 1993; Shimizu, et al., 1989; Kennedy, et al., 1993; Zylan & Brown, 1996; Badiani, et al., 1996; Espejo & Gil, 1997). This study reveals that CHO and fat intake is decreased by restraint stress and is somewhat dependent on the duration of the stress session in conjunction with the level of food deprivation. For example, while 24 h of food deprivation attenuated the effect of 20 min and chronic restraint to inhibit CHO intake, it failed to do so when animals were restrained for 2 h. Further, protein intake was not affected by restraint stress in the majority of the experiments conducted in this dissertation. It may be that different dietary levels of macronutrients account, at least in part, for previous research revealing an elevation (Badiani, et al., 1996; Espejo & Gil, 1997), no effect (Haleem & Parveen, 1996; Kennett, et al., 1985a; Kennett, et al., 1985b) or an inhibition of food intake following stress (Kennett, et al., 1986; Haleem &
Parveen, 1996; Kennett, et al., 1985a; Grignaschi, et al., 1993; Shimizu, et al., 1989; Kennedy, et al., 1993; Zylan & Brown, 1996). When different test diets are combined with different lengths of food deprivation prior to testing, it is quite possible that varying results could be revealed. Finally, very little research has investigated the effect of short stress sessions on food intake. The present study revealed that, through the employment of macronutrient diets, 20 min of restraint inhibit fat and sometimes CHO intake. Given the behavioural nature of these experiments, further conclusions regarding the differential effects of stress duration and severity of food deprivation on macronutrient intake may warrant measurement of brain chemistry.
CHAPTER III: ACUTE STRESS AND DPAT: EXPERIMENT 4

Exposure to 2 h of restraint stress decreases subsequent food intake in the rat (e.g., Grignaschi, et al., 1993; Shimizu et al., 1989; Zurita et al., 1996). Further studies investigating the neurochemical mechanisms involved have established a central role for serotonin (5-HT) in the effect of restraint stress on food intake. The feeding inhibition induced by acute restraint stress is blocked by centrally acting 5-HT antagonists, while xylamidine, a 5-HT antagonist that does not act centrally, fails to elicit this effect (Grignaschi, et al., 1993). The 5-HT1A receptor agonist, 8-hydroxy-dipropylaminotetralin (DPAT), is able to block the feeding inhibition due to restraint stress (Grignaschi, et al., 1993). This effect is most likely due to the binding of DPAT to presynaptic 5-HT1A receptors in the raphe nuclei of the brainstem which, when activated, act to reduce 5-HT release throughout most of the brain (Zifa & Fillion, 1992).

As described earlier, increased brain 5-HT decreases CHO intake while sparing protein intake (Leibowitz et al., 1989). Further, increased brain 5-HT reduces fat intake (Blundell & Lawton, 1995; Mullen & Martin, 1992).

The following experiment investigated the effect of 2 h of restraint stress on macronutrient (CHO, protein and fat) intake in the rat. Based on findings from previous studies it was hypothesized that 2 h of restraint stress would decrease CHO (Shimuzu, et al., 1989; Zurita, et al., 1996) and fat (Blundell & Lawton, 1995; Mullen & Martin, 1992) intake through an elevation of central 5-
HT activity. Further, this experiment attempted to determine whether a reduction in overall 5-HT levels though peripheral application of the 5-HT1A agonist, DPAT, would counteract the effect of restraint stress on food intake.

Grignaschi and colleagues (1993) found that 100 μg/kg and to a greater extent 300 μg/kg DPAT attenuated the effect of 1 h immobilization stress to inhibit feeding. Based on these findings, we chose to employ doses of 100 μg/kg and 300 μg/kg DPAT in this study.

Research on the effect of DPAT on food intake has shown that low doses of DPAT (less than 500 ng/kg) fail to increase food intake in non-stressed, food-deprived animals (Dourish, Hutson, Kennett & Curzon, 1986; Hutson, Dourish & Curzon, 1986). As mentioned earlier, while some restraint experiments do not employ food deprivation (Kennett, et al., 1986; Shimizu, et al., 1989; Zurita, et al., 1996; Zylan & Brown, 1996), others employ 23-24 h of food deprivation (Grignaschi, et al., 1993). The degree of food deprivation presumably would be reflected in varying neurochemical consequences. Research has revealed that food deprivation influences brain 5-HT levels (Chaouloff, et al., 1997; Hunsicker, et al., 1992; Knott & Curzon, 1974; Loullis, et al., 1979; Nishimura, et al., 1996). Specifically, 24 h of food deprivation increases 5-HT levels in the raphe nucleus of the brainstem without affecting 5-HT1A autoreceptors (Chaouloff, et al., 1997). Given that this area is also important in the effect of DPAT on food intake (Dourish, 1992; Grignaschi, et al., 1993), it may be that more severe food deprivation resulting in an increase in 5-HT would, along with DPAT, increase
binding to the 5-HT autoreceptors in the raphe nuclei which could act to attenuate the stress-induced inhibition of food intake. Recall that stimulation of the 5-HT1A presynaptic receptors within the raphe nuclei by 5-HT1A agonists and high levels of 5-HT result in an inhibited firing of the neurons that project from the these nuclei to most of the forebrain (see Cooper, 1992; Dourish, 1992; see Appendix I). This causes an overall reduction of 5-HT throughout the brain that presumably causes an increase food intake (see Cooper, 1992; Dourish, 1992).

The effect of restraint stress on food intake was examined with DPAT co-administration, following 4 h and 24 h of food deprivation.

3.1 METHODS AND MATERIALS

3.1.1 Participants

234 Female Wistar rats (Animal Care Unit, University of British Columbia, Vancouver, Canada) 5 months of age (350-450 g body weight) were used as subjects in the experiments. Housing conditions were the same as in Section 2. Except during food deprivation and testing, animals had free access to Purina laboratory chow and tap water. All animals were bilaterally ovariectomized at 3 months of age as outlined in Section 2.
3.1.2 Materials

Drugs

Compounds used in the experiments included physiological saline (VWR Canlab) and 8-OH-DPAT HBr (DPAT; RBI, Natick, Maine). DPAT was dissolved in saline to a dose of 100 μg/kg and 300 μg/kg to a volume of 1 ml solution/kg body weight. All compounds were injected IP using a 26 gauge 1/2 inch needle and a 1 cc plastic syringe (VWR Canlab).

Restraint Tubes

The same as those described in Experiments 1-3.

Experimental Diets

The same as those used in Experiments 1-3.

3.1.3 Procedure

Acclimatization Procedure

The same as that employed in Experiments 1-3.

Testing Procedure

On the day of testing, all animals were food-deprived for 4 h (n=120) or for 24 h (n=120) prior to restraint. During this time, animals were allowed free access to water. In order to ensure similarity between groups, a matched-pairs design by body weight was used to define treatment groups. Within each food
deprivation group, animals were randomly assigned to the following six
treatment groups: restraint/saline (n=20), restraint/100 µg/kg DPAT (n=20),
restraint/300 µg/kg DPAT (n=20), control/saline (n=19), control/100 µg/kg
DPAT (n=19), control/300 µg/kg DPAT (n=19). All animals were injected
between 30 to 60 min prior to restraint stress. Injections were timed such that
completion of injections corresponded with the beginning of the dark cycle (1230
h). After injections were completed, the animals assigned to the restraint group
were placed in plastic cylinders and moved to another dark room for the entire
restraint period. Animals in the control group (no restraint) were left in their
home cages for this period. At the end of the 2 h restraint period, restrained
animals were returned to their home cages and all animals received the
macronutrient diets for a period of 40 min. The order of the macronutrients was
the same for all rats. Except for the administration of DPAT, this procedure was
identical to that employed in Experiment 2. Macronutrient intake was measured
as in Experiments 1-3.

Data were analyzed using a repeated-measures MANOVA with CHO,
protein and fat as the three dependent variables. The independent variables
were restraint (restrained or control group), DPAT dose (0, 100 & 300 µg/kg)
and length on food deprivation (4 and 24 h). Simple effects ANOVAs were
conducted where appropriate as outlined in Section 2. All procedures were in
strict accordance with the ethical standards of the American Psychological
Association and the Canadian Council on Animal Care, and were approved by the University of British Columbia Animal Care Ethics Committee.

