

Georgia State University

ScholarWorks @ Georgia State University

Biology Dissertations

Department of Biology

11-28-2007

Neuropeptide Y-Mediated Control of Appetitive and Consummatory Ingestive Behaviors in Siberian Hamsters (Phodopus sungorus)

Megan J. Dailey

Follow this and additional works at: https://scholarworks.gsu.edu/biology_diss



Part of the [Biology Commons](#)

Recommended Citation

Dailey, Megan J., "Neuropeptide Y-Mediated Control of Appetitive and Consummatory Ingestive Behaviors in Siberian Hamsters (Phodopus sungorus)." Dissertation, Georgia State University, 2007.
doi: <https://doi.org/10.57709/1063866>

This Dissertation is brought to you for free and open access by the Department of Biology at ScholarWorks @ Georgia State University. It has been accepted for inclusion in Biology Dissertations by an authorized administrator of ScholarWorks @ Georgia State University. For more information, please contact scholarworks@gsu.edu.

NEUROPEPTIDE Y-MEDIATED CONTROL OF APPETITIVE AND CONSUMMATORY
INGESTIVE BEHAVIORS IN SIBERIAN HAMSTERS (*PHODOPUS SUNGORUS*)

by

MEGAN J DAILEY

Under the Direction of Timothy J Bartness

ABSTRACT

During the past few decades, obesity has risen significantly in the United States with recent estimates showing that 65% of Americans are overweight and 30% are obese. This increase is a major cause for concern because obesity is linked to many secondary health consequences that include type II diabetes, heart disease, and cancer. Current approaches to the obesity problem primarily have focused on controls of food intake and have been largely unsuccessful. Food, however, almost always has to be acquired (foraging) and frequently is stored for later consumption (hoarding). Therefore, a more comprehensive approach that includes studying the underlying mechanisms in human foraging and food hoarding behaviors could provide an additional target for pharmaceutical or behavioral manipulations in the treatment and possibly prevention of obesity.

Neuropeptide Y (NPY) is a particular peptide that provides a potent orexigenic drive to alter foraging, food hoarding (appetitive ingestive behaviors) and food intake (consummatory

ingestive behaviors) in variety of species. NPY is predominantly produced in the arcuate nucleus of the hypothalamus (ARC) and has extensive efferent projections throughout the brain. Two target nuclei of ARC-NPY, the paraventricular nucleus of the hypothalamus (PVH) and perifornical area (PFA), have been shown to mediate the effect of NPY on food intake in laboratory rats and mice, but nothing is known about the effect of ARC-NPY on foraging and food hoarding. In addition, the action of specific NPY receptor subtypes within these two nuclei for these behaviors is unknown. Even though ARC-NPY is one of the main sources of input into the PVH and PFA, it is not known if this NPY fiber projection mediates alterations in appetitive and consummatory ingestive behaviors. Therefore, the purpose of this dissertation is to test 1) if NPY within the PVH or PFA controls appetitive, as well as, consummatory ingestive behaviors, 2) if NPY Y1 receptors within the PVH or PFA differentially control appetitive or consummatory ingestive behaviors, and 3) if NPY from the ARC is necessary for the control of appetitive and consummatory ingestive behaviors.

INDEX WORDS: Obesity, Appetitive, Consummatory, Ingestive Behaviors, Food Hoarding, Neuropeptide Y (NPY)

NEUROPEPTIDE Y-MEDIATED CONTROL OF APPETITIVE AND CONSUMMATORY
INGESTIVE BEHAVIORS IN SIBERIAN HAMSTERS (*PHODOPUS SUNGORUS*)

by

MEGAN J DAILEY

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

in the College of Arts and Sciences

Georgia State University

2007

Copyright by
Megan Jeanne Dailey
2007

NEUROPEPTIDE Y-MEDIATED CONTROL OF APPETITIVE AND CONSUMMATORY
INGESTIVE BEHAVIORS IN SIBERIAN HAMSTERS (*PHODOPUS SUNGORUS*)

by

MEGAN J DAILEY

Committee Chair: Timothy Bartness

Committee: Elliott Albers
Andrew Clancy
Anne Murphy
Larry Young

Electronic Version Approved:

Office of Graduate Studies
College of Arts and Sciences
Georgia State University
December 2007

DEDICATION

I dedicate this to my parents for giving me the opportunity to follow my dreams and achieve happiness.

ACKNOWLEDGEMENTS

I never could have accomplished this on my own. There are many people whose efforts have contributed to this final product, as well as, to my education in general. I would like to thank my committee for believing that I would one day actually finish my dissertation. I greatly appreciate your time, helpful comments and for showing me the direction to get out of here. I would like to thank Kay Song for always being there when I needed someone the most to offer words of wisdom and encouragement. She has stopped the tears from flowing many times. I would like to thank my sidekicks – Kelly Johnson, Maisie Adiviani and Omuwa Braimah. They have been my trusty hands when I have taken on more than I can handle – never letting me down. To the lab, I thank you for allowing my personality to take center stage on more than one occasion (maybe every occasion that I am in the lab). I also would like to thank the many friends, office mates and buddies that have enriched each and every day at GSU. Last, but not least, I would like to thank Tim for putting up with all of me. You are an excellent mentor that is able to push without yelling or the use of unnecessary force. People are only intimidated by you because they respect you and do not want to let you down. It will be difficult to live up to the example you have set.

TABLE OF CONTENTS

| | |
|--|------|
| DEDICATION | iv |
| ACKNOWLEDGEMENTS | v |
| LIST OF FIGURES | viii |
| CHAPTER | |
| 1 GENERAL INTRODUCTION | 1 |
| 2 DOES NPY IN THE PVH OR PFA AFFECT APPETITIVE OR CONSUMMATORY INGESTIVE BEHAVIORS IN SIBERIAN HAMSTERS? | |
| Abstract | 12 |
| Introduction | 13 |
| Methods | 15 |
| Results | 20 |
| Discussion | 26 |
| Figure Captions | 31 |
| Figures | 36 |
| 3 DOES DESTRUCTION OF ALL NPY Y RECEPTORS IN THE PVH ABOLISH POST-FOOD DEPRIVATION INDUCED INCREASES IN FOOD HOARDING AND FORAGING IN SIBERIAN HAMSTERS? | |
| Abstract | 88 |
| Introduction | 89 |
| Methods | 92 |

| | | |
|---|--|-----|
| | Results | 97 |
| | Discussion | 99 |
| | Figure Captions | 103 |
| | Figures | 105 |
| 4 | DOES THE DESTRUCTION OF ARC-NPY ALTER APPETITIVE OR CONSUMMATORY INGESTIVE BEHAVIORS IN SIBERIAN HAMSTERS? | |
| | Abstract | 119 |
| | Introduction | 120 |
| | Methods | 122 |
| | Results | 129 |
| | Discussion | 131 |
| | Figure Captions | 137 |
| | Figures | 140 |
| 5 | CONCLUSION | 165 |
| | REFERENCES | 174 |

LIST OF FIGURES

FIGURE

| | |
|---|----|
| 2.1 Mapping of NPY-PVH injections for the 10 Revolutions/pellet (10 Rev) group | 34 |
| 2.2 Mapping of NPY-PVH injections for the Free Wheel (FW) group | 37 |
| 2.3 Mapping of NPY-PVH injections for the Blocked Wheel (BW) group | 40 |
| 2.4 Mapping of misses for NPY-PVH injections | 42 |
| 2.5 Mapping of NPY-PFA injections for the 10 Revolutions/pellet (10 Rev) group | 44 |
| 2.6 Mapping of NPY-PFA injections for the Free Wheel (FW) group | 47 |
| 2.7 Mapping of NPY-PFA injections for the Blocked Wheel (BW) group | 50 |
| 2.8 Mapping of misses for the NPY-PFA injections | 52 |
| 2.9 Mapping of the Y1 antagonist injections into the PVH for the 10 Revolutions/pellet (10 Rev) group | 54 |
| 2.10 Mapping of the Y1 antagonist injections into the PVH for the Free Wheel (FW) group | 57 |
| 2.11 Mapping of the Y1 antagonist injections into the PVH for the Blocked Wheel (BW) group | 60 |
| 2.12 Mapping of the Y1 antagonist injections into the PFA for the 10 Revolutions/pellet (10 Rev) group | 62 |
| 2.13 Mapping of the Y1 antagonist injections into the PFA for the Free Wheel (FW) group | 65 |

| | |
|--|----|
| 2.14 Mapping of the Y1 antagonist injections into the PFA for the Blocked Wheel group | 68 |
| 2.15 Percent change in foraging for the 10 Revolutions/pellet (10 Rev) group after injections of NPY into the PVH or PFA | 70 |
| 2.16 Percent change in wheel revolutions for the Free Wheel (FW) group after injections of NPY into the PVH or PFA | 71 |
| 2.17 Percent change in food hoarding for the 10 Revolutions/pellet (10 Rev) group after injections of NPY into the PVH or PFA | 72 |
| 2.18 Percent change in food hoarding for the Free Wheel (FW) group after injections of NPY into the PVH or PFA | 73 |
| 2.19 Percent change in food hoarding for the Blocked Wheel (BW) group after injections of NPY into the PVH or PFA | 74 |
| 2.20 Percent change in food intake for the 10 Revolutions/pellet (10 Rev) group after injections of NPY into the PVH or PFA | 75 |
| 2.21 Percent change in food intake for the Free Wheel (FW) group after injections of NPY into the PVH or PFA | 76 |
| 2.22 Percent change in food intake for the Blocked Wheel (BW) group after injections of NPY into the PVH or PFA | 77 |
| 2.23 Percent change in foraging for the 10 Revolutions/pellet (10 Rev) group after injection of a Y1 antagonist into the PVH or PFA | 78 |
| 2.24 Percent change in wheel revolutions for the Free Wheel (FW) group after injection of Y1 antagonist into the PVH or PFA | 79 |

| | | |
|------|---|-----|
| 2.25 | Percent change in food hoarding for the 10 Revolutions/pellet (10 Rev) group after injection of Y1 antagonist into the PVH or PFA | 80 |
| 2.26 | Percent change in food hoarding for the Free Wheel (FW) group after injection of Y1 antagonist into the PVH or PFA | 81 |
| 2.27 | Percent change in food hoarding for the Blocked Wheel (BW) group after injection of Y1 antagonist into the PVH or PFA | 82 |
| 2.28 | Percent change in food intake for the 10 Revolutions/pellet (10 Rev) group after injection of Y1 antagonist into the PVH or PFA | 83 |
| 2.29 | Percent change in food intake for the Free Wheel (FW) group after injection of Y1 antagonist into the PVH or PFA | 84 |
| 2.30 | Percent change in food intake for the Blocked Wheel (BW) after injection of Y1 antagonist into the PVH or PFA | 85 |
| 3.0 | Representative sections of the PVH showing effects of Blank-Saporin (B-SAP) and NPY-Saporin (NPY-SAP) | 105 |
| 3.1 | Average baseline foraging for the 10 Revolutions/pellet group (10 Rev) and wheel revolutions for the Free Wheel (FW) group after injection of Blank-Saporin (B-SAP) or NPY-Saporin (NPY-SAP) into the PVH | 109 |
| 3.2 | Average baseline food hoarding for all groups after injection of Blank-Saporin (B-SAP) or NPY-Saporin (NPY-SAP) into the PVH | 110 |
| 3.3 | Average baseline food intake for all groups after injection of Blank-Saporin (B-SAP) or NPY-Saporin (NPY-SAP) into the PVH | 112 |
| 3.4 | Foraging and wheel revolutions after food deprivation for the PVH injected Blank-Saporin (B-SAP) and NPY-Saporin (NPY-SAP) groups | 114 |

| | |
|---|-----|
| 3.5 Food hoarding after food deprivation for the PVH injected Blank-Saporin (B-SAP) and NPY-Saporin (NPY-SAP) groups | 115 |
| 3.6 Food intake after food deprivation for the PVH injected Blank-Saporin (B-SAP) and NPY-Saporin (NPY-SAP) groups | 117 |
| 4.0 Representative sections of the ARC and PVH showing the effects of Blank-Saporin (B-SAP) and NPY-Saporin (NPY-SAP) | 140 |
| 4.1 Average baseline foraging for the 10 Revolutions/pellet group (10 Rev) and wheel revolutions for the Free Wheel (FW) group after injection of Blank-Saporin (B-SAP) or NPY-Saporin (NPY-SAP) into the ARC | 143 |
| 4.2 Average baseline food hoarding for all groups after injection of Blank-Saporin (B-SAP) or NPY-Saporin (NPY-SAP) into the ARC | 144 |
| 4.3 Average baseline food intake for all groups after injection of Blank-Saporin (B-SAP) or NPY-Saporin (NPY-SAP) into the ARC | 146 |
| 4.4 Foraging and wheel revolutions after food deprivation for the ARC injected Blank-Saporin (B-SAP) and NPY-Saporin (NPY-SAP) groups | 148 |
| 4.5 Food hoarding after food deprivation for the ARC injected Blank-Saporin (B-SAP) and NPY-Saporin (NPY-SAP) groups | 149 |
| 4.6 Food intake after food deprivation for the ARC injected Blank-Saporin (B-SAP) and NPY-Saporin (NPY-SAP) groups | 151 |
| 4.7 Representative sections of the ARC and PVH showing the effects of Phosphate Buffered Saline (PBS) or monosodium glutamate (MSG) treatment | 153 |

| | |
|---|-----|
| 4.8 Average baseline wheel revolutions after Phosphate Buffered Saline (PBS) or monosodium glutamate (MSG) treatment | 156 |
| 4.9 Average baseline food hoarding after Phosphate Buffered Saline (PBS) or monosodium glutamate (MSG) treatment | 157 |
| 4.10 Average baseline food intake after Phosphate Buffered Saline (PBS) or monosodium glutamate (MSG) treatment | 158 |
| 4.11 Wheel revolutions after food deprivation for the Phosphate Buffered Saline (PBS) or monosodium glutamate (MSG) groups | 159 |
| 4.12 Food hoarding after food deprivation for the Phosphate Buffered Saline (PBS) or monosodium glutamate (MSG) groups | 160 |
| 4.13 Food intake after food deprivation for the Phosphate Buffered Saline (PBS) or monosodium glutamate (MSG) groups | 161 |
| 4.14 Representative brain sections showing the compensatory effects after destruction of the ARC | 163 |

CHAPTER 1

GENERAL INTRODUCTION

Obesity is a public health problem worldwide affecting millions of people with the incidence within the United States constantly rising. It is estimated that 65% of Americans are overweight and 30% are obese (Ogden et al., 2006b). The obesity epidemic is a great health concern because it is a major risk factor associated with a variety of pathologic disorders including type II diabetes, heart disease, and cancer (Vague et al., 1980; Satcher, 2001; Gasteyger and Tremblay, 2002). In addition to the cost of our individual health, there is an enormous economic cost. An estimated 9.1% of U.S. healthcare expenditure is directly related to obesity, with recent estimates at \$93 billion per year (Finkelstein, 2003). Therefore, it is of great interest to determine the mechanisms that are responsible for obesity and find a therapy that will prevent or reverse the obese state.