### 3.2 RESULTS

Inspection of Figure 4 indicates that DPAT did not attenuate the inhibitory effect of 2 h restraint stress on CHO intake when animals were food-deprived for 4 h prior to testing, but did attenuate this effect when animals were food-deprived for 24 h at the 300 μg/kg dose of DPAT. As can be seen in Figure 4, restraint inhibited CHO intake and at 300 μg/kg DPAT, CHO intake returned to control levels. However, there is also an inhibition of CHO intake in control animals at the 300 μg/kg DPAT dose (see Figure 4). A MANOVA revealed a significant interaction between all variables \[F(4,412)=2.44, p=.040\]. Analysis of CHO intake indicated a significant interaction between restraint, DPAT dose and length of food deprivation \[F(2,208)=3.31, p=.038\]. Further analyses revealed a significant interaction between restraint and DPAT dose at 24 h of food deprivation \[F(2,208)=4.32, p=.016\] but not at 4 h food deprivation \[F(2,208)=2.68, p=.07\]. Within the 24 h food deprivation group, there were significant differences between restrained and control animals at 0 μg/kg DPAT \[F(1,208)=6.41, p=.012\] and at 100 μg/kg DPAT \[F(1,208)=4.51, p=.035\] but not at 300 μg/kg DPAT \[F(1,208)=0.01, p=.96\].

Figure 5 suggests that 100 μg/kg DPAT increases protein intake in the control group. An ANOVA confirmed a significant interaction between restraint
and length of deprivation on protein intake \[F(1,213)=7.15, p=.008\]. Simple effects analyses revealed that there was a significant effect of restraint at 4 h of food deprivation \[F(1,213)=11.55, p=.001\] but not at 24 h \[F(1,213)=0.14, p=.707\].

Figure 6 shows that restraint inhibited fat intake and animals food-deprived for 24 h ate less overall than animals food-deprived for 4 h prior to testing. Statistical analyses confirmed a significant main effect of restraint \[F(1,220)=43.14, p<.001\] and food deprivation \[F(1,220)=6.76, p=.01\] on fat intake.
Figure 4. The effect of 2 h restraint stress and DPAT at 4 h and 24 h food deprivation on carbohydrate (CHO) intake. Values are represented means ± S.E.M. (* = significantly different from controls)
Figure 5. The effect of 2 h restraint stress and DPAT at 4 h and 24 h food deprivation on protein intake. Values are represented as means ± S.E.M.
Figure 6. The effect of 2 h restraint stress and DPAT at 4 h and 24 h food deprivation on fat intake. Values are represented as means ± S.E.M.
3.3 DISCUSSION

This study revealed that 2 h of restraint stress decreased CHO intake when animals were food-deprived for 4 or 24 h. Further, attempts to block the effect of restraint stress on CHO intake with the 5-HT1A agonist, DPAT, indicated that DPAT was able to attenuate the effect of restraint stress on CHO intake only when animals were food-deprived for 24 h. Animals that were food-deprived for 4 h, while showing inhibited food intake after restraint stress, were not influenced by the administration of DPAT. It may be that the increase in 5-HT levels in the raphe nuclei after 24 h of food deprivation (Chaouloff, et al., 1997) combined with the stimulatory effect of DPAT on 5-HT1A autoreceptors in this area, resulted in a restoration of CHO intake to control levels. It is possible that a higher dose of DPAT would have the same effect as food deprivation to increase 5-HT1A autoreceptor stimulation in the raphe nuclei. However, higher doses of DPAT also appear to stimulate post-synaptic 5-HT1A receptors throughout the brain (Dourish, et al., 1986), which provokes the motoric responses of serotonin behavioural syndrome resulting in an inhibition of food intake (Dourish, et al., 1986). It is also possible that the effect of 24 h food deprivation was simply to enhance the motivational state of the animal to feed in a non-specific manner which, when added to the effects of DPAT, overrode the inhibitory effect of restraint stress on food intake. However, the data suggest that control animals that were food-deprived for 24 h prior to testing consumed less CHO than controls in the 4 h deprivation group. It may be that the
reduction in overall food intake allowed DPAT to counteract the effect of restraint on CHO intake. In either case, these data suggest that low doses of DPAT alone are not sufficient to overcome the effect of 2 h restraint stress on CHO intake.

Animals that were food-deprived for 24 h prior to restraint inhibited their intake of CHO and fat, but not protein intake, while animals that were food-deprived for 4 h prior to restraint reduced their intake of all three macronutrients. Inspection of Figure 5 suggests that the significant effect on protein by restraint may partially be due to an increase in intake by control animals in the 100 μg/kg DPAT group as well as an inhibition of intake due to restraint. However, the effect of 100 μg/kg DPAT to increase protein intake in control animals was not significant. It may be that the inhibition of protein intake due to restraint stress at 4 h and not 24 h of food deprivation reflects differential activation of the opiate system. Central administration of the opiate antagonist naloxone decreases deprivation-induced feeding when animals are deprived of food for 24 h (Levine, Grace & Billington, 1990) and protein intake is increased by morphine administration (Bhakthavatsalam & Leibowitz, 1986; Gosnell, Krahn & Majchzak, 1990). Further, acute restraint stress has been shown to increase the display of morphine-induced behaviours (Woolfolk & Holtzman, 1995; Zurita & Molina, 1999). It is possible that 24 h but not 4 h of food-deprivation is sufficient to stimulate the opiate system. Further support for this notion is that control animals food-deprived for 24 h prior to testing consumed less CHO and fat than animals deprived for 4 h. The finding that
protein intake was not influenced by DPAT administration in either experiment suggests that 5-HT activity was not selectively involved in the effect of food deprivation and restraint stress on protein intake.

Research on the regulation of protein intake has yielded inconsistent findings (Thibault & Booth, 1999). It may be that the regulation of this macronutrient is via mechanisms involving the regulation of amino acid levels independently of changes in neurotransmitter levels. Given the importance of protein intake in the maintenance of the organism, it seems likely that the regulation of intake of this macronutrient is influenced differently than the intake of CHO and fat. If this is the case, 24 h of food deprivation could modify protein intake so that the inhibition due to stress is attenuated and animals are motivated to ingest protein at control levels (overriding the effect of restraint stress). Indeed, additional research has revealed that protein intake is differentially influenced by the deprivation state of the animal (Baker, Booth, Duggan & Gibson, 1987; Marks-Kaufman, 1982).

Two h of restraint stress inhibited fat intake regardless of the deprivation state of the animal. Further, DPAT was unable to counteract this effect. The inhibitory effect of stress on fat intake was expected because acute restraint stress acts to increase 5-HT activity in the brain (Clement, Schafer, Ruwe, Gemsa & Wesemann, 1993; Inoue, Tsuchia & Koyama, 1994; Joseph & Kennett, 1983) and increased 5-HT activity has been shown to decrease fat intake (Blundell & Lawton, 1995; Mullen & Martin, 1992). However, if the impact of stress on fat
intake was due to 5-HT mechanisms, DPAT should have been able to attenuate this effect. This suggests that the effect of restraint stress on fat intake may be due to non-serotonergic mechanisms. It is possible that the adrenergic system plays a role in this effect. Researchers have found that acute restraint stress decreases hypothalamic NE concentrations (Hellriegel & D'Mello, 1997; Konstandi, Johnson, Lang, Malamas & Marselos, 2000). Further, animals exposed to mild deprivation (2-3 h) show a decrease in \( \alpha_2 \) adrenergic receptor density in the PVN (Jhanwar-Uniyal & Leibowitz, 1986). As well, experiments investigating the effects of longer periods of deprivation (48 h) reveal more extreme effects on \( \alpha_2 \) receptors in the PVN (Jhanwar-Uniyal & Leibowitz, 1986) and 24 h of food deprivation decreases plasma NE levels (De Boer, et al., 1989). Fat intake is increased by stimulation of the adrenergic system (Leibowitz, Shor-Posner, Maclow & Grinker, 1986; Orthen-Gambill & Kanarek, 1982). Therefore, it may be that the effect of restraint stress and food deprivation to inhibit fat intake is due to a reduction in the functioning of the adrenergic system particularly within the PVN.

Overall, the results of this study have several implications for the effect of restraint stress on neurochemical systems. The deprivation state of the animal has an impact on the effect of stress on macronutrient intake and the ability of serotonergic compounds to counteract the effect. Further, it appears that while 5-HT at least partially mediates the effect of stress on CHO intake, it may play less of a role in mediating the effect of stress on fat or protein intake. Further
experiments on the neurotransmitters involved in the effect of stress on fat and protein intake may implicate NE or opiates, both of which have been revealed to play a role in food intake (Slangen, 1974; Leibowitz, 1988; Borsini, Bendotti, Thurlby & Samanin, 1982; Zurita, et al., 1996).
CHAPTER IV: CHO-RESTRICTION, MACRONUTRIENT INTAKE AND SEROTONIN

Experiments in Chapters 2 and 3 involve the development of a stress paradigm that has the specific effect of decreasing CHO intake in a manner that can be attenuated by reducing 5-HT levels in the brain. Chapter 4 employs a CHO-restriction paradigm that is hypothesized to have the opposite effect on CHO intake. The experiments conducted in this chapter will develop a model of CHO-restriction that reveals an increase in CHO intake, and test whether this effect can be attenuated by increasing 5-HT activity through pharmacological means.


It was hypothesized that by chronically restricting CHO intake in rats a reduction in brain tryptophan levels, and ultimately brain 5-HT, would result. Further, when CHO-restricted animals were presented with a self-selection
Stress, serotonin and macronutrient intake

macronutrient meal of CHO, fat and protein, they would preferentially consume CHO in an attempt to elevate reduced tryptophan and 5-HT levels. Finally, if this elevated CHO intake is due to reduced brain 5-HT levels, then administration of a compound that enhances brain 5-HT activity (such as fluoxetine, a 5-HT reuptake inhibitor) prior to exposing animals to the three-choice diet of CHO, protein and fat should attenuate CHO intake in the chronically CHO-restricted group so that intake is similar to controls. Indeed, increased activity at postsynaptic 5-HT receptors reduces the amount of food eaten during a meal (Simansky, 1996) and, administration of fluoxetine decreases total caloric intake in rats (Lawton & Blundell, 1993), with further experiments demonstrating a selective decrease in CHO intake and an increase in protein intake (Paez & Leibowitz, 1993). Moreover, in severely food-deprived rats, treatment with fluoxetine increased post-fasting anorexia, and decreased CHO, lipid and protein intake (Duhault, Lacour, Espinal & Rolland, 1993). Fluoxetine has also been clinically successful with many bulimics (Leibowitz, 1990) and has been shown to decrease the frequency of bingeing and purging in BN (Brewerton, 1995).

While several studies have investigated the effect of restricted feeding paradigms in animals on subsequent food intake, these models have focused on restricting total food intake. In light of the evidence showing a correlation between CHO intake and brain 5-HT levels, and the importance of 5-HT in satiety, investigating the effects of CHO-restriction on macronutrient intake is warranted. Experiments 5-7 define the specific conditions required to reveal an
effect of CHO-restriction on subsequent CHO over-consumption. Experiment 8 investigates the potential role of 5-HT in this effect through the application of fluoxetine prior to presenting the choice meal of CHO, protein or fat.

4.1 GENERAL METHODS AND MATERIALS

4.1.1 Experimental Diets

The CHO-restricted diet and control diet was composed of proportions of three separate macronutrient diets (CHO, protein and fat). The relative levels of macronutrients in the control diet were determined from collecting data on animals (Wistar females, 4 months of age and ovariectomized at 3 months of age) maintained on these macronutrients offered in separate dishes for 10 days. From these data we determined the relative percentage of each macronutrient consumed. This allowed us to develop a control diet containing 40% protein, 45% CHO and 15% fat providing animals with the normal amount of each macronutrient. The CHO-restricted diet varied in composition depending on the experiment. It is important to note that, because the effect of CHO intake on brain 5-HT is due to the proportion of CHO ingested relative to protein, when CHO proportions were modified in the following experiments, protein proportions were shifted to accommodate the change in CHO proportion. The proportion of fat in any of the diet regimes always remained the same. Once we had established the percentage of each macronutrient that animals would consume in a maintenance situation, we then modified the CHO-restricted diet from the
“normal” intake in an attempt to define the point at which CHO-restriction would result in a significant impact on subsequent CHO intake. During each of the experiments, animals were placed on the CHO-restricted or control diet for 14 days. Pilot data collected in our laboratory revealed that 14 days were sufficient to reveal an impact of CHO-restriction on macronutrient intake. Diets were mixed in the laboratory and placed within the animal’s cage in a large petri dish. During the 14 day period, animals had access to water but no other food was available. On day 15, animals were exposed to a 3-choice macronutrient diet (CHO, protein and fat) provided in three petri dishes within a metal tray.

All recipes for the macronutrient diets were obtained from Leibowitz and colleagues (1989) and are the same as those employed in Experiments 1-4.

4.1.2 Materials

The CHO-restricted and control diets were placed in large petri dishes, 4” (10.16 cm) in diameter. On test day, the components of the diets were placed in 2” (5.08 cm) diameter petri dishes and three dishes (CHO, protein and fat) were placed in metal trays (made to fit three petri dishes snugly). Each rat was exposed to one of these trays of individual macronutrients. All diets were weighed using an analytical balance, accurate to the nearest 0.05 g.
4.1.3 CHO-restriction Procedure

Animals were singly housed three weeks prior to the test day. Two weeks prior to the test day the standard rat chow was removed from the cages and animals were placed on the CHO-restricted or control diets for 14 days. Animals were randomly assigned to two groups (CHO-restricted and control) matched for body weight in order to control for individual differences in food intake between groups. Diet was replenished each day so that animals were not deprived of food. If an animal lost weight or appeared ill it was removed from the experiment and placed on laboratory chow immediately (this occurred in about 10% of animals). Water was available ad libitum throughout the diet exposure period.

In order to minimize the effect of injection stress on the test day, animals were subjected to intraperitoneal (IP) injections of saline vehicle three times during the diet exposure period (approximately once every four days). Dishes that the diet was placed in were washed every four days and animals were weighed throughout the diet exposure period in order to monitor the health of the rats. 4 (Experiments 5 and 6) or 24 (Experiments 7 and 8) hours prior to testing (on the fourteenth day of diet exposure), animals were food-deprived. Water was available ad libitum throughout the food deprivation and testing period.
4.1.4 Testing Procedure

Macronutrient diets were introduced to the animals’ home cages at 1300h during the middle phase of the light cycle. Colonies were maintained on a 12/12 h light-dark cycle with lights on at 0700 h. Testing was conducted during the middle part of the light cycle because food consumption is naturally low at this time and may allow an effect of CHO-restriction on macronutrient intake to be revealed. The length of the test period varied depending on the experiment.

The order of the macronutrients was the same for all rats. Each diet dish was weighed on an analytical balance before and after the exposure period. Differences in weights were then calculated for each macronutrient. Spillage that occurred beneath the cages was not collected because baseline studies revealed that it was relatively negligible and similar across treatment groups. However, if an animal upset its dish of CHO, protein or fat (spilling all or a portion of that macronutrient from the dish) the score for that macronutrient for that animal was excluded from the statistical analysis. Further, if there was any mixing of macronutrients between dishes, scores for these dishes were excluded from analysis. Approximately 5-7% of measures for individual macronutrients were lost due to dishes being upset or mixing of macronutrients between dishes. The amount of food consumed was calculated as g per 100 g body weight. A score was determined for protein, CHO and fat intake for each animal.

Data were analyzed using a MANOVA with simple effects ANOVAs and Tukey’s pairwise comparisons where appropriate. Significance was determined
at p<.05. All procedures were in strict accordance with the ethical standards of the American Psychological Association, the Canadian Council on Animal Care and were approved by the University of British Columbia Animal Care Ethics Committee.

4.2 EXPERIMENT 5

The purpose of this experiment was to determine a test duration to measure intake of the three-choice macronutrient diet. We were interested in finding a test length that was sufficient to reveal a difference in CHO intake between the control and CHO-restricted group. Animals were exposed to the three-choice macronutrient diet for 30 or 60 min.

4.2.1 Methods and Materials

Participants

55 Female Wistar rats (Animal Care Unit, University of British Columbia, Vancouver, Canada) 4 months of age (300-350 g body weight) at the beginning of CHO-restriction were used as subjects. Animals were ovariectomized at 3 months of age using a combination of 75 mg/kg ketamine and 7 mg/kg xylazine anesthesia. Animals were singly housed in standard hanging wire mesh cages during the experiment and group-housed in standard triple hanging wire mesh cages at all other times. When not employed in the experiment, animals were maintained on standard Purina rat chow. Temperature conditions in the colony
were maintained at 21 +/- 1°C. All animals were bilaterally ovariectomized as outlined in Section 2.

**Procedure**

In this experiment, the CHO-restricted diet consisted of 10% CHO, 75% protein and 15% fat. Animals were randomly assigned to two treatment groups: CHO-restricted (n=30) or control (n=25) matched for body weight. Animals were maintained for 14 days on either the control (40% protein, 45% CHO and 15% fat) or 10% CHO-restricted diet. Animals were tested at two different time intervals: 30 (CHO-restricted n=15, control n=13) and 60 min (CHO-restricted n=15, control=12). All animals were food-deprived for 4 h prior to presentation of the test diet.

Data were analyzed using a repeated-measures MANOVA with CHO, protein and fat as the three dependent variables. The independent variables were CHO-restriction (CHO-restricted and controls) and test length (30 and 60 min). Simple effects ANOVAs were conducted where appropriate as outlined in Section 2.

**4.2.2 Results and Discussion**

Inspection of Figure 7 reveals that the difference in CHO intake between the 30 and 60 min test was due to an elevated intake of CHO by control animals at 60 minutes. Fourteen days of CHO-restriction and a 30 min test period are
sufficient to reveal a difference between CHO-restricted and control animals in CHO consumption. Further, a 60 min test period seems to be required to reveal a facilitatory effect of CHO-restriction on fat intake (Figure 9). This effect may be due to a shift from the 5-HT to opiate systems regulating intake between 30-60 min (Shor-Posner, Azar, Filart, Tempel & Leibowitz, 1986a).