Obesity is caused by an imbalance between energy intake and expenditure and is influenced by both genetic and environmental factors. Although humans appear to be genetically programmed to conserve energy and eat beyond current energy requirements whenever food is plentiful, the rapid increase in obesity cannot simply be explained by genetics alone. We are constantly bombarded with visual and auditory stimuli that motivate us to acquire and eat more food than is needed to maintain normal energy balance. An environment with easy access to high calorie, low cost foods combined with decreased physical activity has resulted in the chronic positive energy balance that leads to weight gain. An understanding of the mechanisms regulating energy intake and expenditure could hopefully lead to a pharmaceutical or behavioral manipulation that will inhibit the motivation to acquire and eat more food than is needed to maintain energy balance and, thus, may curtail the growing obesity epidemic.

Energy balance is normally achieved by adjustments in energy intake, storage, and expenditure in order to meet daily energy requirements. This includes changes in both the appetitive ingestive behaviors of foraging, transport and hoarding of food and the consummatory ingestive behavior of eating (Craig, 1918; Bartness, 1997). Current approaches to the obesity problem primarily focus on the mechanisms controlling the consummatory ingestive behavior of food intake and have been largely unsuccessful. Food, however, almost always has to be acquired (foraging) and frequently is stored for subsequent consumption (hoarding; (Vander Wall, 1990)). Even though humans do not have to expend much energy to acquire food, as do other animals, we still have to expend some energy and time to get food and alterations in how we acquire our food are seen under different physiological conditions. For example, if you go to the grocery store hungry, you will bring home more food than when you are full (Dodd et al., 1977; Beneke and Davis, 1985; Mela et al., 1996). Moreover, obese people bring home more high fat foods and more calories per person than lean people (Ransley et al., 2003g). Once food is acquired and stored in our refrigerators/freezers and pantries, we are more likely to eat this stored food than go out and acquire additional food (Ransley et al., 2003f). Therefore, understanding the underlying mechanisms in human foraging and food storing (hoarding) behaviors could provide an additional target for pharmaceutical or behavioral manipulations in the treatment and possibly prevention of obesity.

One way to reveal the underlying mechanisms controlling specific appetitive and consummatory ingestive behaviors is to use a real-world species and test it in semi-naturalistic environmental conditions, thereby studying phenomena at face value because of the evolutionary pressures shaping the natural behavior and physiology of the animals. Studying a wide variety of species that alter their energetic strategy in response to varied energetic demands will broaden our understanding of the hormonal and neuropeptide control of specific ingestive behaviors. One animal

model that has offered great insight into the mechanisms controlling both phases of ingestion is the Siberian hamster (*Phodopus sungorus*). Siberian hamsters exhibit changes in appetitive (foraging and food hoarding) and consummatory (food intake) ingestive behaviors in a variety of challenges both in the wild and in the laboratory (Flint, 1966; Bartness and Clein, 1994; Wood and Bartness, 1996; Bartness, 1997; Day et al., 1999; Day and Bartness, 2001; Day et al., 2002; Day and Bartness, 2003; Day and Bartness, 2004; Day et al., 2005; Keen-Rhinehart and Bartness, 2005). Even though the appetitive ingestive behavior of food hoarding appears to be an important part of the energetic repertoire of many species, Siberian hamsters are natural hoarders and are equipped with special structures for carrying food (cheek pouches) allowing them to transport significant amounts of foraged food (Vander Wall, 1990). Changes in food hoarding in this species can occur independently of changes in food intake. For example, food deprived-refed hamsters increase foraging and food hoarding, with little or no change in food intake (Day and Bartness, 2003). This differential expression of appetitive (foraging/hoarding) and consummatory (food intake) ingestive behaviors suggests that at least a partially independent physiology subserves each ingestive behavior. Thus, Siberian hamsters are an ideal species to study the effects of environmental and physiological challenges on foraging, food hoarding and food intake, including the role of neuropeptides related to energy balance in mediating these behaviors.

Foraging is an important appetitive behavior that is ignored or omitted in most studies of energy balance. In the wild, animals must expend significant energy to search for a utilizable food source. Depending upon their energetic status and energy demand, they partition the foraged food for immediate or future use. Food can be consumed and oxidized immediately, or stored for later use as body fat or as a food hoard. Most previous laboratory investigations of energy balance include unlimited access to food placed in close proximity to the animal. Under these utopian

conditions, there is an artificial restriction on expressing important appetitive ingestive behaviors. In studies of the effect of foraging effort (requiring animals to run a prescribed number of wheel revolutions in order to earn food pellets) on energetic strategies of Siberian hamsters using a unique foraging/hoarding caging system (described later in this section), food hoarding is increased by low levels of energy expenditure and reduced to control levels with higher foraging efforts (Day and Bartness, 2001). Thus, foraging alone can alter the energetic strategy of animals. Therefore, the additional requirement of animals to forage for their food appears to be important to understanding how animals allocate their time and energy in order to fulfill their energy needs. Thus, all my studies presented here examined the allocation of energy to both appetitive and consummatory ingestive behaviors in Siberian hamsters where the increased energy expended due to foraging is taken into account. This was accomplished by using a simulated burrow system that incorporates a wheel-running requirement for the delivery of food pellets (foraging/hoarding apparatus) and measuring the changes in foraging, food hoarding, and food intake of Siberian hamsters.

Although several neuronal populations within the brain contribute to energy balance, neuropeptide Y (NPY) is one particular peptide that provides a potent orexigenic drive to alter both appetitive and consummatory ingestive behaviors in variety of species (Kalra et al., 1999; Schwartz et al., 2000; Day et al., 2005). NPY neurons are largely restricted to the arcuate nucleus of the hypothalamus (ARC; (Chronwall et al., 1985g; White and Kershaw, 1989)), with low levels of expression within the compact zone of the dorsomedial hypothalamus (DMH; (Li et al., 1998)) and the brainstem catecholamine cell groups A1, C1-C3 (Everitt et al., 1984i). ARC-NPY neurons have extensive efferent projections to numerous hypothalamic regions (de Quidt and Emson, 1986; Broberger et al., 1998) that play an important role in the regulation of energy balance, such as the paraventricular nucleus (PVH), DMH, perifornical area (PFA) and the lateral hypothalamic area

(LHA), as well as the brainstem (de Quidt and Emson, 1986). Because the ARC has a reduced blood brain barrier, ARC-NPY neurons are positioned to sense peripheral metabolic and hormonal signals (*ie.* leptin, ghrelin, insulin, and glucose) and to relay this information to other brain regions to drive changes in appetitive and consummatory ingestive behaviors (Sawchenko, 1998; Watts, 2000). When animals are faced with negative energy balance, such as during food deprivation, ARC-NPY gene expression is increased (Brady et al., 1990; Mercer et al., 1995). Central injection of NPY into the third ventricle increases food intake in laboratory rats (Morley et al., 1987a), and it increases food hoarding to a greater extent than food intake in Siberian hamsters (Day et al., 2005). Thus, the NPY neurons within the hypothalamus function in the control of appetitive and consummatory ingestive behaviors to maintain energy balance.

Although the major source of NPY appears to be the ARC of the hypothalamus (Chronwall et al., 1985f; White and Kershaw, 1989), NPY expression also is present in brainstem catecholamine cell groups A1, C1-C3 (Everitt et al., 1984h) and NPY-immunoreactive terminals within the hypothalamus originate from these neurons (Everitt et al., 1984g; Broberger et al., 1998). The role of brainstem NPY in the regulation of ingestive behaviors has been questioned because they are thought to make-up only a small proportion of the NPY-immunoreactive fibers within the hypothalamic nuclei that are involved in the regulation of ingestive behaviors (Bai et al., 1985; Broberger et al., 1998; Broberger et al., 1999c). In rodent models, however, in which the ARC neurons have been compromised, such as adult rodents that become obese due to neonatal monosodium glutamate (MSG) treatment and the anorectic *anx/anx* mutant mice, there is still an abundance of NPY-immunoreactive fibers present in the PVH and DMH (Broberger et al., 1998; Broberger et al., 1999a). In these models, it has been hypothesized that NPY inputs to the PVH from the brainstem may be increased to compensate for the loss of ARC-NPY neurons

(Broberger et al., 1998; Broberger et al., 1999b). Other studies also suggest that there may be a local effect of NPY within the brainstem. For example, fourth ventricular injections of NPY stimulate food intake in rats to the same extent as third ventricular injections (Corp et al., 2001). In decerebrate rats, when all connections between the forebrain and brainstem are severed, the isolated brainstem is still capable of altering consummatory ingestive behaviors when energetically challenged (Grill and Kaplan, 2001; Harris et al., 2006b). Although these data suggest that the brainstem may contain local circuits that are capable of solely controlling ingestive behaviors, no one has looked at the brainstem control of foraging and food hoarding and if site-specific injections of NPY within the brainstem affects these appetitive ingestive behaviors.

Changes in appetitive and consummatory ingestive behaviors may be controlled by separate NPY receptors. Of the five receptor subtypes that have been cloned for NPY, four have been localized within the rodent brain, including the NPY Y1, Y2, Y4 and Y5 (Parker and Herzog, 1999). Although the exact role of each of the NPY receptor subtypes in regulating ingestive behaviors is unclear, the Y1 and Y5 receptor subtypes appear to be the most directly involved in the regulation of appetitive and consummatory ingestive behaviors (Chamorro et al., 2002a). The Y2 receptor is expressed on ARC-NPY neurons and is considered an autoreceptor that can regulate ingestive behaviors through the modulation of endogenous NPY release (Batterham et al., 2002). Y1 receptor agonists, stimulate hyperphagia (*i.e.*, increase food intake) in laboratory rats (O'Shea et al., 1997d), whereas antagonists significantly reduce the hyperphagia induced by centrally-administered NPY or food deprivation (O'Shea et al., 1997a; Wieland et al., 1998; Morgan et al., 1998). Antagonists to Y5 reduce food intake in laboratory rats and Y5 receptor knockout mice eventually become hyperphagic and present an obese phenotype (Schaffhauser et al., 1997; Marsh et al., 1998). There also is evidence for the involvement of these NPY receptor subtypes in ingestive behaviors of Siberian

hamsters. Specifically, the Y1 receptor appears to be most responsible for the increase in food hoarding and perhaps foraging (appetitive ingestive behaviors), whereas the Y5 receptor subtype may play a greater role in food intake (consummatory behavior) based on third intracerebroventricular (icv) injections of the Y1 or Y5 receptor agonists to Siberian hamsters tested using the foraging/hoarding apparatus (Day et al., 2005). In addition, recent pilot data from the Bartness lab suggests the NPY receptors to be involved in selectively regulating these behaviors because pretreatment with intra-PVH Y1 receptor antagonist blocked NPY-induced increases in hoarding when the agonist was subsequently injected into the PVH (D. Day and T. Bartness, unpublished observations). The Y5 antagonist did not affect the NPY-induced increase in ingestive behaviors when administered directly into the PVH, however. Thus, we have some data to suggest the role of Y1 and Y5 NPY receptor subtypes in foraging, food hoarding, and food intake in energy balance of Siberian hamsters and some indication of the importance of these receptors specifically in the PVH.

Although there are numerous pathways that are involved with ingestive behaviors, the ARC to PVH pathway is considered key for the regulation of ingestive behaviors. ARC neurons are primary targets for the many peripheral metabolic feedback signals and play an important role in transforming these hormonal signals to a neuronal signal that is then transmitted to the PVH and other brain areas. The PVH is an important integration site for numerous circuits involved in energy homeostasis, which in turn generates an appropriate response to modulate ingestive behaviors. The PVH receives a dense innervation of NPY fibers and, more specifically, those originating from ARC-NPY neurons and from brainstem nuclei (Broberger et al., 1998). As stated above, the PVH has been shown in our lab and others to be a key site for the direct action of NPY. Physiological doses of NPY directly administered into the PVH potentially stimulates food intake in a dose-

dependent manner in rats (Stanley and Leibowitz, 1985). In addition, NPY release within the PVH has been shown to change appropriately during the pre- and postmeal periods in response to food deprivation and food restriction (Yoshihara et al., 1996a; Yoshihara et al., 1996c; Jain et al., 1998). In our laboratory, pilot data (described previously) suggests an effect of NPY in the PVH on appetitive ingestive behaviors, as well as consummatory ingestive behaviors. This pilot study, though, used exogenous administration of NPY or receptor agonists at high doses. It is necessary to test the effect of physiological doses of NPY in the PVH on appetitive ingestive behaviors in our model and if these behaviors can be inhibited by specific Y receptor antagonists that block the effect of endogenous NPY after food deprivation. Even without definitive results on the role of NPY in the PVH in controlling appetitive ingestive behaviors, it is clear that the PVH is a main hypothalamic site for the action of NPY in controlling consummatory ingestive behavior.

The PVH serves as an integrator and link between the neuroendocrine and autonomic nervous systems where NPY may be able to affect numerous circuits involved in energy homeostasis and generate appropriate responses to modulate ingestive behaviors. NPY Y receptors are found throughout the PVH of rodents and are colocalized with many other neuropeptides or hormones that are important regulators of energy balance and endocrine axes (Parker and Herzog, 1999). A subpopulation of corticotropin-releasing hormone (CRH) neurons express the Y5 receptor, but not the Y1 receptor (Campbell, 2000; Li, 2000). Y1 positive nerve terminals, however, are in close proximity to CRH neurons and may suggest that NPY has both pre- and post-synaptic actions on these neurons (Li, 2000). Y1 receptors are expressed on thyrotropin-releasing hormone (TRH) neurons within the PVH (Broberger et al., 1999c). In addition, the Y5 receptor is expressed on both oxytocinergic (OT) and arginine vasopressin (AVP) neurons within the PVH that are important for autonomic control of energy expenditure (Watts, 2000; Campbell, 2001). These data provide

morphological evidence for the role of NPY in the PVH influencing energy metabolism through actions affecting downstream hypothalamic-pituitary axes and the autonomic nervous system through NPY Y receptor-mediated mechanisms.

There are extensive NPY projections between the hypothalamus and brainstem that appear to play distinctive roles in controlling ingestive behaviors. Bilateral neural transections at the level of the dorsal tegmentum in the mesencephalon, which transect ascending fibers from the brainstem to many areas of the brain including the hypothalamus, markedly decrease NPY concentrations in the medial preoptic area, median eminence, PVH and DMH indicating that a substantial number of neurons from the brainstem, including NPY neurons, project to these four nuclei (Sahu et al., 1988c). In contrast, these same neural transections produced no alteration in NPY concentrations within the suprachiasmatic nucleus, ARC and ventromedial nucleus of the hypothalamus suggesting that NPY innervation to these nuclei may be derived mainly from ARC-NPY neurons or other sources outside of the brainstem (Sahu et al., 1988d). In addition, these neural transections of ascending brainstem fibers attenuate the effectiveness of NPY to increase food intake when injected into the 4th ventricle (Sahu et al., 1988b; Steinman et al., 1994c), suggesting that the effect of NPY on increasing food intake requires intact brainstem to forebrain structures. By contrast, these neural transections reduced the effects of third ventricular injections of low doses of NPY on food intake, (Sahu et al., 1988a; Steinman et al., 1994b), suggesting that the effect of NPY in the hypothalamus on increasing food intake does not require brainstem input. When neural transections are made more medially in the mesencephalon than those previously described and transect mostly descending projections from the forebrain to the brainstem, the effectiveness of NPY to increase food intake was attenuated after injections into the third ventricle, but not the fourth (Steinman et al., 1994a). This suggests that the effect of NPY in the hypothalamus requires descending projections to the brainstem. Thus, the

neuronal projections between the hypothalamus and brainstem appear to have specific roles in regulating NPY-mediated control of ingestive behaviors, but it is still necessary to outline and functionally define specific projections involved in both appetitive and consummatory ingestive behaviors.

Specific NPY projections between the hypothalamus and brainstem have been selectively destroyed to test their effects on food intake in rats. Saporin, a type 1 ribosomal inactivating protein, can be targeted to destroy specific populations of neurons by conjugation with antibodies that are selectively internalized by the targeted cell population (Wiley and Kline IV, 2000). When the brainstem catecholamine neurons that express NPY and project to the hypothalamus are destroyed by saporin conjugated to antidopamine- β -hydroxylase (D-SAP), the normal glucoprivic feeding response is impaired and the glucoprivation-induced increase in expression of NPY mRNA in the ARC is eliminated (Fraley and Ritter, 2003). On the other hand, when NPY neurons of the ARC are destroyed by saporin conjugated to NPY (NPY-SAP), the lesion does not impair the increased feeding response after glucoprivation (Bugarith et al., 2005h). This differential response from the two NPY-producing neurons within the brainstem or the hypothalamus suggests there may be separate roles for each circuit in the control of consummatory ingestive behaviors, but these studies omitted any investigation of these circuits in the control of appetitive ingestive behaviors. Even if both hypothalamic and brainstem NPY play a role in both phases of ingestion, they may differentially control ingestive behaviors based on specific energetic challenges.

Dissertation Goals

The purpose of this dissertation is to test the effect of NPY on appetitive and consummatory ingestive behaviors. In Chapter 2, I discuss two specific hypothalamic sites, the PVH and PFA,

where NPY may differentially control the appetitive ingestive behaviors of foraging and food hoarding and consummatory ingestive behavior of food intake. In Chapter 3, I investigate the effects of destroying all NPY Y receptor-containing neurons in the PVH on post-food deprivation induced increases in food hoarding. In Chapter 4, I test the role ARC-NPY plays in controlling appetitive and consummatory ingestive behaviors under baseline conditions and after an energetic challenge of food deprivation. Collectively, the conclusions of these studies will be discussed in Chapter 5.

The studies within this dissertation will help to understand the role of NPY in controlling appetitive ingestive behaviors, as well as, provide additional information on the importance of hypothalamic NPY, specific target nuclei of NPY and specific NPY Y receptor subtypes that are involved in controlling both appetitive and consummatory ingestive behaviors. Thus, overall this dissertation should provide further insight into factors that contribute to energy balance and new ways in which to behaviorally or pharmaceutically target treatments of obesity.

CHAPTER 2

DOES NPY IN THE PVH OR PFA AFFECT APPETITIVE OR CONSUMMATORY INGESTIVE BEHAVIORS IN SIBERIAN HAMSTERS?

Abstract

During just the past few decades, obesity has risen significantly worldwide. Current approaches to the obesity problem primarily have focused on controls of food intake and have been largely unsuccessful. Food, however, almost always has to be acquired (foraging) and frequently is stored for subsequent consumption (hoarding). Studying the underlying mechanisms in human foraging and food hoarding behaviors could provide an additional target for treatments of obesity. Neuropeptide Y (NPY) is a potent orexigenic peptide known to alter foraging and food hoarding (appetitive ingestive behaviors), as well as food intake (consummatory ingestive behavior). Site-specific injections of NPY into the paraventricular nucleus of the hypothalamus (PVH) or perifornical area (PFA) increase food intake in rats, but it is not known if NPY within these areas also affects foraging or food hoarding. Therefore, I tested a) if NPY within the PVH or PFA stimulates appetitive or consummatory ingestive behaviors in Siberian hamsters and b) if the antagonism of the NPY Y1 receptor subtype, known to play a role in hoarding in our animals, inhibits the increase in food hoarding normally seen after food deprivation. This was accomplished by injecting three doses NPY directly into the PVH or PFA and measuring foraging, hoarding, and intake 1, 2, 4 and 24 h after injection. A subset of the animals were then food deprived for 56 h and injected with a Y1 antagonist before refeeding and ingestive behaviors measured at time points previously stated. Collectively, NPY increased both appetitive and consummatory ingestive behaviors after injection into the PVH or

PFA and antagonism of the NPY Y1 receptor inhibited the post food deprivation-induced increase in food hoarding.

Introduction

Obesity is an increasingly important health problem that is the result of energy intake chronically exceeding energy expenditure. During just the past few decades, obesity has risen significantly in the United States with recent estimates showing that 65% of Americans are overweight and 30% are obese (Ogden et al., 2006a). This increase in obesity is a major cause for concern because obesity is linked to many secondary health consequences that include type II diabetes, heart disease, and cancer (Vague et al., 1980; Satcher, 2001; Gasteyger and Tremblay, 2002). Much attention has been paid to decreasing energy intake through dieting or increasing energy expenditure through exercise as a means for combating obesity. This approach has not been very effective because people have a difficult time committing to such changes. We live in an environment where we are constantly bombarded with perceptual signals that motivate us to acquire and eat more food than is needed to maintain normal energy balance. Easy access to inexpensive, high caloric density foods has only exacerbated the motivation to obtain and eat more food than is necessary. Understanding the mechanisms that regulate such behavior would enable researchers to devise alternate means, such as drug therapy, to fight the obesity epidemic.

Ingestive behavior is comprised of both appetitive and consummatory behaviors (Craig, 1918). Appetitive ingestive behaviors motivate us to obtain and store food (foraging, hoarding), while consummatory ingestive behavior is the actual eating of the acquired food (Craig, 1918). Most research has only focused on the control of consummatory behaviors with little or no attention paid to appetitive ingestive behaviors. Because we can only consume food we have already acquired

or stored, it is important to identify specific peptides involved in controlling both phases of ingestion. This should result in a better understanding of the behaviors that cause individuals to constantly be in a state of positive energy balance.

Although several neuronal populations within the brain contribute to energy balance, Neuropeptide Y (NPY) is one peptide that provides a potent orexigenic drive to alter both appetitive and consummatory ingestive behaviors in variety of species (Kalra et al., 1999; Schwartz et al., 2000; Bartness and Day, 2003). Central injections of NPY into the third ventricle increase food intake (consummatory ingestive behavior) in laboratory rats (Morley et al., 1987b) and increase food hoarding (appetitive ingestive behavior) to a greater extent than food intake in Siberian hamsters (Day et al., 2005). Third ventricular injections of peptides, though, affect many brain nuclei so their sites of action are unknown with such intracerebroventricular (icv) injections. Site-specific injections of NPY administered into the paraventricular nucleus of the hypothalamus (PVH) or the perifornical area (PFA) elicit a potent increase in food intake in rats (Stanley and Leibowitz, 1985; Stanley et al., 1993), suggesting these two sites as the main loci of the effect of NPY to alter ingestive behaviors.

Both the PVH and PFA possess NPY Y receptors and contain numerous NPY-immunoreactive fibers (Parker and Herzog, 1999). It may be that NPY elicits changes in appetitive or consummatory ingestive behaviors by acting differentially on specific Y receptor subtypes within these two areas. NPY Y1 receptor agonists increase food intake in laboratory rats (O'Shea et al., 1997c), whereas antagonists significantly reduce the increase in food intake induced by centrally-administered NPY or food deprivation (O'Shea et al., 1997a; Wieland et al., 1998; Morgan et al., 1998). Similarly, a Y1 receptor agonist injected into the PVH or PFA elicits a strong dose-dependent increase in food intake (Stanley et al., 1992). NPY Y1 receptor also appears to be

responsible for the increase in food hoarding in Siberian hamsters seen after injections of NPY based on third icv injections of a NPY Y1 receptor agonist (Day et al., 2005). This suggests that the effect of NPY to alter appetitive or consummatory ingestive behaviors may be mediated specifically by NPY Y1 receptors and that the PVH and PFA are two sites of this effect. Given the ability of NPY injected into the PVH or PFA to stimulate food intake in rats and that this consummatory response can be attenuated after injection of antibodies to Y1 receptor (Stanley and Leibowitz, 1985; Stanley et al., 1993), the purpose of this study was to test: a) whether the increase in food hoarding after icv injection of NPY in Siberian hamsters may be mediated by the PVH and/or PFA and b) if post-food deprivation increases in food hoarding can be blocked by injections of a NPY Y1 receptor antagonist administered into the PVH or PFA. This was accomplished by injecting saline or three doses of NPY into the PVH or PFA of Siberian hamsters and measuring foraging, food hoarding or food intake 1, 2, 4, and 24 h after injection. A subset of the animals were then food deprived and injected with either a vehicle or Y1 receptor antagonist before refeeding and behavioral measures taken at the same time points.

Methods

Animals and Housing

Adult male Siberian hamsters ~3 months old and weighing 35-46 g were obtained from our breeding colony. The colony was established in 1988 and its genealogy was described recently (Day and Bartness, 2001). Hamsters were group-housed and reared from birth in a 16:8h light-dark cycle (lights-on at 2030). Room temperature was maintained at 21 ± 2 °C and relative humidity was 50 ± 10 %. All procedures were approved by the Georgia State University

Institutional Animals Care and Use Committee and were in accordance with the Public Health Service and United States Department of Agriculture guidelines.

Sixty-four animals were acclimated for 1 wk in our hoarding/foraging apparatus as previously shown and described (Day and Bartness, 2001). Briefly, two cages were connected with a convoluted polyvinyl-chloride tubing system (38.1 mm inner diameter and ~1.52 m long), with corner and straightways for both horizontal and vertical climbs. The top or “food cage” was 456 x 234 x 200 mm (length x width x height) equipped with a water bottle and running wheel. The bottom or “burrow cage” was 290 x 180 x 130 mm and was covered to simulate the darkness of a natural burrow. The burrow cage contained Alpha-Dri (Specialty Papers, Kalamazoo, MI) bedding and cotton nesting material. The animals were fed 75 mg pellets (Purified Rodent Diet; Research Diets, New Brunswick, NJ) and tap water were available *ad libitum* during this period.

At the end of this acclimation period, all animals were removed from the foraging apparatus and housed in a single-shoebox cage with food and water available *ad libitum*. Guide cannulae were then surgically implanted in all hamsters (see *Cannula Implantation* for details). After a 1 wk postsurgical recovery period, all hamsters were transferred back to the foraging/hoarding apparatus and baseline measures were taken.

Training and Baseline Measures

Hamsters were trained to forage for their food based on procedures previously published (Day and Bartness, 2001). In brief, hamsters were given free access to food for 2 d while they adapted to the running wheel. In addition to the free food, a 75 mg food pellet was dispensed upon completion of every 10 wheel revolutions. Wheel revolutions were counted using a magnetic detection system and monitored by a computer-based hardware/software system (Med

Associated, Lancaster, NH). On the third day, the free food condition was replaced by a response-contingent condition in which only every 10 wheel revolutions triggered the delivery of a pellet. This condition was in effect for the remaining 5 d of the 1 wk-long training period. The hamsters were then separated into 3 foraging groups that were matched for percent change in body mass and average hoard size. The three foraging groups were 10 revolutions/pellet (10 Rev), Free Wheel/Free Food (FW; food was available non-contingently [not earned]), but the running wheel was active [locomotor activity control group]) or Blocked Wheel/Free Food (BW; food was available non-contingently [not earned], but the running wheel was blocked [sedentary control group]).

Cannula Implantation

The animals were anesthetized with isoflurane and the fur at the top of the head was removed to expose the area to be incised. A guide cannula (26-gauge stainless steel; Plastics One, Roanoke, VA) was unilaterally implanted stereotaxically targeted for the PVH (AP - 0.03cm, ML -.03cm, and DV -.55cm) and for the PFA (AP -0.04 cm, ML -0.045 cm, DV -0.6 cm). Specifically, the skull was trephined at the specific coordinates and the cannula was lowered into place. The guide cannula was secured to the skull using 3/16 mm jeweler's screw and dental acrylic. A removable dummy cannula was placed into the guide cannula throughout the experiment except when it was removed for the injections.

Injection Protocol

Injections consisted of either vehicle (sterile 0.15 M NaCl) or one of three doses (0.176, 0.352, or 0.704 nmol) of NPY (American Peptide, Sunnyvale, CA) via an internal cannula (33-

gauge stainless steel, Plastics One) that penetrated below the top of the skull 0.6 cm into the PVH and 0.65 cm into the PFA. The inner cannula was connected to a microsyringe via polyethylene tubing and the injection volume for the vehicle or NPY was 100 nl. Each animal in the 3 foraging groups received all doses of NPY or its vehicle in a counterbalanced schedule to control for possible order effects of peptide administration. They were injected twice a week with a three day interval between each injection to serve as a washout period across a 2 wk period.

Two hours before the onset of the dark cycle, food was removed from the pouches of the hamsters, they were placed in clean burrow cages and access to the tubes was blocked. Animals were restrained by hand during the 30 s injection period and the injection needle remained in place ~ 30 s before withdrawal. Hamsters were returned to their respective cages and access to the tubes was reinstated. Foraging, food hoarding, and food intake were measured 1, 2, 4, and 24 h postinjection.

Food deprivation protocol

After the completion of the NPY or vehicle injection cycles and last washout period, half of the animals were fasted for 56 h. In our previous studies of food hoarding, we have used food deprivation lengths ranging from 12 to 56 h (Institutional Animals Care and Use Committee approved), with the latter length appearing somewhat severe and/or nonphysiological. In the utopian conditions of the laboratory, however, Siberian hamsters are almost 50 % body fat compared with ~25 % in nature (Weiner, 1987). Short food deprivation lengths of 12-24 h are minimally energetically challenging in these animals and stimulation of food hoarding is

minimal (Clein MR and Bartness, TJ, unpublished results). Therefore, we selected 56 h food deprivation to trigger the behavior nearly maximally.

Before refeeding, half of the food deprived and non-food deprived animals received either a Y1 antagonist (BIBO 3304; dose; gift from Boehringer Ingelheim) or vehicle control (0.15 M saline, 10 % Dimethyl sulfoxide, 2.5 % glacial acetic acid) injected unilaterally into the PVH or PFA as described previously (see *Injection Protocol* for details). Hamsters were returned to their respective cages and foraging, food hoarding and food intake were measured 1, 2, 4 and 24 h postinjection.

Cannula Verification

After the end of all injection cycles, 100 nl of methylene blue dye was injected to confirm placement of the cannula in the PVH. The animals were transcardially perfused with 0.9 % saline followed by 4 % paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were placed in fixative overnight and then transferred to a 30 % sucrose solution for 48 h. Coronal brain sections were sliced (80 μ m) using a microtome. Sections were then mounted on glass slides and stained with cresyl violet. Animals whose cannula placement was not within the PVH were considered misses and their data were not included in the analyses.