A MANOVA revealed a significant interaction between diet regime (CHO-restricted versus control), length of test period (30 versus 60 min test) and macronutrient intake \[F(2,80)=10.11, p<.001\]. Analyses for separate macronutrients revealed a significant interaction between diet regime and length of test period for CHO intake \[F(1,40)=4.33, p=.044\] and fat intake \[F(1,40)=14.90, p<.001\] but not for protein intake \[F(1,40)=2.78, p=.103;\] Figures 7-9]. Simple effects analyses revealed that at 30 min, the CHO-restricted group consumed significantly more CHO than the control group \[F(1,40)=10.60, p=.002\], while at 60 minutes there was no difference in CHO intake between groups \[F(1,40)=0.02, p=.88\] (see Figure 7). At 60 min, the CHO-restricted group consumed significantly more fat than the control group \[F(1,40)=23.02, p<.001;\] Figure 9].
Figure 7. The effect of chronic exposure to a 10% CHO-restricted diet for 14 days prior to testing on CHO intake for a 30 and 60 min test period. Values are represented as mean ± S.E.M. (* = significantly different from control group)

Figure 8. The effect of chronic exposure to a 10% CHO-restricted diet for 14 days prior to testing on protein intake for a 30 and 60 min test period. Values are represented as mean ± S.E.M.
Figure 9. The effect of chronic exposure to a 10% CHO-restricted diet for 14 days prior to testing on fat intake for a 30 and 60 min test period. Values are represented as mean ± S.E.M. (* = significantly different from control group)

4.3 EXPERIMENT 6

The purpose of this experiment was to determine whether 15% or 20% CHO-restriction for 14 days would influence CHO and possibly fat intake. The question we attempted to answer here is: Can a 15% and/or 20% CHO-restriction diet have an impact on subsequent macronutrient intake? These data will allow us to determine whether a 10% CHO level is required to reveal an over-consumption of CHO on test day, or if a less severe CHO-restriction regime can produce the same effect. As mentioned earlier, baseline intake experiments revealed that rats consume 45% of their calories as CHO when allowed to self-select for macronutrients. Therefore, it is possible that a 15 or 20% CHO-
restriction is severe enough to reveal an increase in CHO consumption. Such information will help define the parameters of the CHO-restriction paradigm.

4.3.1 Methods and Materials

Participants

40 Female Wistar rats (Animal Care Unit, University of British Columbia, Vancouver, Canada) 4 months of age (300-350 g body weight) were used as subjects. Other conditions were the same as those described in Experiment 5.

Procedure

Animals were maintained for 14 days on the control diet regime (40% protein, 45% CHO and 15% fat; n=10), 15% CHO regime (70% protein, 15% CHO and 15% fat; n=15) or 20% CHO regime (65% protein, 20% CHO and 15% fat; n=15). All animals were food-deprived for 4 h prior to testing. The test period was 30 min in length.

Data were analyzed using a repeated-measures MANOVA with CHO, protein and fat as the three dependent variables. The independent variable was CHO-restriction (15% CHO-restricted, 20% CHO-restricted and controls). Simple effects ANOVAs and Tukey’s tests were conducted where appropriate.
4.3.2 Results and Discussion

Overall, neither 15% nor 20% CHO-restriction diets revealed a strong effect on macronutrient intake when compared to control animals (Figure 10). A MANOVA test revealed a main effect of diet regime \( [F(2,33)=5.89, p=.007] \), however there was no interaction between diet regime and macronutrient intake \( [F(4,66)=2.14, p=.09] \). A Tukey’s test revealed that there was a significant difference in overall macronutrient intake between animals maintained on the control diet when compared to the 15% CHO-restriction regime \( (p=.006) \). Inspection of Figure 10 reveals that this appears to be due to an increase in fat intake in the 15% CHO-restricted animals, however this effect was not sufficient to produce a significant interaction between diet regime and macronutrient intake. It appears that a 10% CHO-restriction diet is required to produce an increase in subsequent macronutrient intake. Therefore, the following experiments employed an exposure diet of 10% CHO.
Figure 10. The effect of chronic exposure to a 15% or 20% CHO-restricted diet for 14 days prior to testing on macronutrient intake for a 30 min test period compared to a control diet of 45% CHO. Values are represented as mean ± S.E.M.

4.4 EXPERIMENT 7

In pilot studies involving peripheral drug injections conducted in our laboratory, preliminary data indicated that injections per se acted to inhibit CHO over-consumption in CHO-restricted animals. In an attempt to overcome the effect of injection stress on food intake we food-deprived test animals for 24 h prior to exposure to injections and the choice macronutrient diet.
4.4.1 Methods and Materials

Participants

70 Female Wistar rats (Animal Care Unit, University of British Columbia, Vancouver, Canada) 5 months of age (300-350 g body weight) were used as subjects. Other conditions were the same as those described in Experiment 5.

Procedure

Animals were placed on control (40% protein, 45% CHO and 15% fat; n=35) or 10% CHO-restricted (10% CHO, 75% protein and 15% fat; n=35) diet for 14 days. During that time, exposure injections were administered on two different days in order to habituate the animals to the injections. Exposure injections involved administering physiological saline (VWR Canlab) IP using a 26 gauge 1/2 inch needle and a 1 cc plastic syringe (VWR Canlab) at a volume of 1 ml/kg body weight.

Food was removed from the animals’ cages 24 h prior to testing. On day 15, animals were injected with saline 1 h prior to presentation with the 3-choice macronutrient diet. Intake was measured at 30 min.

Data were analyzed using a repeated-measures MANOVA with CHO, protein and fat as the three dependent variables. The independent variable was CHO-restriction (CHO-restricted and controls). Simple effects ANOVAs were conducted where appropriate.
4.4.2 Results and Discussion

Figure 11 shows that when animals are food-deprived for 24 h prior to testing, there is no effect of injection stress to inhibit food intake and CHO-restriction increases CHO and protein intake while having no effect on fat intake. A MANOVA analysis confirmed a significant interaction between diet regime (CHO-restriction versus control) and macronutrient intake \[F(2,108)=3.62, p=.03\]. Analyses conducted on the separate macronutrients revealed a significant effect of diet regime on CHO intake \[F(1,58)=31.07, p<.001\] and protein intake \[F(1,54)=7.59, p=.008\] but not on fat intake \[F(1,58)=2.51, p=.12\] as shown in Figure 11. This is the first time an effect on protein intake has been revealed in these experiments. It is possible that food deprivation for 24 h prior to testing increases appetite for protein. If this is the case, subsequent experiments employing the same paradigm should reveal an effect on protein.
Figure 11. The effect of chronic exposure to a 10% CHO-restricted and control diet for 14 days prior to testing on macronutrient intake for a 30 min test period after a 24 h food deprivation. Values are represented as mean ± S.E.M. (* = significantly different from controls)

4.5 Experiment 8

The results of Experiments 5-7 suggest that exposing female rats to a 10% CHO diet for a two-week period will result in an increase in CHO intake. The purpose of this experiment was to investigate the potential role of 5-HT in the effect of CHO-restriction on subsequent macronutrient intake.
4.5.1 Methods and Materials

Participants

55 Female Wistar rats (Animal Care Unit, University of British Columbia, Vancouver, Canada) 4 months of age (300-350 g body weight) were used as subjects. All other conditions were the same as those described in Experiment 5.

Drugs

Animals were ovariectomized using a combination of 75 mg/kg ketamine and 7 mg/kg xylazine anesthesia. Compounds used in the experiments included physiological saline (VWR Canlab) and crystalline fluoxetine (Sigma, Chicago). Fluoxetine was dissolved in saline to a dose of 3 mg/kg and 6 mg/kg to a volume of 1 ml solution/kg body weight. All compounds were injected IP using a 26 gauge 1/2 inch needle and a 1 cc plastic syringe (VWR Canlab).

Procedure

Food was removed from the animals’ cages 24 h prior to testing. On the day of testing, animals were randomly assigned (matched for body weight) to four treatment groups: control (n=13), CHO-restricted/0 mg/kg fluoxetine (n=14), CHO-restricted/3 mg/kg fluoxetine (n=14), and CHO-restricted/6 mg/kg fluoxetine (n=14). Animals were injected between 1230h-1300h and returned to their home cages.
Data were analyzed using simple effects ANOVAs for each macronutrient. The independent variables were: the control group, CHO-restriction/0 mg/kg fluoxetine, CHO-restriction/3 mg/kg fluoxetine and CHO-restriction/6 mg/kg fluoxetine. Tukey’s tests were conducted where appropriate.