Statistical analysis

Behavioral measures were analyzed using a three-way mixed model ANOVA with repeated measures (3 x 4 x 4; foraging group x drug x time) using Number Crunching Statistical Software v 2000 (Kaysville, UT). Duncan's new multiple range tests were used for post hoc tests

when appropriate. Differences among groups were considered statistically significant if $P_s < 0.05$. Exact probabilities and test values were omitted for simplicity and clarity of the presentation.

Results

Experiment 1: Does NPY increase foraging, food hoarding or food intake in Siberian hamsters after injection into either the PVH or PFA?

Wheel Revolutions. (PVH) Wheel running in the FW group, a test for locomotor activity effects of NPY, was significantly decreased by all three doses of NPY into the PVH at 0-1 h and additional decreases by the highest dose at 2-4 h and 4-24 h compared with vehicle ($P_s < 0.05$; Fig. 2.2a). No cumulative differences were seen for NPY 0-24 h post injection compared to vehicle (Fig. 2.2a).

(PFA) Wheel running in the FW group was significantly decreased after PFA NPY injections at 1-2 h for the low dose of NPY and 2-4 h for the high dose followed by increases in wheel running for all NPY doses at 4-24 h compared with vehicle ($P_s < 0.05$; Fig. 2.2b). No cumulative differences were seen for NPY 0-24 h post injection compared to vehicle (Fig. 2.2b).

Foraging. (PVH) Foraging in the 10 Rev group was significantly decreased after PVH NPY injections at 0-1 h for all doses of NPY with additional decreases seen with the highest dose at 1-2 h and 2-4 h compared with vehicle ($P_s < 0.05$; Fig. 2.1a). There was a cumulative decrease in foraging at the highest dose of NPY 0-24 h post injection compared with vehicle ($P_s < 0.05$; Fig. 2.1a).

(PFA) Foraging in the 10 Rev group was significantly decreased after PFA NPY injections at 1-2 h for all doses of NPY followed by significant increases at 2-4 h for all doses

and 4-24 h for both the middle and high NPY doses compared with vehicle ($P_s < 0.05$; Fig. 2.1b). There was a cumulative increase in foraging with the highest NPY dose 0-24 h post injection compared with vehicle ($P_s < 0.05$; Fig. 2.1b).

Food Hoarding. (PVH) In the 10 Rev group, food hoarding was significantly increased after PVH NPY injections for all three doses of NPY at 0-1 h and 4-24 h, with additional significant increases in food hoarding at 2-4 h for both the middle and high NPY doses compared with vehicle ($P_s < 0.05$; Fig. 2.3a). There was a cumulative increase in food hoarding 0-24 h post injection for both the middle and high NPY doses compared with vehicle ($P_s < 0.05$; Fig. 2.3a). In the FW group, food hoarding was significantly increased after PVH NPY injections for all three doses across all times compared with vehicle ($P_s < 0.05$; Fig. 2.4a). There was a cumulative increase in food hoarding 0-24 h post injection for all three NPY doses compared with vehicle ($P_s < 0.05$; Fig. 2.4a). In the BW group, food hoarding was significantly increased after PVH NPY injections with all three doses of NPY at 4-24 h, with the lowest dose showing additional increases at 0-1 h and 1-2 h compared with vehicle ($P_s < 0.05$; Fig. 2.5a). There were cumulative increases for all three doses of NPY 0-24 h post injection compared with vehicle ($P_s < 0.05$; Fig. 2.5a).

(PFA) In the 10 Rev group, food hoarding was significantly increased after PFA NPY injections with all three doses of NPY at 0-1 h and 4-24 h with additional increases seen with the lowest and middle doses of NPY at 2-4 h compared with vehicle ($P_s < 0.05$; Fig. 2.3b). There was a cumulative increase in food hoarding with all three doses of NPY at 0-24 h post injection compared with vehicle ($P_s < 0.05$; Fig. 2.3b). In the FW group, food hoarding was significantly increased with all three doses of NPY at 2-4 h and 4-24 h with the middle and high doses

showing additional increases at 0-1 h and 1-2 h compared with vehicle ($P_s < 0.05$; Fig. 2.4b).

There was a cumulative increase in food hoarding for all three doses of NPY at 0-24 h compared with vehicle ($P_s < 0.05$; Fig. 2.4b). In the BW group, food hoarding was significantly increased with all three doses of NPY at 4-24 h with each dose showing additional increases at the other times compared with vehicle, but in a varied fashion. Specifically, increases in food hoarding were seen with the lowest NPY dose at 1-2 h, the middle dose at 2-4 h, and the highest dose at 0-1 h, 1-2 h, and 2-4 h compared with vehicle ($P_s < 0.05$; Fig. 2.5b). There was a cumulative increase in food hoarding for the middle and high NPY doses at 0-24 h compared with vehicle ($P_s < 0.05$; Fig. 2.5b).

Food Intake. (PVH) In the 10 Rev group, food intake was significantly increased after PVH NPY injections with all three doses of NPY at 0-1 h compared with vehicle ($P_s < 0.05$; Fig. 2.6a). In the FW and BW groups, food intake was significantly increased with all three NPY doses at 0-1 h and 1-2 h compared with vehicle ($P_s < 0.05$; Figs. 2.7a and 2.8a). There were no increases in cumulative food intake from 0-24 h for any NPY dose for any foraging group compared to vehicle (Figs. 2.6a, 2.7a, 2.8a).

(PFA) In the 10 Rev group, food intake was significantly increased after PVH NPY injections for all three doses of NPY at 2-4 h, with the low and middle doses showing additional increases at 0-1 h and the highest dose at 4-24 h compared with vehicle ($P_s < 0.05$; Fig. 2.6b). There was a cumulative increase in food intake from 0-24 h post injection with the highest NPY dose compared with vehicle ($P_s < 0.05$; Fig. 2.6b). In the FW group, food intake was significantly increased with all three doses of NPY at 0-1 hr, with the middle dose showing additional increases at 1-2 h and the highest dose at 1-2 h and 2-4 h compared with vehicle ($P_s < 0.05$; Fig.

2.7b). There were significant increases in cumulative food intake with all three NPY doses at 0-24 h post injection compared with vehicle ($P_s < 0.05$; Fig. 2.7b). In the BW group, food intake was significantly increased with all three NPY doses at 0-1 h, 1-2 h, and 2-4 h post injection followed by a significant decrease in food intake at 4-24 h compared with vehicle ($P_s < 0.05$; Fig. 2.8b). There were no cumulative differences in food intake with any NPY dose in the BW group compared to vehicle (Fig. 2.8b).

Experiment 2: Does the antagonism of NPY Y1 receptor in the PVH or PFA block post-food deprivation increases in food hoarding in Siberian hamsters?

Wheel Revolutions. (PVH) After PVH injection of the Y1 antagonist or vehicle, there was an increase in wheel running in food deprived animals compared with the non-food deprived animals at all times ($P_s < 0.05$; Fig. 2.10a). The Y1 antagonist significantly exaggerated this food deprivation-induced increase in wheel running at 0-1 h compared with their vehicle injected counterparts ($P_s < 0.05$; Fig. 2.10a). The Y1 antagonist also significantly exaggerated the post food deprivation induced-increase in cumulative wheel running 0-24 post injection compared with vehicle injected counterparts ($P_s < 0.05$; Fig. 2.10a).

(PFA) After PFA injection of the Y1 antagonist or vehicle, there was a decrease in wheel revolutions at 0-1 h, 1-2 h, and 2-4 h when food deprived animals were compared with non-food deprived animals ($P_s < 0.05$; Fig. 2.10b). The vehicle-injected-food deprived animals significantly increased wheel running at 4-24 h compared with their non-food deprived counterparts ($P_s < 0.05$; Fig. 2.10b). The Y1 antagonist significantly inhibited this post food deprivation-induced increase in wheel running at 4-24 h compared with vehicle injected counterparts ($P_s < 0.05$; Fig. 2.10b). The Y1 antagonist also significantly decreased cumulative

wheel running after food deprivation 0-24 h post injection compared with vehicle injected counterparts ($P_s < 0.05$; Fig. 2.10b).

Foraging. (PVH) After PVH injection of the Y1 antagonist or vehicle, there were food deprivation-induced increases in foraging compared with non-food deprived counterparts ($P_s < 0.05$; Fig. 2.9a). There was no difference in foraging between the food deprived/Y1 antagonist treated animals and the food deprived/vehicle treated animals at any time (Fig. 2.9a).

(PFA) After PFA injection of the Y1 antagonist or vehicle, there were food deprivation-induced increases in foraging compared with non-food deprived counterparts ($P_s < 0.05$; Fig. 2.9b). The Y1 antagonist significantly inhibited this post food deprivation-induced increase in foraging at 0-1 h and 1-2 h compared with vehicle injected counterparts ($P_s < 0.05$; Fig. 2.9b). The Y1 antagonist significantly exaggerated the post food deprivation-induced increase in foraging at 2-4 h and 4-24 h post injection, compared with the vehicle injected counterparts ($P_s < 0.05$; Fig. 2.9b).

Food hoarding. (PVH) After PVH injection of Y1 antagonist or vehicle, there were food deprivation-induced increases in food hoarding in all groups compared with non-food deprived counterparts ($P_s < 0.05$; Figs. 2.11a, 2.12a, 2.13a). The Y1 antagonist significantly inhibited this post food deprivation-induced increase in food hoarding at the 0-1h compared with vehicle injected counterparts in the 10 Rev group only ($P_s < 0.05$; Fig. 2.11a). The Y1 antagonist also decreased cumulative food hoarding at 0-24 h post injection compared with vehicle injected counterparts in the 10 Rev group ($P_s < 0.05$; Fig. 2.11a).

(PFA) After PFA injection of Y1 antagonist, there was a food deprivation-induced increase in food hoarding in the 10 Rev group compared with their non-food deprived animals ($P_s < 0.05$; Fig. 2.11b). The Y1 antagonist significantly inhibited the post-food deprivation increase in food hoarding at 0-1 h, 1-2 h, and 4-24 h compared with vehicle injected counterparts ($P_s < 0.05$; Fig. 2.11b). The Y1 antagonist significantly exaggerated the post food deprivation-induced increase in food hoarding at 2-4 h post injection compared with the vehicle injected counterparts ($P_s < 0.05$; Fig. 2.11b). In the FW group, the Y1 antagonist significantly inhibited the post food deprivation-induced increase in food hoarding at all times compared with vehicle injected counterparts ($P_s < 0.05$; Fig. 2.12b). In the BW group, the Y1 antagonist significantly inhibited the post food deprivation-induced increase in food hoarding at 0-1 h and 2-4 h compared with the vehicle injected counterparts ($P_s < 0.05$; Fig. 2.13b). The Y1 antagonist significantly inhibited the cumulative post food deprivation-induced increase in food hoarding 0-24 h post injection compared with vehicle injected counterparts ($P_s < 0.05$; Fig. 2.13b).

Food intake. (PVH) After PVH injection of the Y1 antagonist or vehicle, there were food deprivation-induced increases in food intake in all groups compared with non-food deprived animals ($P_s < 0.05$; Fig. 2.14a, 2.15a, 2.16a). In the 10 Rev group, the Y1 antagonist significantly exaggerated this post food deprivation-induced increase in food hoarding at 1-2 h and 2-4 h, with a significant cumulative increase in food intake compared with the vehicle injected counterparts ($P_s < 0.05$; Fig. 2.14a). In the FW group, the Y1 antagonist significantly inhibited this post food deprivation-induced increase in food intake at 0-1 h compared with vehicle injected counterparts ($P_s < 0.05$; Fig. 2.15a). The Y1 antagonist significantly decreased cumulative food intake compared with the vehicle injected counterparts ($P_s < 0.05$; Fig. 2.15a). In the BW group, the Y1

antagonist significantly inhibited the post-food deprivation increase in food intake at 0-1 h, but no cumulative difference in food intake was seen 0-24 h post injection compared to vehicle injected counterparts ($P_s < 0.05$; Fig. 2.16a).

(*PFA*) After PFA injection of the Y1 antagonist or vehicle, there was a post food deprivation-induced increase in food intake in the 10 Rev group compared with non-food deprived animals ($P_s < 0.05$; Fig. 2.14b). There was no effect of the Y1 antagonist on this post food deprivation-induced increase in food intake compared to vehicle injected counterparts (Fig. 2.14b). In the FW group, there was a post food deprivation-induced increase in food intake at 0-1 h post injection compared with non-food deprived animals ($P_s < 0.05$; Fig. 2.15b). The Y1 antagonist significantly inhibited the post food deprivation-induced increase in food intake at the 1-2 h compared with vehicle injected counterparts ($P_s < 0.05$; Fig. 2.15b). In the BW group, the Y1 antagonist significantly inhibited the post-food deprivation increase in food intake at the 0-1 h and 1-2 h followed by additional decreases in food intake at 2-4 h and 4-24 h compared with vehicle injected counterparts ($P_s < 0.05$; Fig. 2.16b). In the BW group, the Y1 antagonist significantly decreased cumulative food intake 0-24 h post injection compared with vehicle injected counterparts ($P_s < 0.05$; Fig. 2.16b).

Discussion

The major findings of the present experiment were that: a) NPY injected into the PVH or PFA increases food hoarding to a greater extent than foraging or food intake b) the Y1 receptor appears to play a role in controlling food hoarding in both the PVH and PFA based on the ability of the Y1 receptor antagonist to inhibit post food deprivation increases in food hoarding and

c) the Y1 receptor plays an additional role in controlling foraging in the PFA based on the ability of the Y1 receptor antagonist to inhibit post food deprivation increases in foraging.

The greater increases in food hoarding than foraging or food intake after injections of NPY into the PVH and PFA mimic the result seen after third ventricular injections of NPY in Siberian hamsters (Day et al., 2005). In fact, the increase in food hoarding averaging ~200-800 % across all time points after PVH and PFA NPY injections are the same as the increases seen after third ventricular injections (Day et al., 2005). Because NPY injected into other areas of the hypothalamus (*eg.* dorsomedial hypothalamus, medial preoptic area, lateral hypothalamus and ventromedial hypothalamus) do not produce the same robust increases in food hoarding in Siberian hamsters (unpublished observations), these two loci may be the primary mediators of the effect of NPY on food hoarding. Although NPY had the same effect of increasing food hoarding in the PVH and PFA, there was a differential effect of NPY on foraging and food intake. NPY decreased foraging ~25-50 % across all time points after injections into the PVH, whereas PFA NPY increased foraging ~50-400 % at later time points. NPY PVH injections increased food intake ~200-300 % only at the earliest time point, whereas NPY PFA injections increased food intake ~25-600 % across time. It is not known why there is this differential effect of NPY on foraging and food intake between the PVH and PFA, but this could be explained by NPY stimulating different Y receptor subtypes in each area that may then result in varying downstream effects on appetitive and consummatory ingestive behaviors. This idea is supported by the finding that a Y1 agonist injected into the third ventricle of Siberian hamsters increases food hoarding, whereas the a Y5 agonist increases food intake (Day et al., 2005). The PVH and PFA express four of the five receptor subtypes that have been cloned for NPY, the Y1, Y2, Y4 and Y5 receptors (Campbell, French-Mullen, 2001). Therefore, there may be a differential

expression of Y receptor subtypes within the PVH or PFA that when stimulated by NPY would result in varying ingestive behaviors.