4.5.2 Results and Discussion

As Figure 12 shows, CHO-restricted animals consumed significantly more CHO than animals maintained on the control diet, and fluoxetine attenuated this effect \(F(3,47)=4.53, p=.007\). Specifically, Tukey’s tests revealed a significant difference between the 6 mg/kg fluoxetine group and the CHO-restricted group receiving 0 mg/kg fluoxetine. Further, the 6 mg/kg fluoxetine group was not different in its CHO consumption when compared to the control group. Therefore, treatment with fluoxetine inhibited the effect of CHO-restriction as animals in both the 3 and 6 mg/kg fluoxetine groups ate significantly less than those in the 0 mg/kg group. As Figures 13 and 14 reveal, CHO-restriction and fluoxetine treatment had no effect on protein \(F(3,41)=1.59, p=.21\) or fat \(F(3,43)=0.90, p=.45\) intake. However, it should be noted that the pattern of protein and fat intake between the treatment groups was similar to that seen with CHO intake as CHO-restriction enhanced intake and fluoxetine treatment attenuated this effect.
Figure 12. The effect of 14 days of CHO-restriction with and without fluoxetine on subsequent CHO intake. Values are represented as mean ± S.E.M. (groups with same letter are significantly different from each other)

Figure 13. The effect of 14 days of CHO-restriction with and without fluoxetine on subsequent protein intake. Values are represented as mean ± S.E.M.
The results of this study confirm that chronic CHO-restriction increases CHO intake and fluoxetine attenuates this effect. Specifically, at a dose of 6 mg/kg fluoxetine counteracted the effect of CHO-restriction on CHO intake. Overall, fat and protein intake were not consistently influenced by CHO-restriction. An effect of CHO-restriction to increase protein intake was seen in Experiment 7 but not replicated in Experiment 8. And fat intake was only increased in Experiment 5 after a 60 min, but not 30 min, test period. Finally, Experiment 8 revealed a similar pattern of CHO-restriction and fluoxetine administration on fat and protein intake (see Figures 12-14), however this was not significant.
These data suggest that chronic CHO-restriction specifically elevates CHO appetite while having little effect on appetite for fat or protein. Therefore, it appears that organisms have the capacity to regulate intake of specific macronutrients such that, when deprivation occurs, motivation to consume that macronutrient is increased. This is supported by the findings that chronic protein restriction results in a preference to consume protein when a choice diet is offered (Dibattista & Holder, 1998; Piquard, et al., 1978). It seems that dietary self-selection is closely adapted to the nutritional and other physiological requirements of the organism (Dibattista & Holder, 1998; Piquard, et al., 1978).

These experiments also suggest that the effect of chronic CHO-restriction on subsequent CHO intake is mediated by 5-HT and that caloric restriction does not seem to be necessary to reveal this effect. Chronic CHO-restriction results in a specific increase in CHO appetite, and CHO appetite is closely linked to brain 5-HT levels (Rouch, Nicolaidis & Orosco, 1999; Leibowitz, et al., 1989; Islam, et al., 1994). Chronic CHO-restriction may lead to a reduction in brain 5-HT levels, which results in an increase in CHO intake when a choice diet is offered. Moreover, the 5-HT reuptake inhibitor, fluoxetine, was able to counteract the effect of chronic CHO-restriction on CHO appetite while having no effect on fat or protein intake. Indeed, studies investigating the effect of fluoxetine on CHO intake have revealed that fluoxetine inhibits CHO intake (Lawton & Blundell, 1993). Fluoxetine may influence CHO intake by increasing 5-HT release in the PVN (Paez & Leibowitz, 1993). Research has revealed that 5-HT administered
into the PVN decreases CHO intake while having no effect on protein or fat intake (Leibowitz, et al., 1989). The effect of 5-HT within the PVN to induce satiety may be due to activation of 5-HT1B and 5-HT2C postsynaptic receptors within this nucleus (Simansky, 1996). Other researchers have suggested that 5-HT activity within the PVN specifically influences CHO appetite, while extra-PVN sites may modulate more general aspects of satiety (Simansky, 1996). Finally, given that the animals in these experiments were not restricted in their overall caloric intake, the effect of CHO-restriction to increase CHO appetite through 5-HT appears to be independent of the amount of calories consumed. However, it should be pointed out that, while the caloric content of the CHO-restricted and control diets was the same, 24 h intakes were not recorded, so it is possible that CHO-restricted animals consumed less diet (and therefore, less calories) than control animals. Further, while both treatment groups gained weight during the 14 day exposure period, control animals gained an average of 30 grams, while CHO-restricted animals gained an average of 17 grams.

As indicated in Experiments 7 and 8, in order to reveal an effect of fluoxetine on CHO over-consumption animals had to be food-deprived for 24 h prior to testing. As noted in Section 2, the duration of food deprivation has significant effects on 5-HT levels (Chaouloff, et al., 1997; Hunsicker, et al., 1992; Knott & Curzon, 1974; Louillis, et al., 1979; Nishimura, et al., 1996). 24 h of food deprivation increases 5-HT levels in the raphe nucleus of the brainstem without affecting 5-HT1A autoreceptors (Chaouloff, et al., 1997). These findings suggest
that 24 h of food deprivation should increase CHO appetite through a reduction of overall brain 5-HT through its effects on 5-HT within the raphe nucleus. Further, 24 h of food deprivation appeared to increase CHO and protein but not fat intake when compared to 4 h food deprivation (Experiments 5 and 7). However, this increase in CHO and protein intake was more severe in CHO-deprived animals suggesting that 24 h food deprivation may enhance the effect of CHO-restriction on selective macronutrient preference.
CHAPTER V: GENERAL CONCLUSIONS

While there are several forms of stress that influence food intake in humans and other species, this dissertation focused on two forms of stress that affect food intake through serotonergic mechanisms: physical restraint stress and dietary stress. The purpose of this dissertation was to further define the conditions under which these forms of stress may have opposing effects on CHO intake in the female rat and to determine whether 5-HT is involved in that effect.

Experiments 1-3 addressed the issue of whether different lengths of restraint stress (20 min, 2 h or 2 h/day for 5 day) differentially affect macronutrient intake. Results from these experiments revealed that at 4 h of food deprivation, 20 min, 2 h and chronic (2 h/day for 5 days) restraint stress significantly inhibited CHO and fat intake. However, after 24 h of food deprivation, the impact of 20 min and chronic restraint on CHO intake was attenuated, while the inhibition of fat intake remained.

Experiment 4 addressed the issue of whether 5-HT is involved in the effect of a 2 h restraint session on CHO intake. As revealed in Experiments 1-3, after 4 and 24 h of food deprivation, 2 h of restraint decreased CHO and fat intake. Further, DPAT was able to counteract the effect of 2 h of restraint on CHO intake after 24 but not 4 h of food deprivation, and did not significantly influence the inhibition of fat intake.
In Experiments 1-3 three lengths of restraint stress were employed to
determine their effect on macronutrient intake at two different lengths of food
deprivation, 4 h and 24 h. Results of these studies showed that while 2 h of
restraint stress inhibited CHO and fat intake at 4 and 24 h of food deprivation, 20
min and chronic stress inhibited CHO intake only at 4 h of food deprivation, and
fat intake after both 4 and 24 h of food deprivation. In Experiment 4, 2 h of
restraint stress was employed at 4 and 24 h of food deprivation along with DPAT
(a 5-HT agonist) in order to determine the impact of increasing 5-HT activity on
restraint-induced inhibition of CHO and fat intake. This experiment revealed that
while DPAT was able to counteract the effect of 2 h of restraint on CHO intake
after 24 h of food deprivation, it was unable to do so after 4 h of food
deprivation. Finally, DPAT administration did not influence the stress-induced
inhibition of fat intake. Based on these findings, testing the effect of DPAT on
the effect of 20 min and chronic restraint was not warranted, as DPAT only
attenuated the effect of restraint on CHO intake after 24 h of food deprivation
and neither 20 min nor chronic stress revealed a significant inhibition of CHO
intake after 24 h of food deprivation.

Experiments 5-8 addressed the question: Can dietary stress that limits
the intake of CHO without limiting overall caloric intake influence subsequent
macronutrient intake and if so, does 5-HT play a role in this effect? Results
indicated that a 14 day 10% CHO-restriction regime increases CHO intake during
a 30 min test after 4 and 24 h of food deprivation, and administration of
fluoxetine was able to counteract this effect. Further, fat intake was only elevated when animals were tested for 60 min, and protein intake was increased in one of the experiments but this effect was not replicated.

Comparisons of the different stress paradigms employed in this dissertation indicate certain patterns. When dietary stress was employed, CHO intake was elevated after 4 and 24 h and an increase in brain 5-HT activity (by fluoxetine administration) attenuated this effect when animals were food-deprived for 24 h. All lengths of restraint stress (20 min, 2 h and chronic) inhibited CHO intake when animals were food-deprived for 4 h, while 24 h of food deprivation resulted in a decrease in CHO intake only after 2 h of restraint. Finally, a reduction in 5-HT activity (through the administration of a low dose of DPAT) attenuated this effect. Combined, these data suggest that chronic CHO-restriction may act to increase CHO intake through a reduction in 5-HT levels and 2 h of restraint stress may act to inhibit CHO intake through an increase in 5-HT levels. Indeed, dieting and restrained eating decrease tryptophan activity (Wolfe, et al., 1997) and acute stress increases brain 5-HT levels (Haleem & Parveen, 1996; Kennett, et al., 1985b).