The Y1 antagonist significantly inhibited post-food deprivation induced increases in food hoarding at early time points after injection into both the PVH and PFA. It was only in the PFA, however, that there was a release from the inhibition of food hoarding at later times and no cumulative effects on food hoarding 0-24 h after injection of the Y1 antagonist or vehicle. The Y1 antagonist also inhibited the post food deprivation-induced increase in foraging at early time points after injection into the PFA, but not into the PVH, with a release from this inhibition in foraging at later time points. The release of inhibition of foraging and food hoarding seen after injections of the Y1 antagonist into the PFA, but not the PVH, indicates that the Y1 receptor may mediate different responses to NPY between the two sites. This is further supported by the finding that NPY injected into the PVH decreases foraging and the Y1 antagonist in the PVH has no effect on foraging. In addition, the Y1 antagonist increased food intake in the PVH with no effect in the PFA. Therefore, the Y1 receptor may mediate different ingestive responses within the two nuclei.

A single injection of NPY into either the PVH or PFA is able to induce hamsters to hoard in 1 h what they normally would hoard in a 24 h period and the Y1 antagonist is able to almost completely inhibit the post food deprivation-induced increase in food hoarding at 1 h. Although foraging and food intake also were affected by injections of NPY into the PVH and PFA, the effect was not as robust as the increases in food hoarding. Food hoarding was the only behavior in all three foraging groups (10 Rev, FW and BW) that was stimulated by NPY over the 24 h period. Because human food hoarding is an important ingestive component to overall energy balance and NPY and the Y1 receptor show robust effects on food hoarding in this study, NPY

and the Y1 receptor in humans may provide an additional target for pharmaceutical manipulation that could result in an alteration of ingestive behaviors that leads to positive energy balance.

Most previous laboratory investigations of energy balance include unlimited access to food placed in close proximity to the animal. Under these utopian conditions, there is an artificial restriction on expressing important appetitive ingestive behaviors of foraging and food hoarding. Foraging alone can alter the energetic strategy of animals. For example, food hoarding is increased by low levels of energy expenditure and reduced to control levels with higher foraging efforts in Siberian hamsters (Day and Bartness, 2001). Therefore, the additional requirement of animals to forage for their food appears to be important to understanding how animals allocate their time and energy in order to fulfill their energy needs. In the present study, the requirement of animals to forage for their food altered the effect of NPY on all three behaviors measured. Specifically, NPY in the PVH and PFA produced increases in food hoarding to a lesser degree in the 10 Rev (foraging) group than the FW group. After injections of NPY into the PVH, there also was a reduced increase in food intake in the foraging group compared with the FW. After injections of NPY into the PFA, food intake was increased to a greater extent in the foraging group than the FW group. In the BW group, the sedentary control condition (no access to a running wheel), the effect of NPY on food hoarding and food intake was not as great as either the 10 Rev or FW groups. Because NPY decreased foraging in the 10 Rev group, the animals in this group had less pellets available to them to allocate to hoarding or eating, compared with the free food available to the FW group. This further supports the notion that foraging for food does alter the amount of food either hoarded or eaten, an effect seen in previous studies of Siberian hamsters (Day and Bartness, 2001). Because humans show differences in foraging behavior based on different physiological conditions and foraging affects

the amount of food hoarded or eaten, it is necessary to include a foraging component in studies of appetitive and consummatory ingestive behaviors.

Collectively, the present study provides evidence supporting a role of NPY in the PVH and PFA in controlling the appetitive ingestive behaviors of foraging and food hoarding, in addition to its known role in controlling food intake. These data also show that NPY and the Y1 receptor mediate more robust changes in food hoarding, than foraging or food intake. In addition, this study further supports the use of models that allow for the expression of both appetitive and consummatory ingestive behaviors because, as evidenced here, new roles for neuropeptides involved in ingestive behaviors may be found if the proper model is applied.

Figure Captions

Figure 2.1. Mapping of the NPY-PVH injections for the 10 Revolutions/pellet (10 Rev) group with the corresponding greatest increases/decreases in foraging (a), food hoarding (b) and food intake (c).

Figure 2.2. Mapping of the NPY-PVH injections for the Free Wheel (FW) group with the corresponding greatest increases/decreases in wheel revolutions (a), food hoarding (b) and food intake (c).

Figure 2.3. Mapping of the NPY-PVH injections for the Blocked Wheel (BW) group with the corresponding greatest increases/decreases in food hoarding (a) and food intake (b).

Figure 2.4. Mapping of the misses for NPY-PVH injections with the corresponding greatest increases/decreases in food hoarding (a) and food intake (b).

Figure 2.5. Mapping of the NPY-PFA injections for the 10 Revolutions/pellet (10 Rev) group with the corresponding greatest increases/decreases in foraging (a), food hoarding (b) and food intake (c).

Figure 2.6. Mapping of the NPY-PFA injections for the Free Wheel (FW) group with the corresponding greatest increases/decreases in wheel revolutions (a), food hoarding (b) and food intake (c).

Figure 2.7. Mapping of the NPY-PFA injections for the Blocked Wheel (BW) group with the corresponding greatest increases/decreases in food hoarding (a) and food intake (b).

Figure 2.8. Mapping of the misses for the NPY-PFA injections with the corresponding greatest increases/decreases in food hoarding (a) and food intake (b).

Figure 2.9. Mapping of the Y1 antagonist injections into the PVH for the 10 Revolutions/pellet (10 Rev) group with the corresponding greatest increases/decreases in foraging (a), food hoarding (b) and food intake (c).

Figure 2.10. Mapping of the Y1 antagonist injections into the PVH for the Free Wheel (FW) group with the corresponding greatest increases/decreases in wheel revolutions (a), food hoarding (b) and food intake (c).

Figure 2.11. Mapping of the Y1 antagonist injections into the PVH for the Blocked Wheel (BW) group with the corresponding greatest increases/decreases in food hoarding (a) and food intake (b).

Figure 2.12. Mapping of the Y1 antagonist injections into the PFA for the 10 Revolutions/pellet (10 Rev) group with the corresponding greatest increases/decreases in foraging (a), food hoarding (b) and food intake (c).

Figure 2.13. Mapping of the Y1 antagonist injections into the PFA for the Free Wheel (FW) group with the corresponding greatest increases/decreases in wheel revolutions (a), food hoarding (b) and food intake (c).

Figure 2.14. Mapping of the Y1 antagonist injections into the PFA for the Blocked Wheel (BW) group with the corresponding greatest increases/decreases in food hoarding (a) and food intake (b).

Figure 2.15. Mean \pm SEM percent change in foraging for the 10 Revolutions/pellet (10 Rev) group after injections of NPY into the PVH (a) or PFA (b). *Ps<0.05 compared with vehicle control.

Figure 2.16. Mean \pm SEM percent change in wheel revolutions for the Free Wheel (FW) group after injections of NPY into the PVH (a) or PFA (b). *Ps<0.05 compared with vehicle control.

Figure 2.17. Mean \pm SEM percent change in food hoarding for the 10 Revolutions/pellet (10 Rev) group after injections of NPY into the PVH (a) or PFA (b). *Ps<0.05 compared with vehicle control.

Figure 2.18. Mean \pm SEM percent change in food hoarding for the Free Wheel (FW) group after injections of NPY into the PVH (a) or PFA (b). *Ps<0.05 compared with vehicle control.

Figure 2.19. Mean \pm SEM percent change in food hoarding for the Blocked Wheel (BW) group after injections of NPY into the PVH (a) or PFA (b). *Ps<0.05 compared with vehicle control.

Figure 2.20. Mean \pm SEM percent change in food intake for the 10 Revolutions/pellet (10 Rev) group after injections of NPY into the PVH (a) or PFA (b). *Ps<0.05 compared with vehicle control.

Figure 2.21. Mean \pm SEM percent change in food intake for the Free Wheel (FW) group after injections of NPY into the PVH (a) or PFA (b). *Ps<0.05 compared with vehicle control.

Figure 2.22. Mean \pm SEM percent change in food intake for the Blocked Wheel (BW) group after injections of NPY into the PVH (a) or PFA (b). *Ps<0.05 compared with vehicle control.

Figure 2.23. Mean \pm SEM percent change in foraging for the 10 Revolutions/pellet (10 Rev) group after injection of a Y1 antagonist into the PVH (a) or PFA (b). Values are means \pm SEM. *Ps<0.05 compared with non-food deprived controls.

Figure 2.24. Mean \pm SEM percent change in wheel revolutions for the Free Wheel (FW) group after injection of a Y1 antagonist into the PVH (a) or PFA (b). *Ps<0.05 compared with non-food deprived controls.

Figure 2.25. Mean \pm SEM percent change in food hoarding for the 10 Revolutions/pellet (10 Rev) group after injection of a Y1 antagonist into the PVH (a) or PFA (b). * $P_s < 0.05$ compared with non-food deprived controls.

Figure 2.26. Mean \pm SEM percent change in food hoarding for the Free Wheel (FW) group after injection of a Y1 antagonist into the PVH (a) or PFA (b). * $P_s < 0.05$ compared with non-food deprived controls.

Figure 2.27. Mean \pm SEM percent change in food hoarding for the Blocked Wheel (BW) group after injection of a Y1 antagonist into the PVH (a) or PFA (b). * $P_s < 0.05$ compared with non-food deprived controls.

Figure 2.28. Mean \pm SEM percent change in food intake for the 10 Revolutions/pellet (10 Rev) group after injection of a Y1 antagonist into the PVH (a) or PFA (b). * $P_s < 0.05$ compared with non-food deprived controls.

Figure 2.29. Mean \pm SEM percent change in food intake for the Free Wheel (FW) group after injection of a Y1 antagonist into the PVH (a) or PFA (b). * $P_s < 0.05$ compared with non-food deprived controls.

Figure 2.30. Mean \pm SEM percent change in food intake for the Blocked Wheel (BW) group after injection of a Y1 antagonist into the PVH (a) or PFA (b). * $P_s < 0.05$ compared with non-food deprived controls.

Figure 2.1a

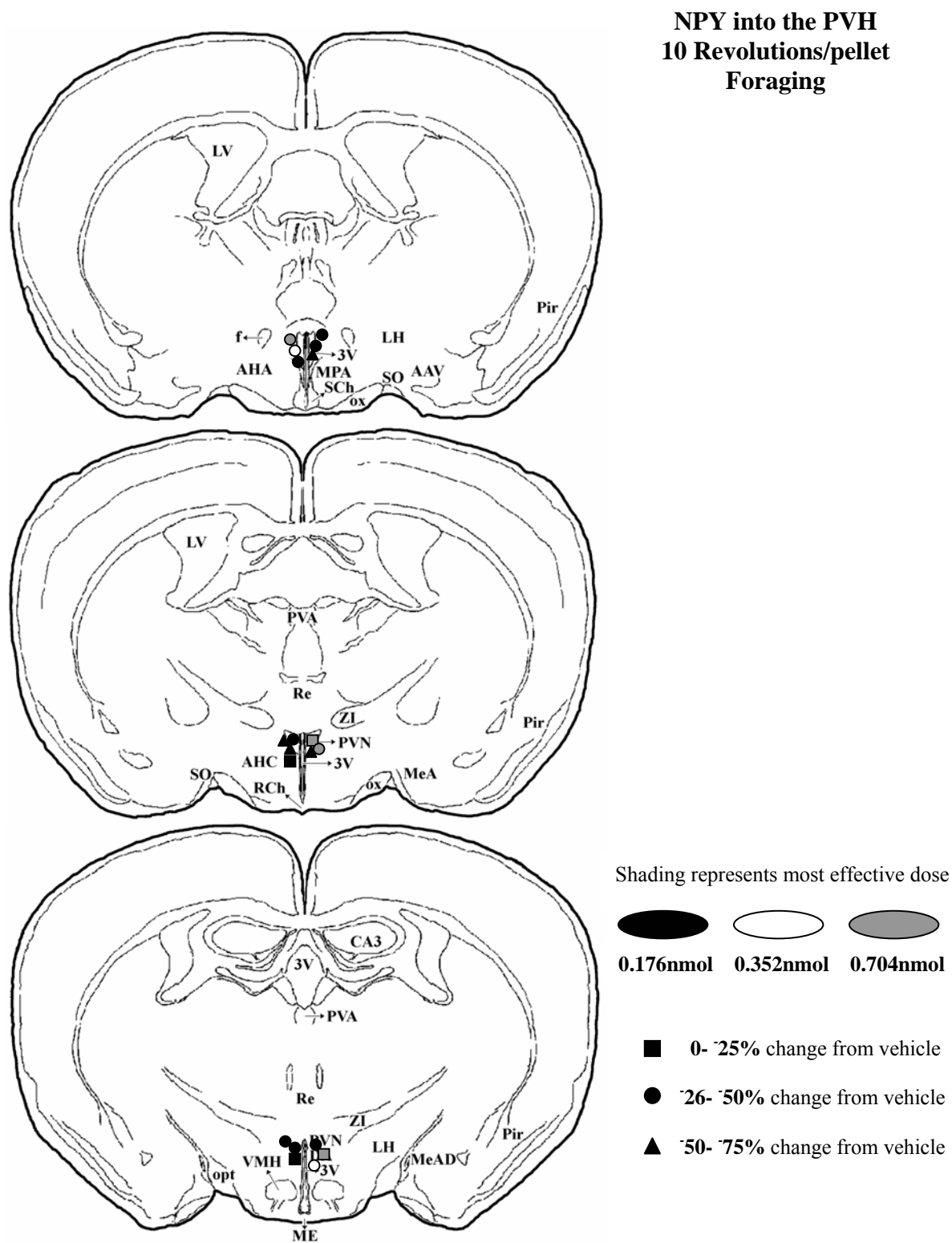
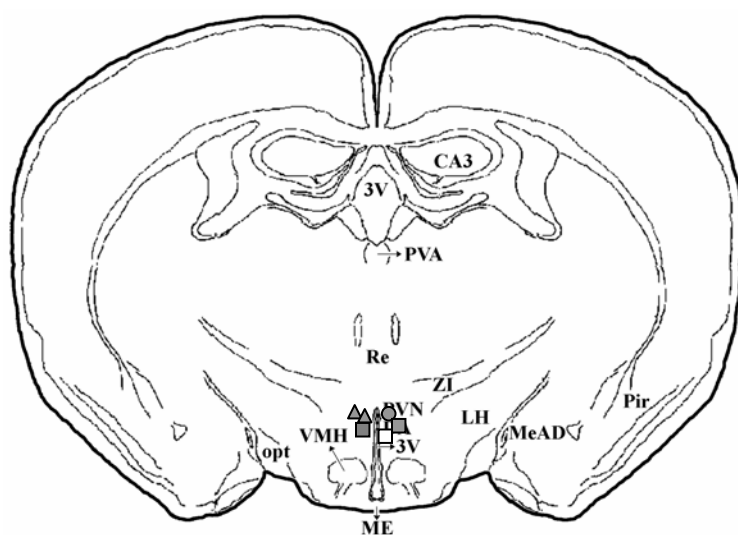
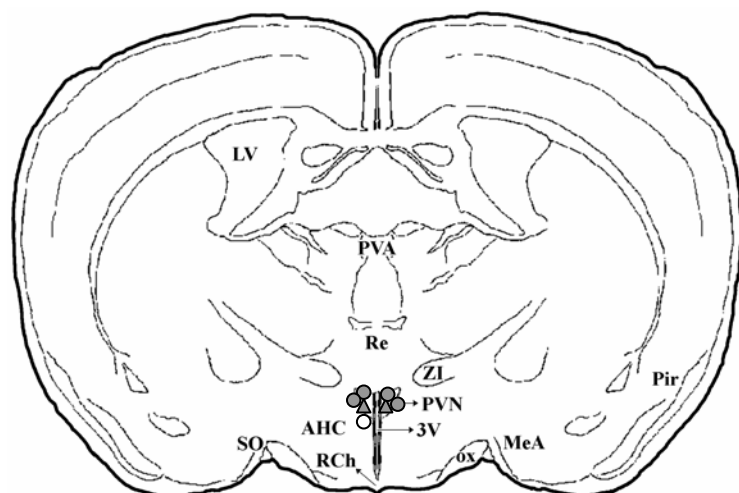
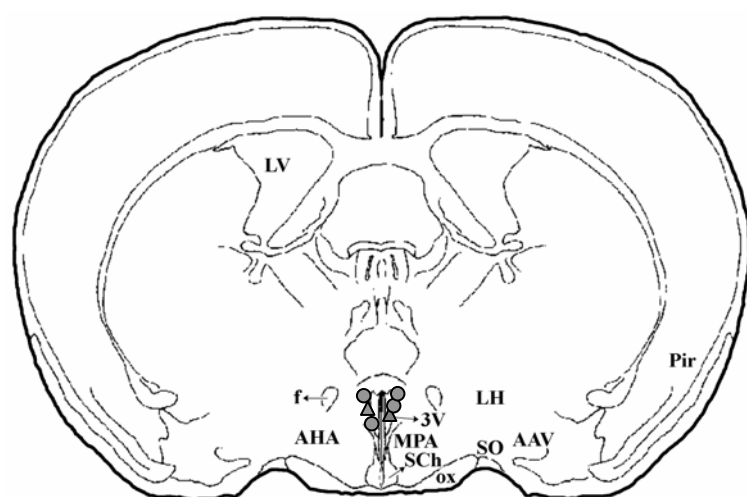