The influence of stress on fat intake revealed some interesting results as well. Overall, the effect of dietary and restraint stress on fat intake does not seem to be due to serotonergic mechanisms. In the chronic CHO-restriction experiments, fat intake was increased after 60 but not 30 min of feeding. As well, at 60 min, CHO intake returned to normal. Therefore, after 60 min of
testing there appears to be a preferential shift in consumption from CHO to fat. The impact of dietary stress on fat intake may be due to opiate mechanisms. Research has shown that a low dose (2 mg/kg) of morphine (an opiate agonist) increases food intake in satiated rats and reveals a preferential increase in fat intake and a reduction of CHO intake in food-restricted rats (Shor-Posner, et al., 1986a). It is likely that neurotransmitters such as opiates, NE and 5-HT work together to influence naturally occurring macronutrient intake patterns during the early part of the dark cycle, when food-seeking behaviour and ingestion are most pronounced in the rat. At dark onset there is a sharp increase in α2 noradrenergic receptors in the PVN (Leibowitz, 1988) as well as an increase in opioid levels (Kerdelhue, Karteszi, Pasqualini, Reinberg, Mezey & Palkovits, 1983; Wesche & Frederickson, 1979). At this time 5-HT levels are at their lowest in the circadian cycle (Wesemann & Weiner, 1990). At dark onset, rats preferentially consume CHO, with a shift towards protein-predominant meals later in the dark cycle (Tempel, et al., 1989). Further, even a short food deprivation period of 2 h causes a compensatory feeding response, particularly fat and CHO intake (Tempel, et al., 1989). Norepinephrine stimulation (Leibowitz, Brown, Tretter & Kirchgessner, 1985) and low levels of 5-HT (Leibowitz, et al., 1989) increase CHO intake while decreasing protein intake, and increased opiate activity increases protein intake in satiated rats and increases fat intake in food-restricted rats (Shor-Posner, et al., 1986a). It may be that this shift from CHO to protein (and fat if food deprivation proceeds food intake) is due to a change in
hypothalamic neurochemistry, with noradrenergic activation followed by 5-HT and opiate activation. Therefore, dietary stress may increase opiate activity resulting in an elevation of fat intake at 60 min. The fact that at 30 min CHO intake was elevated could be due to reduced 5-HT (and likely elevated NE) levels affecting macronutrient intake immediately and an opiate mechanism acting more slowly to influence food intake at a later time point.

In restraint stress experiments, fat intake was inhibited at all stress durations and regardless of length of food deprivation prior to testing. Further, DPAT administration was not able to counteract this effect after 2 h of restraint stress. Research has revealed that 2 h, but not 1 h, of restraint is sufficient to induce stress-induced anhedonia through an activation of the opiate system that has been measured through the reduced intake of a palatable 1% sucrose solution (Zurita, et al., 1996). As mentioned earlier, pharmacological activation of the opiate system increases fat intake in food-deprived rats (Shor-Posner, et al., 1986a). It may be that an increase in endogenous opiate activity through restraint stress influences behaviour differently than exogenous application of opiate agonists (Zurita, et al., 1996). In addition, restraint stress experiments employ palatable sucrose solutions to investigate the role of opiate mechanisms. Finally, fat intake is influenced by central NE activity (Leibowitz, et al., 1986; Orthen-Gambill & Kanarek, 1982). Given the impact of opiates and NE on fat intake, it is possible that restraint stress affects fat intake through one or both of these systems. Given the pervasiveness of the inhibitory effect of restraint stress
Stress, serotonin and macronutrient intake 88

on fat intake (all durations and regardless of level of food deprivation) in these experiments and the fact that DPAT was unable to counteract this effect in the 2 h restraint paradigm, it is possible that the effect of restraint stress on fat intake is due to activation of the opiate system or inhibition of the adrenergic system. Future research in this area could investigate the role that opiates and NE play in the effect of dietary and restraint stress on fat intake.

In contrast to CHO and fat intake, protein intake was not consistently affected by any of the stress paradigms employed in this dissertation. Indeed, only one of the experiments revealed an effect on protein intake. In Experiment 7, chronic CHO-restriction and 24 h of food deprivation resulted in an increase in protein intake. However, Experiment 8 did not reveal an effect on protein intake. It should be noted that there was often some indication of protein being elevated by CHO-restriction and inhibited by restraint stress. Therefore, it seems that protein intake is affected by restraint stress and CHO-restriction. It may be that time of testing attenuated the effect of these stress regimes on protein intake. The CHO-restriction experiments conducted in Experiments 5-8 involved testing during the light part of the light:dark cycle which is a period of minimal activity and food intake for the rat. The restraint stress experiments outlined in Sections 2 and 3 tested animals at dark onset which is a period known for its preferential consumption of CHO and fat and minimal protein appetite (Leibowitz, et al., 1989). It is possible that the same stressors would have affected protein
intake if testing took place later in the dark cycle when the intake of protein is preferred (Leibowitz, et al., 1989).

Overall, the effect of 24 h of food deprivation on macronutrient intake depended on the paradigm employed. In Experiments 5-8, 24 h of food deprivation did not attenuate the effect of CHO-restriction to increase CHO intake. In Experiment 1, 24 h food deprivation decreased the effect of 20 min of restraint stress on CHO intake while having no effect on fat intake. As well, 24 h of food deprivation inhibited overall food intake in the chronic restraint (2 h/day for 5 days) experiment described in Experiment 3. Finally, in Experiments 2 and 4, 24 h of food deprivation did not attenuate the effect of 2 h of restraint stress to inhibit CHO and fat intake, although it appears to have enhanced the effect of DPAT to increase CHO intake in restrained animals. Studies investigating the impact of food deprivation on behaviour and brain neurochemistry show that 24 h of food deprivation increases the motivation to eat possibly through a reduction in brain 5-HT levels (Chaouloff, et al., 1997; Hunsicker, et al., 1992; Knott & Curzon, 1974; Loullis, et al., 1979; Nishimura, et al., 1996) which should increase CHO and possibly fat intake. Results from this dissertation suggest that when a stressor acts to decrease 5-HT levels (CHO-restriction) or is relatively mild (20 min restraint), 24 h of food deprivation acts in a motivational capacity to increase food intake. Finally, as revealed in Experiment 4, 24 h of food deprivation may enhance the effects of a compound that inhibits brain 5-HT allowing animals to feed at control levels following a 2 h restraint session,
however it's impact on 5-HT levels alone were not sufficient to override the
effect of restraint stress on CHO intake.

Although corticosterone levels are modified by stress, it is unclear whether
this hormone was involved in the results obtained in this study. While
corticosterone levels are elevated by restraint stress (Ottenweller, et al., 1994),
it's effect is to increase food intake, particularly CHO intake (Leibowitz, 1992).
CHO intake was never increased in these experiments and was inhibited in the
majority of experiments. However, if the corticosterone release induced by
restraint stress increased brain 5-HT levels (Saphier, et al., 1995; Tejani-Butt &
Labow, 1994), a decrease in CHO intake would be expected. Finally, while diet
restriction can increase corticosterone levels (Garcia-Belenguer, et al., 1993;
Gursoy, et al., 2001; Heiderstadt, et al., 2000) this is only found in regimes that
are much more severe in nature than the 14 day CHO-restriction employed here.
For example, researchers have revealed that lengthy periods of caloric restriction
(e.g., 8 weeks, Gursoy, et al., 2001; 4 weeks, Garcia-Belenguer, et al., 1993; 37
days, Heiderstadt, et al., 2000) or relatively extreme restrictions in food intake
(50% restriction of ad libitum food intake, Garcia-Belenguer, et al., 1993) is
required to reveal in increase in plasma corticosterone levels. Finally, extended
periods of food deprivation (72 to 96 h) are required to reveal an increase in
serum corticosterone levels (Mitev, et al., 1993).
5.1 IMPLICATIONS

The relationship between mood disturbances and eating in humans has been the focus of much research over the past several decades. It appears that mood state strongly influences food intake. Normally functioning individuals who eat a diet high in CHO are reported to be less depressed, while people who eat a diet high in protein are more likely to be depressed (De Castro, 1987). Further, there is a high comorbidity between eating disorders and mood disturbances, especially anxiety and depression (e.g., Brewerton, Lydiard, Herzog, Brotman, O'Neil & Ballenger, 1995; see Brewerton, 1995). It appears that while anxiety may act to increase eating (Powell & Thelen, 1996), endogenous depression is associated with decreased food intake (e.g., Gold, et al., 1988; Kazes, et al., 1994). Seasonal Affective Disorder and Premenstrual Syndrome, mood disorders that share symptoms of depression, lethargy and an inability to concentrate, also share the symptom of a need to consume large amounts of carbohydrates (Wurtman, 1990). In addition, women with BN are more anxious before a binge than after compensatory behaviour, with no overall change in level of depression (Powell & Thelen, 1996). Further, while acute stress has been shown to increase food intake in normally functioning women (Weinstein, et al., 1997) further studies have found this effect to be positively correlated with physiological responsiveness to stress (Rutledge & Linden, 1998).

While a variety of neurochemical systems have been investigated in both AN and BN, 5-HT has received a considerable amount of attention in recent
years. In studies investigating the role of 5-HT in eating disorders, women who have long term recovery from AN or BN have been shown to have increased 5-HIAA levels when compared to controls (Kaye, Gwirtsman, George & Ebert, 1991; Kaye, Greeno, Moss, Fernstrom, Fernstrom, Lilenfeld, et al., 1998). 5-HIAA is a 5-HT metabolite whose increase may reflect an overactive 5-HT system that could contribute to behavioural constraint, obsessionality and inhibition of appetite (Soubrie, 1986; Spoont, 1992). Further, increased 5-HT activity could make people specifically vulnerable to developing an eating disorder as well as certain core symptoms of these disorders, including anxious dysphoria, obsessional thinking, perseveration, and cognitive distortions (Kaye, Strober, Stein & Gendall, 1999). These processes could be further enhanced by malnutrition-triggered changes in hypothalamic neuropeptides that modulate aversively conditioned learning (Demitrack, Lesem, Listwak, Brandt, Jimerson & Gold, 1990). Extreme dieting, by its effects on plasma tryptophan, the precursor of 5-HT, could be a means of reducing brain 5-HT functional activity (Kaye, Gwirtsman, George, Obanzek, Brewerton, Jimerson, et al., 1988) and thus briefly reversing dysphoric effects (Steinberg, Tobin & Johnson, 1990).

Previous research has shown that dieting often triggers the beginning of BN (Kaye, Klump, Frank & Strober, 2000). Dieting may then cause a reduction in 5-HT that, in bulimics, may contribute to binge eating and dysphoric mood (Jimerson, Lesem, Kaye & Brewerton, 1992; Kaye, et al., 1988). Further, ongoing stress may play a role in the development of AN and BN. Indeed, Kaye
and colleagues (2000) suggest that an inability to cope effectively with stress may result in dysfunctional eating behaviours. The 5-HT system in individuals with BN may be inherently unstable and poorly modulated resulting in erratic fluctuations rather than more precise compensation that buffers the effects of dieting or stress as is found in women without bulimia (Kaye, et al., 2000). Kaye and colleagues (2000) have hypothesized that women with BN may have an inherent modulatory defect in 5-HT function that does not allow them to respond appropriately to the stress of dieting. In women with BN, brain tryptophan levels may be reduced during the restrictive phase of their disorder. This could interact with an already compromised 5-HT system resulting in an increased motivation to binge. Therefore, women with BN may binge as a means of self-modulating 5-HT (Jimmerson, et al., 1992) as binge episodes may increase the level of brain tryptophan resulting in increased 5-HT synthesis and release (Fernstrom & Faller, 1978; Fernstrom & Wurtman, 1971).

Bulimia has been characterized as a biphasic disorder involving a cycling between a restrictive or dieting phase and a binge phase while average body weight is maintained (American Psychiatric Association, 1994). Two AN patient subgroups have been identified by consummatory behaviour: a restricting type, in whom a relatively enduring pattern of dietary restriction is characteristic, and a binge eating type, in whom episodes of binge eating or purging coincide with dietary restriction and subnormal body weight (American Psychiatric Association, 1994). The restraint stress paradigms employed in Experiments 1-4 may provide
a model for the restriction of food intake in AN and BN, while the CHO-restriction and subsequent over-consumption noted in Experiments 5-8 may provide a model for the restriction and binge phases of AN (binge type) and BN. Results from Experiments 5-8 may provide additional support for Kaye’s theory of the role of 5-HT in the binge phase of BN and AN (Kaye, et al., 2000; Jimerson, et al., 1992). If, during the restrictive phase of the disorder, bulimics and anorexics reduce their intake of CHO-rich foods, they may decrease their brain 5-HT. A reduction in brain 5-HT levels increases CHO-appetite that could result in a “binge” on CHO-rich foods. Further, when women with BN are prescribed fluoxetine, this may act to elevate their brain 5-HT levels such that the impact of the restrictive phase of the disorder does not have the same effect on CHO-craving and no binge is observed. Indeed, research investigating the effect of fenfluramine (a 5-HT agonist) on CHO craving in obese women has revealed that fenfluramine decreases CHO intake (Wurtman, Wurtman, Mark, Tsay, Gilbert & Growdon, 1985). Further, acute tryptophan depletion has been shown to increase food intake in women with BN when compared to a control group of women (Weltzin, Fernstrom, Fernstrom, Neuberger & Kaye, 1995). Acute tryptophan depletion has also been shown to increase negative mood states (e.g., depression and anxiety), increase concern with body image and result in a subjective loss of control of eating in women who have recovered from BN (Smith, Fairburn & Cowen, 1999) as well as in women who are actively bulimic (Kaye, et al., 2000). Therefore, it may be that chronic depletion of tryptophan
as a result of dieting (Wolfe, et al., 1997) precipitates the development of BN in women who are predisposed to develop this disorder. If this is the case, then the model described here may be of value for research attempting to further define various aspects of BN. This model may be of value in further defining the effects of various pharmacological treatments as well as behavioural modifications of diet intake and stress factors on bingeing behaviours in women with bulimia and other disorders that involve binge eating.

The development of AN (Donohue, 1984) and BN (Powell & Thelen, 1996) have been reported to be related to stress. Potential causes of AN are childhood trauma (often sexual abuse), a predisposition to develop an eating disorder (genetics, labile 5-HT system), stress and societal pressures (see Kaye, et al., 2000). Potential causes of BN are dieting and childhood trauma (see Kaye, et al., 2000). Moreover, BN is more likely to occur in women who have a family history of affective disorders and/or alcoholism (see Kaye, et al., 2000).

McKinney (1974) proposed four criteria for animal models in psychiatry: (1) similarity in inducing conditions, (2) similarity of behavioural states produced, (3) common underlying neurobiological mechanisms, and (4) reversal by clinically effective treatment techniques. Because the etiology of bulimia and anorexia are multidimensional and very complex (see Polivy & Herman, 2002) it is impossible to create a model that includes all these factors and is useful for experimental research (Polivy & Herman, 2002). Therefore, animal models of AN or BN often resort to modeling bulimic or anorexic behaviour, either by naturally
occurring examples or experimental use of lesioning, drug treatment, or environmental manipulation. The value of the restraint paradigms may lie in the inhibition of specific macronutrients in response to stress. While the value of a chronic CHO-restriction paradigm may lie in its similarity to BN and AN (binge type) in terms of the relative proportions of macronutrients consumed during the restrictive and binge-phases of the disorder and the involvement of 5-HT in the subsequent CHO over-consumption. However, given that animals with functional 5-HT systems were employed and the CHO over-consumption was acute, it is unlikely to be representative of the underlying neurochemical dysfunction that predisposes one to and drives the chronicity of BN.

The paradigms described in this dissertation may help to tease apart the multiple biological and behavioural aspects of the development and maintenance of eating disorders. One of the complications of determining the biological underpinnings of AN and BN is the fact that biological functioning is influenced by the nutritional state of the individual. Indeed, malnutrition and aberrant eating behaviours have been shown to influence neurotransmitter levels and the functioning of these systems. If we attempt to separate the possible causes of eating disorders into several categories, the literature shows that there may be a heritable trait that one is born with, a traumatic incident in the individual’s past and/or chronic stress in addition to dieting behaviour. It may be of value to investigate each potential antecedent of eating disorders separately. By using animals whose 5-HT systems are functional initially we may be able to determine
the relative impact of environmental stressors on the development of dysfunctional eating behaviour and whether environmental stress alone is sufficient to cause and maintain disordered eating. Investigations of this nature may allow us to determine the relative impact of mild stress on the development of eating disorders and may provide directions for further understanding of the etiology of these disorders.

5.2 DIRECTIONS FOR FUTURE RESEARCH

While the findings reported in this study provide some indication of the neurochemical mechanism through which restraint stress and CHO-restriction influence macronutrient intake, there are several other experiments that would be worth pursuing.