Figure 2.1b



NPY into the PVH
10 Revolutions/pellet
Food Hoard

Shading represents most effective dose




 0.176nmol 0.352nmol 0.704nmol

■ 200-500% change from vehicle

● 501-800% change from vehicle

▲ 801-1100% change from vehicle

Figure 2.1c

NPY into the PVH
10 Revolutions/pellet
Food Intake

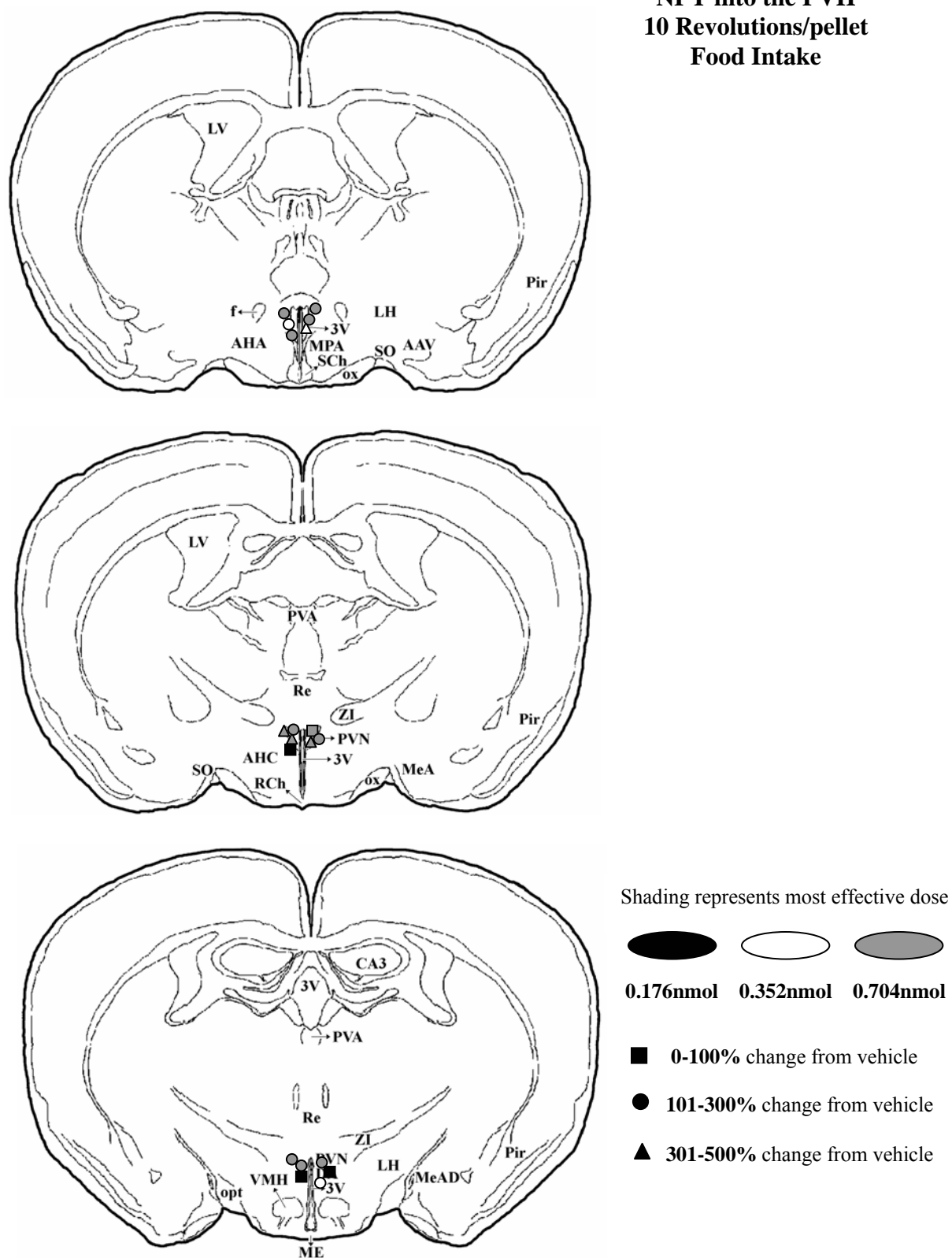
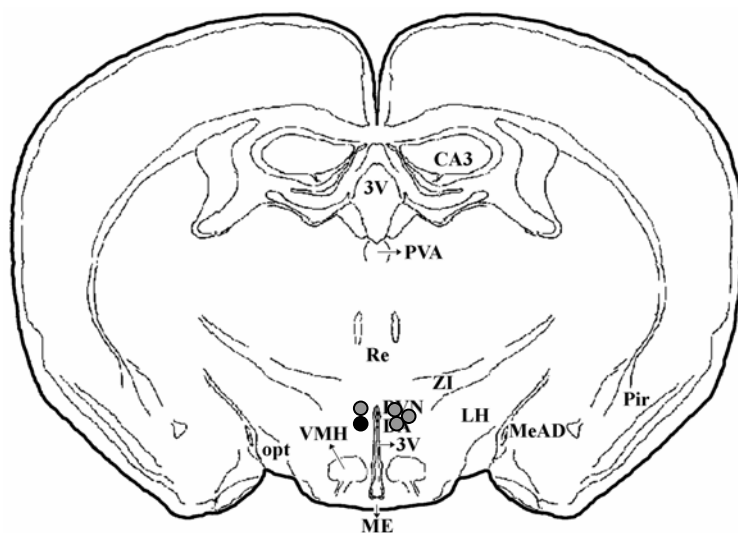
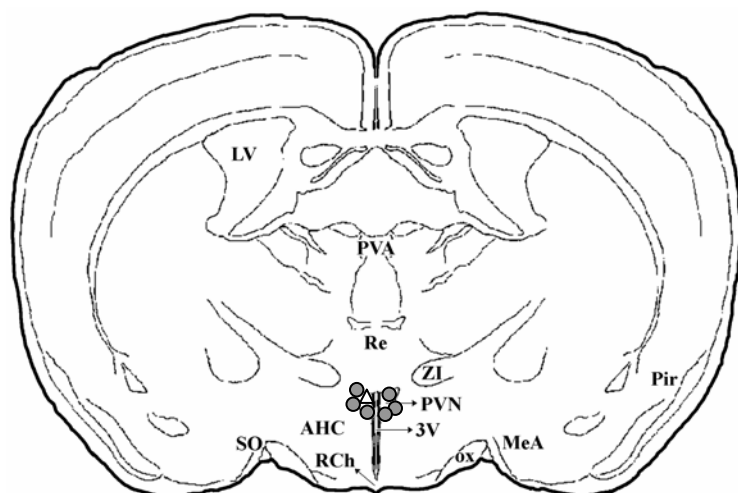
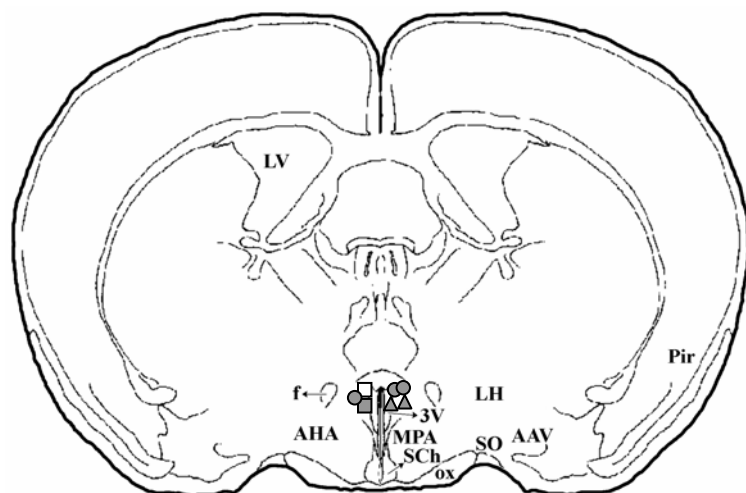


Figure 2.2a

NPY into the PVH
Free Wheel
Wheel Revolutions



Shading represents most effective dose

0.176nmol 0.352nmol 0.704nmol

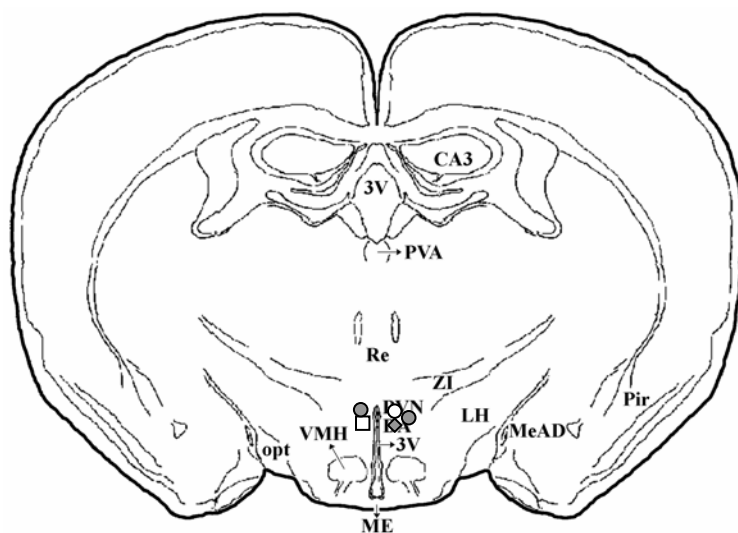
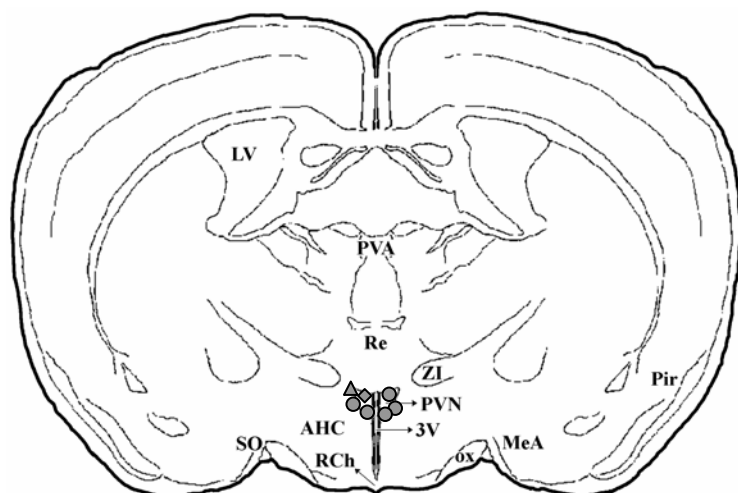
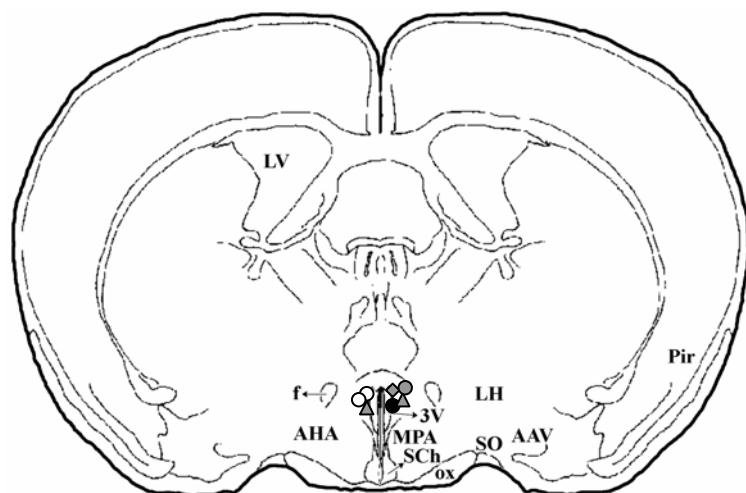
■ 0-25% change from vehicle