As mentioned earlier, this is the first study to investigate the effect of restraint stress on macronutrient intake in the female rat. It is possible that some of the unexpected effects of 24 h food deprivation and the differences between chronic and 2 h of restraint stress (Experiments 2-4) could be specific to the female rat. However, before conclusions can be made regarding the differential response to restraint stress in the male and female rat, similar experiments need to be performed in the male rat. Given that restraint increases corticosterone release more in the female than the male (Haleem & Parveen, 1996; Mendelson & McEwen, 1991) and that stress appears to influence 5-HT activity through an increase in corticosterone release (Mendelson & McEwen,
1991; Saphier, et al., 1995; Tejani-Butt & Labow, 1994) it is possible that performing a similar experiment in male rats would yield divergent results.

As well, research has revealed that the 5-HT systems in female rats and women are significantly different than those of males (Kennett, et al., 1986; Walsh, et al., 1995). Evidence suggests that the 5-HT systems of female rats are more labile than those of males (Kennett, et al., 1986). Therefore, exposure to a diet modification paradigm that influences 5-HT (as in Experiments 5-8) may affect males and females differently. It would be of interest to carry out similar experiments in male rats to further define any differences between genders in the response to stress.

In addition, the serotonergic drugs employed in this study had an effect on overall brain 5-HT activity. Several 5-HT receptor subtypes may be important in the effect of restraint and dietary stress on CHO intake. Serotonin may influence food intake by acting initially within the raphe nucleus of the brainstem to stimulate 5-HT1A presynaptic receptors which, when stimulated, inhibit the release of 5-HT in terminal regions throughout the forebrain (Cooper, 1992). It appears that selective suppression of CHO intake may be mediated by 5-HT2A/2C receptors either alone or together or by 5-HT1B receptors in conjunction with 2A receptors (Lawton & Blundell, 1993). Research has revealed that 5-HT2A receptors influence feeding by disrupting the continuity of a meal, while 5-HT2C receptors influence the rate of food intake and 5-HT1B receptors regulate meal size (Simansky, 1996). It would be of considerable value to
determine whether agonists or antagonists known to selectively bind to specific post-synaptic receptors in the brain could reveal similar effects. Given that co-activation of 5-HT1B and 5-HT2C receptors appear to stimulate normal satiety (Simansky, 1996) such studies might investigate the effect of agonists selective to these receptor subtypes to inhibit the CHO over-consumption induced by CHO-restriction and antagonists to attenuate the effect of 2 h restraint stress on CHO intake. Moreover, it would also be of great interest to locate the area of the brain in which these forms of stress act to influence macronutrient intake. As outlined previously, research suggests that the PVN, SCN and VMH of the hypothalamus are likely sites (see Leibowitz, 1992).

Further, it is unclear whether corticosterone is involved in the effect of restraint stress to inhibit CHO intake. While corticosterone has been shown to increase CHO intake (see Leibowitz, 1992), it has also been shown to increase 5-HT activity in the brain (Saphier, et al., 1995; Tejani-Butt & Labow, 1994). Studies employing corticosterone antagonists administered prior to exposure to the stressor would help to determine if corticosterone is involved in the effect of restraint stress to inhibit CHO intake. If corticosterone is active in the effect of restraint stress on CHO intake then blocking its activity would attenuate the effect of restraint stress on feeding.

Recall that fat intake was inhibited with all lengths of restraint stress and was not influenced by food deprivation or DPAT administration. Further, after a 60 minute test period, fat intake was increased in CHO-restricted animals.
Experiments investigating the role of opiates or NE in this effect may provide some insight into the mechanism through which stress influences fat intake. Given that opiate agonists have been shown to decrease food intake, and specifically fat intake (Shor-Posner, et al., 1986a). Further, fat intake is increased by stimulation of the adrenergic system (Leibowitz, et al., 1986; Orthen-Gambill & Kanarek, 1982) and hypothalamic NE is decreased in response to stress (Hellriegel & D'Mello, 1997; Konstandi, et al., 2000). Additional experiments employing opiate antagonists or NE agonists in restraint stress paradigms and opiate agonists or NE antagonists in CHO-restriction paradigms would provide some indication of whether these systems are involved in the effect of stress on fat intake.

While the majority of experiments reported here suggest that stress has no effect on protein intake, this may or may not be due to the time of testing chosen for these experiments. The lack of effect on protein could be a statistical artifact due, at least in part, to low levels of protein intake in control animals. Indeed, there was often a trend towards an effect of CHO-restriction or restraint stress similar in direction to that revealed with CHO intake. In the rat, protein is the preferred macronutrient in the last third of the dark cycle (Tempel, et al., 1989). In the CHO-restriction experiment, animals were tested during the light part of the cycle, while the restraint experiments tested animals at the beginning of the dark cycle. It may be that the lack of effect on protein intake was due to the time of testing and not the effect of stress on protein intake per se. Indeed,
in all the experiments reported in this study, protein intake was low for both experimental and control groups indicating a lack of preference for this macronutrient. Further experiments testing animals at a later phase of the dark cycle would provide some indication regarding the effect of stress on protein intake.

The effect of restraint stress, CHO-restriction and food deprivation may have affected the palatability of the macronutrients to the rats. Research has shown that 24 h of food deprivation enhances the preferences for nonsweet nutrients over sweet nutrients (Sclafani & Ackroff, 1993). Further, acute and chronic restraint stress have differential effects on the palatability of sweet and salty solutions (Howell, Harris, Clarke, Youngblood, Ryan & Gilbertson, 1999). One h of restraint stress decreased preference for sweet but not salty solutions, while chronic restraint (3 h/day for 3 days) had no effect on preference for sweet or salty solutions (Howell, et al., 1999). Further, there are gender differences in preferences for sweet and salty foods in rats (Clarke & Ossenkopp, 1998). Female rats show a greater preference for sweet and salty foods when compared to males (Clarke & Ossenkopp, 1998). These studies indicate that future research investigating palatability shifts between stressed and nonstressed animals is warranted. As well, because stress and gender influence palatability of sweet and salty foods, exposing male rats to the stress paradigms described here could yield differential results.
Finally, additional experiments could be conducted to further the development of these paradigms as valid models of eating disorders. For example, given that one of the characteristics of an eating disorder is its chronicity, it would be of interest to develop a paradigm that produced chronic cycling between food restriction and over-consumption. For example, it may be possible to develop a chronic cycling between restrained eating and bingeing through applying the CHO-restriction in a cyclic nature over several months.

Finally, in order to develop a more complete model of AN or BN, it may be of value to combine the CHO-restriction paradigm with chronic restraint stress in an attempt to reveal an overall stress induced caloric restriction in addition to CHO-restriction. It may also be of interest to chronically restrict animals on fat consumption in order to determine its effect on subsequent macronutrient intake.

Overall, the findings reported in this study provide the researcher with some paradigms through which to continue to study the relationship between stress, macronutrient intake and neurochemistry. Further investigation into the mechanisms through which restraint stress and CHO-restriction influence subsequent macronutrient intake may help to further research into the neurochemical underpinnings of AN, BN and related disorders.
REFERENCES


Stress, serotonin and macronutrient intake


Stress, serotonin and macronutrient intake

eating after food deprivation. *Pharmacology, Biochemistry & Behavior*,

25, 381-392.


*Journal of Comparative and Physiological Psychology*, 54, 294-301.

Simansky, K.J. (1996). Serotonergic control of the organization of feeding and


action of the serotonin (5-HT) uptake inhibitor sertraline in rats:

comparison with directly acting 5-HT agonists. *Brain Research Bulletin*,

25, 953-960.

Slangen, J.L. (1974). The role of hypothalamic noradrenergic neurons in food


bulimia nervosa following acute tryptophan depletion. *Archives of General

Psychiatry*, 56, 171-176.

Soubrie, P. (1986). Reconciling the role of central serotonin neurosis in human


processing: Implications for human psychopathology. *Psychological


(1989). Patterns of extracellular 5-hydroxyindole-acetic acid (5-HIAA) in


APPENDIX I: MECHANISM OF 5-HT1A AUTORECEPTOR ACTIVATION IN THE BRAINSTEM TO REDUCE 5-HT RELEASE THROUGHOUT THE FOREBRAIN

Basic anatomy:

The Dorsal and Median Raphe Nuclei in the brainstem contain 5-HT1A autoreceptors (presynaptic), which decrease 5-HT release into the synapse when stimulated. The cell bodies (and dendrites) of many of the 5-HT neurons that project throughout the forebrain are also housed within the Dorsal and Median Raphe Nuclei.

Steps through which activation of 5-HT1A autoreceptors in the brainstem result in decreased 5-HT release throughout most of the forebrain:

1A autoreceptors (presynaptic) in the Dorsal and Median Raphe Nuclei of the brainstem are stimulated by 5-HT1A agonists or 5-HT

↓

Decreased 5-HT release into synapses

↓

No 5-HT to activate 5-HT neurons whose cell bodies are housed within the Dorsal and Median Raphe Nuclei (these neurons do not fire)

↓

These 5-HT neurons whose terminals release 5-HT throughout most of the forebrain are inhibited from firing

↓

Decreased 5-HT release throughout most of the forebrain