● 26-50% change from vehicle




▲ 51-75% change from vehicle

Figure 2.2b

NPY into the PVH
Free Wheel
Food Hoard



Shading represents most effective dose




 0.176nmol 0.352nmol 0.704nmol

■ 100-500% change from vehicle

● 501-1000% change from vehicle

▲ 1001-1500% change from vehicle

◆ 1501%-2000% change from vehicle

Figure 2.2c

NPY into the PVH
Free Wheel
Food Intake

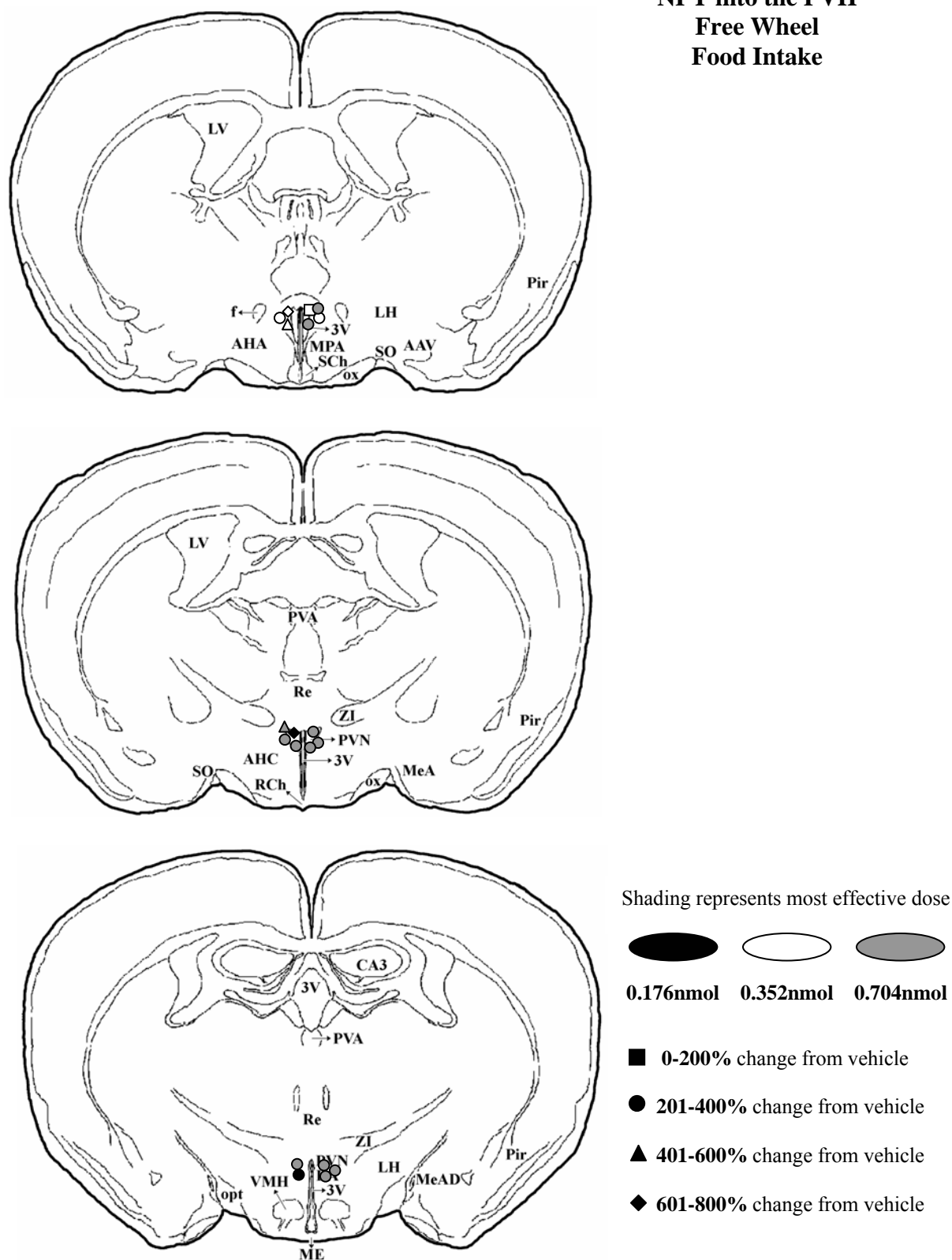


Figure 2.3a

NPY into the PVH
Blocked Wheel
Food Hoard

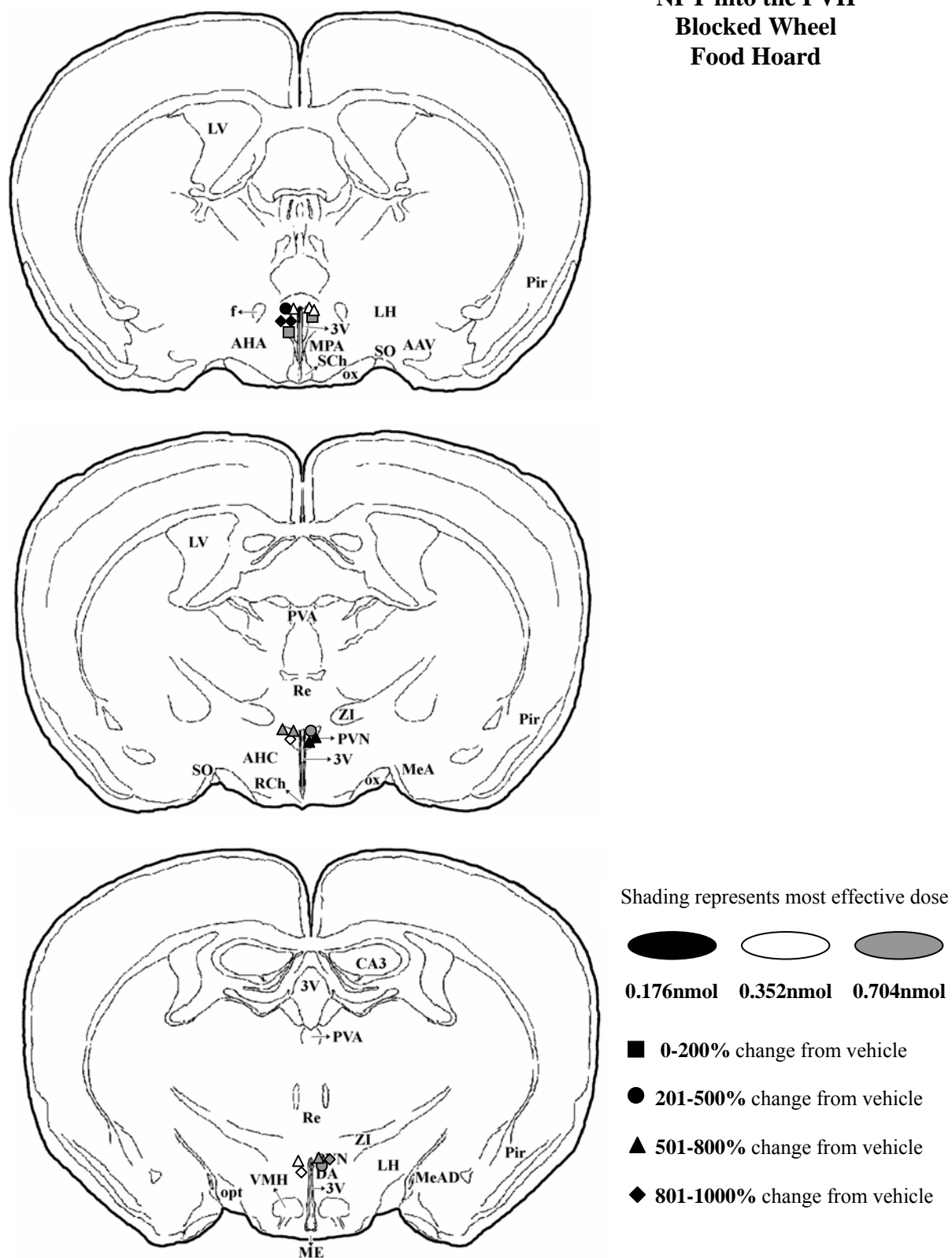


Figure 2.3b

NPY into the PVH
Blocked Wheel
Food Intake

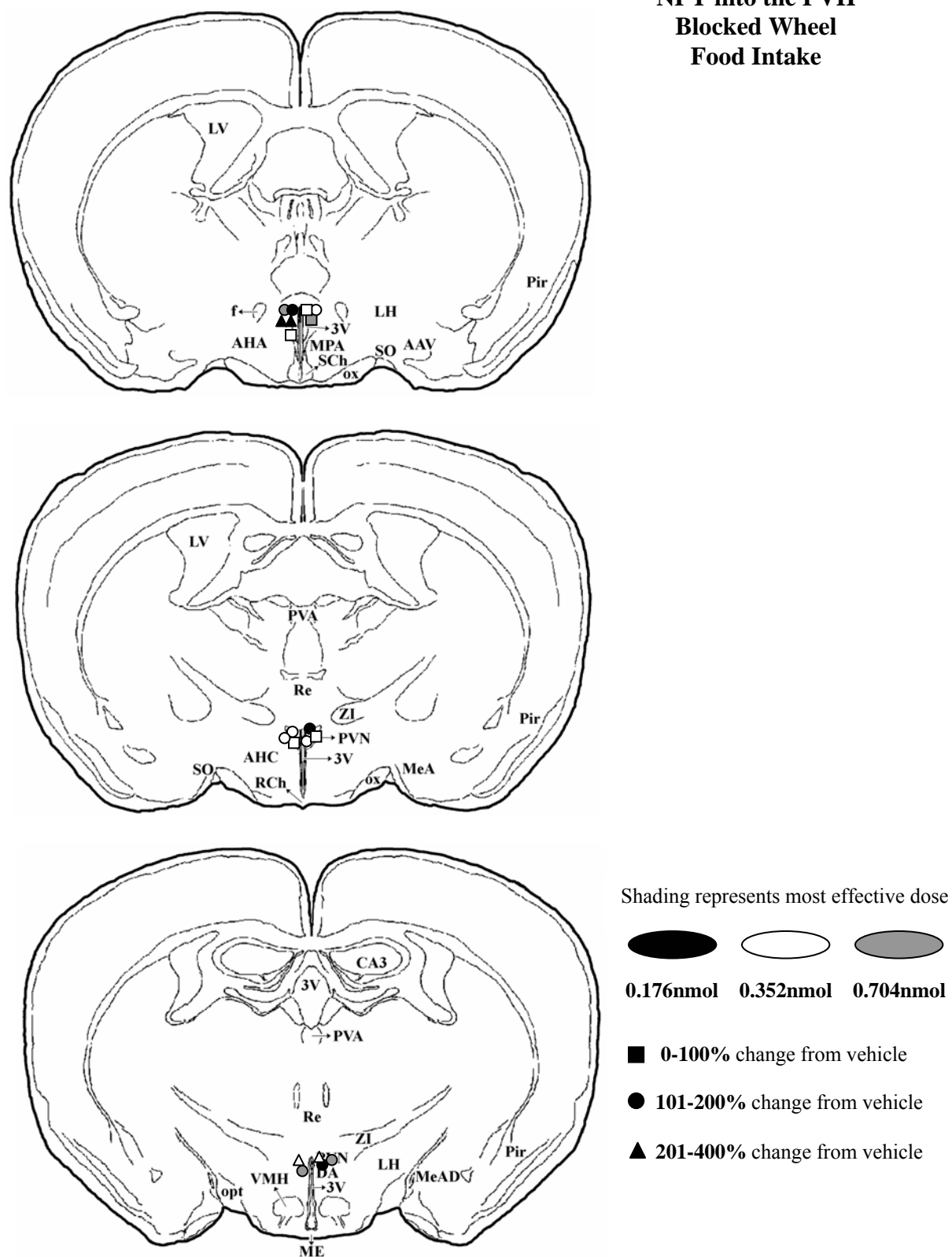
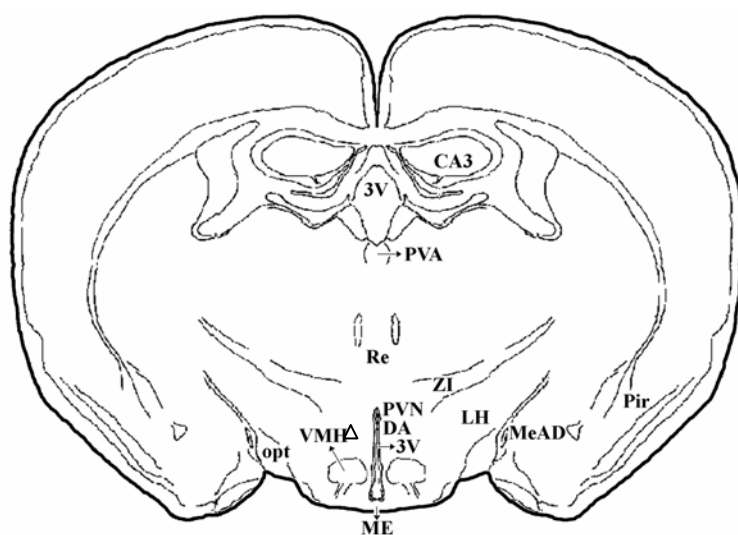
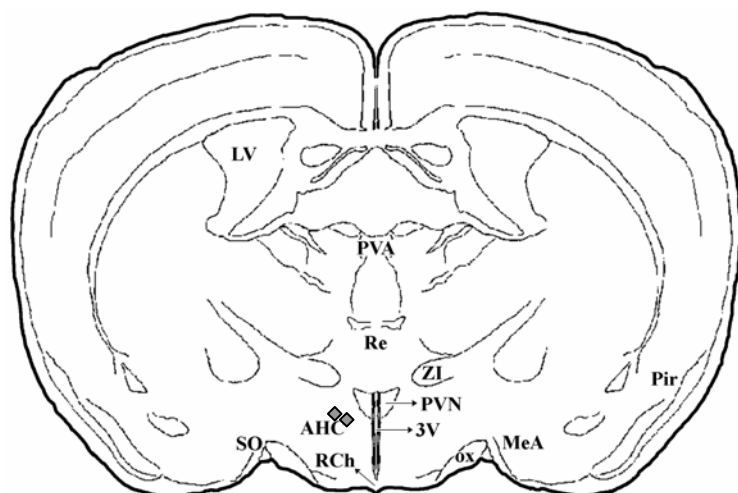
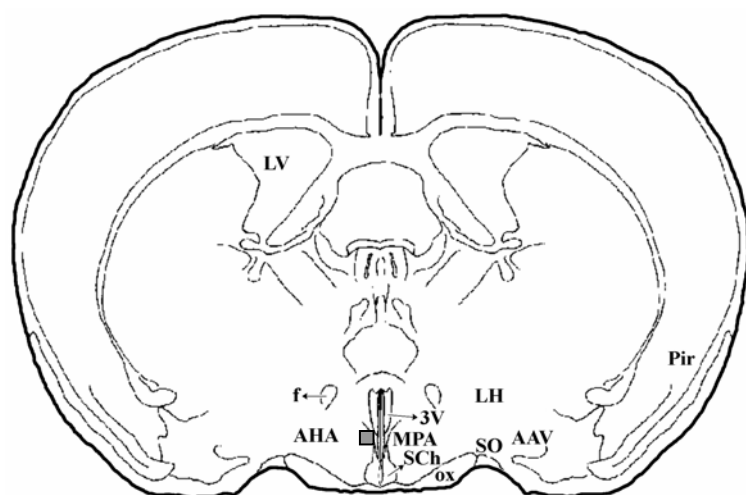





Figure 2.4a

NPY into the PVH
Misses
Food Hoard



Shading represents most effective dose




 0.176nmol 0.352nmol 0.704nmol

■ Below 0% change from vehicle
 ● 1000-2000% change from vehicle
 ▲ 2001-3000% change from vehicle
 ◆ Above 3001% change from vehicle

Figure 2.4b

NPY into the PVH
Misses
Food Intake

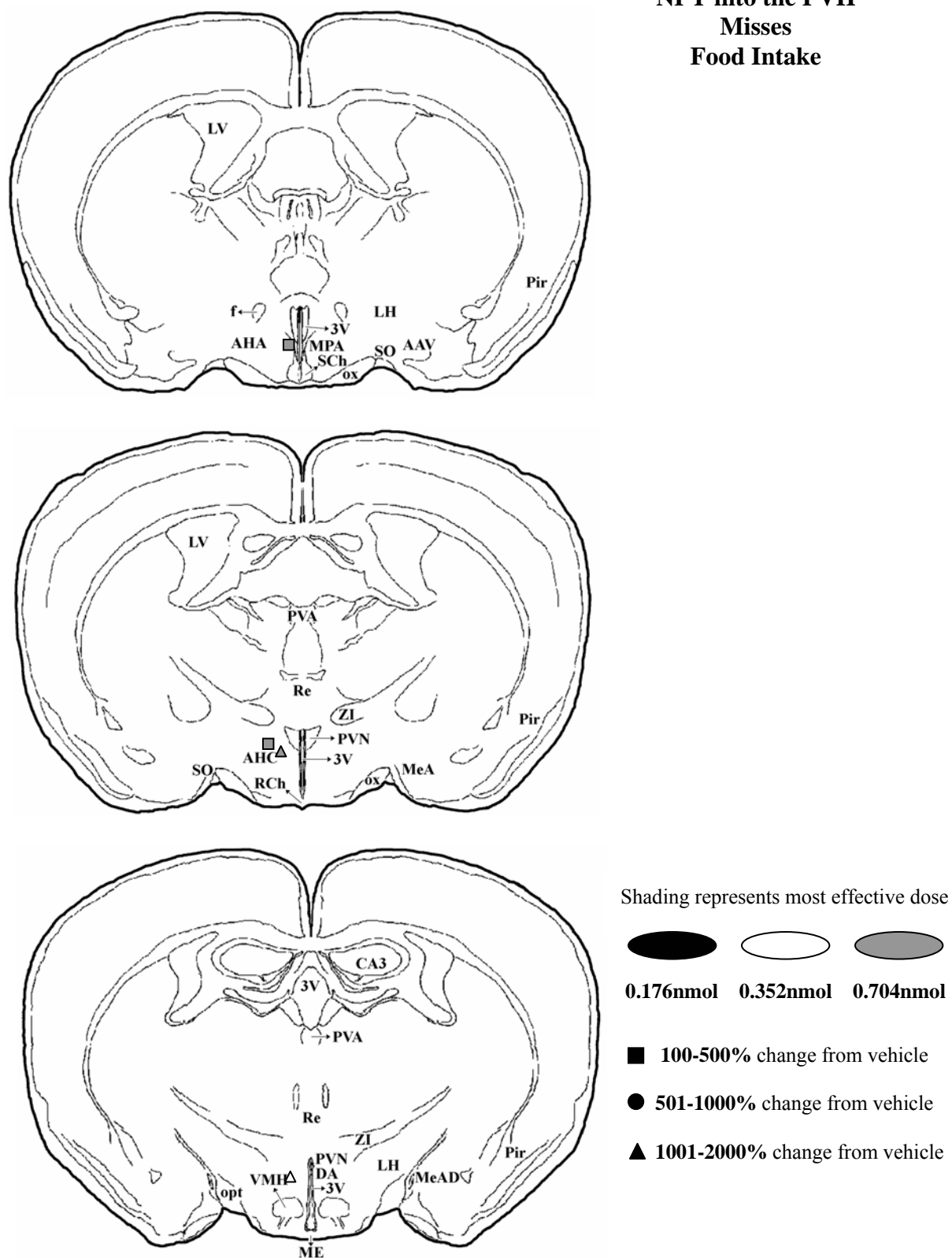
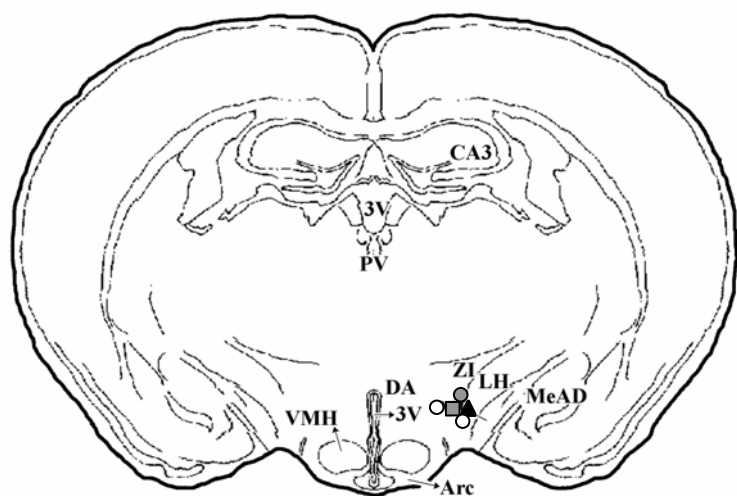
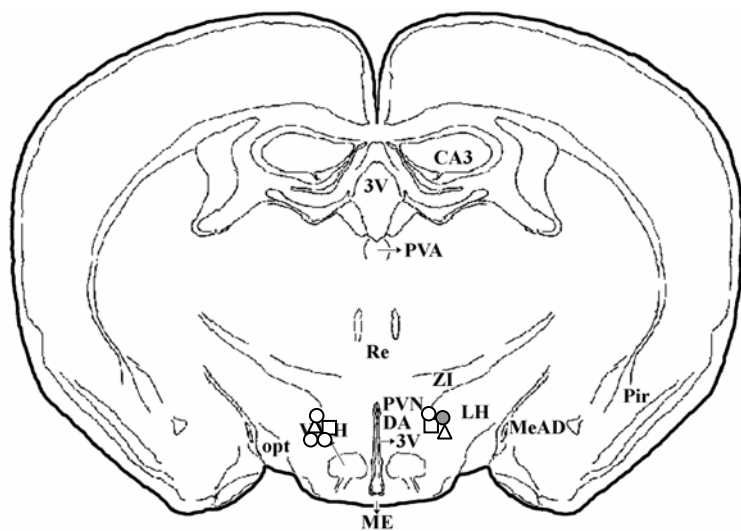
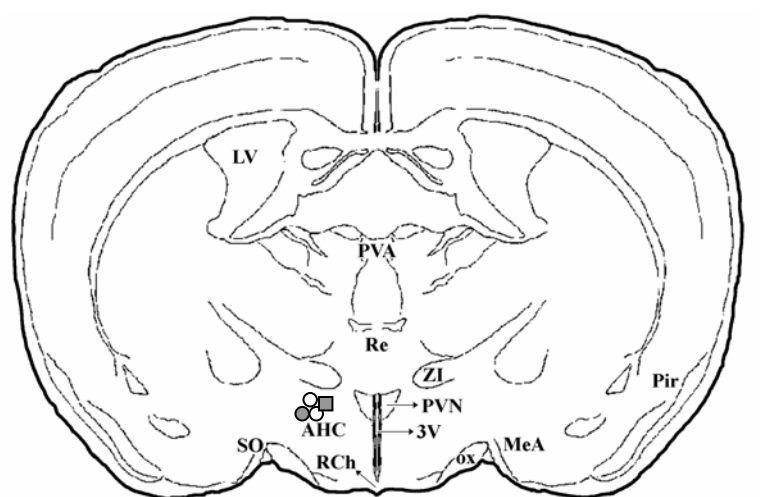


Figure 2.5a



NPY into the PFA
10 Revolutions/pellet
Foraging

Shading represents most effective dose



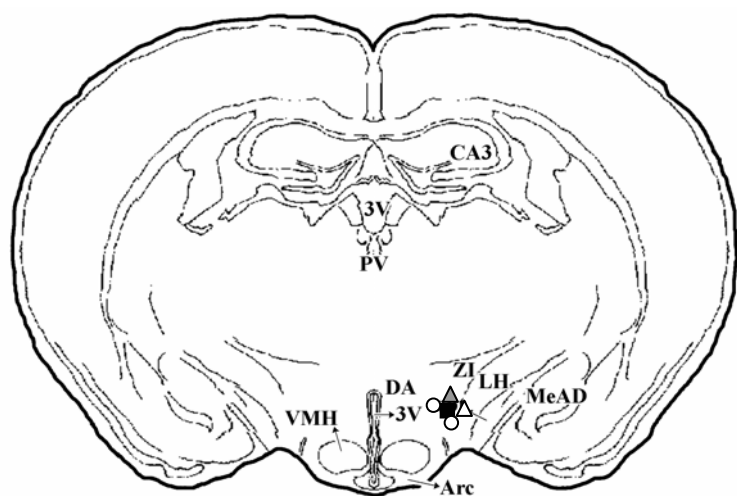
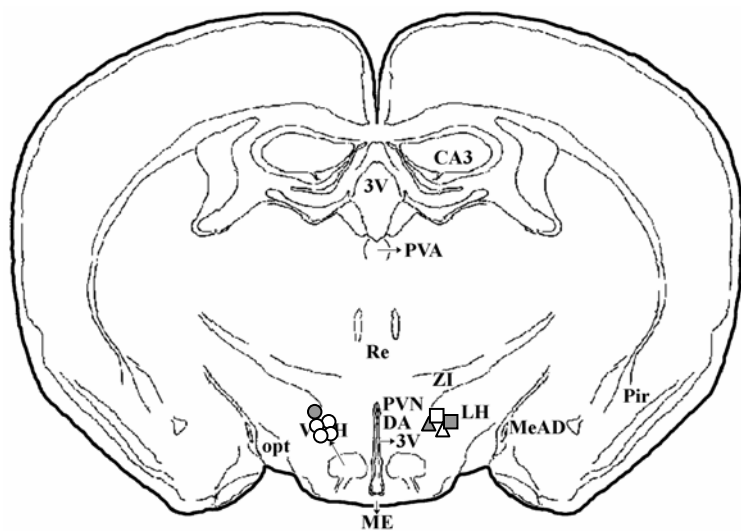
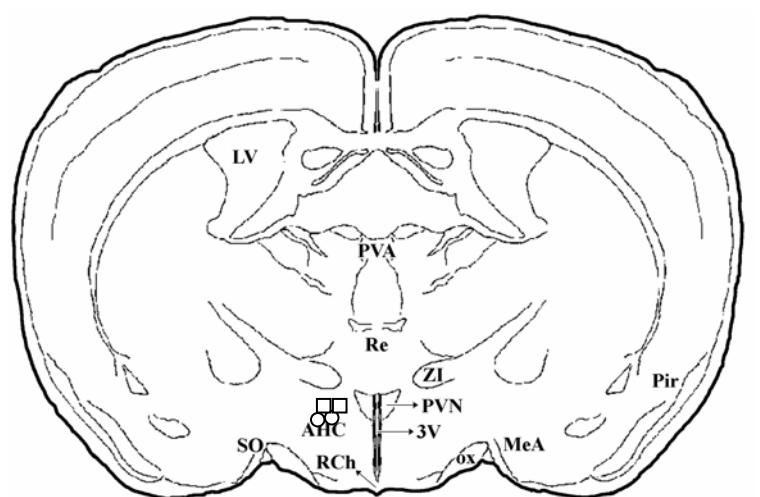
0.176nmol 0.352nmol 0.704nmol

■ 100-500% change from vehicle

● 501-1000% change from vehicle

▲ 1001-2000% change from vehicle

Figure 2.5b



NPY into the PFA
10 Revolutions/pellet
Food Hoard

Shading represents most effective dose



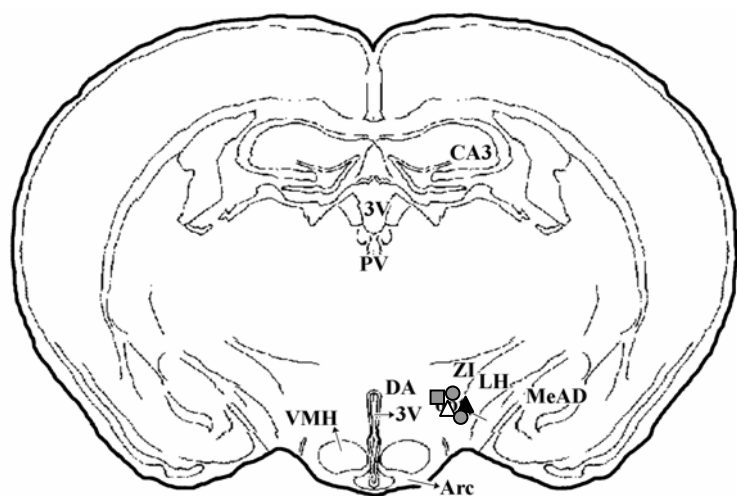
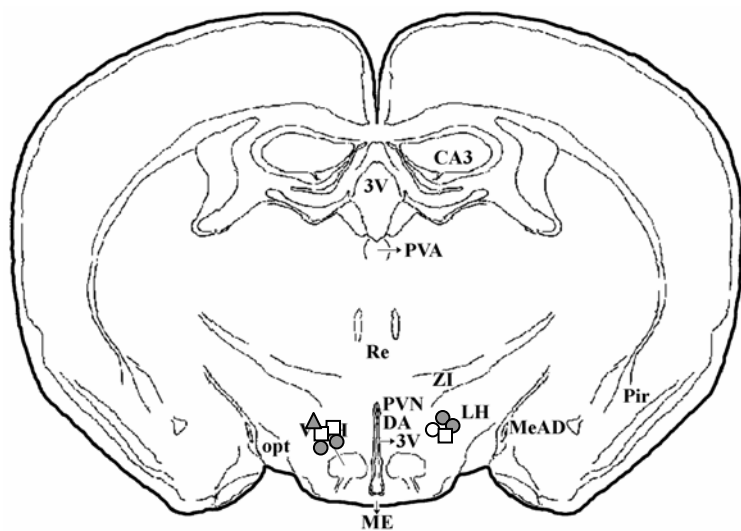
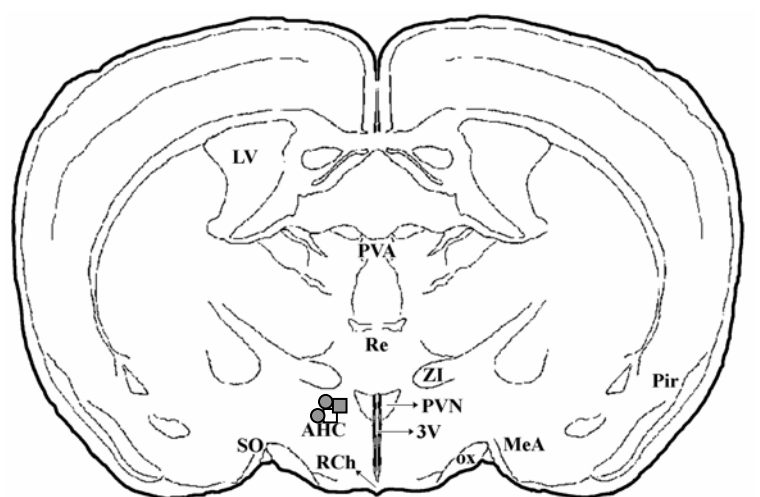
0.176nmol 0.352nmol 0.704nmol

■ 200-400% change from vehicle

● 401-600% change from vehicle

▲ 601-800% change from vehicle

Figure 2.5c



NPY into the PFA
10 Revolutions/pellet
Food Intake

Shading represents most effective dose



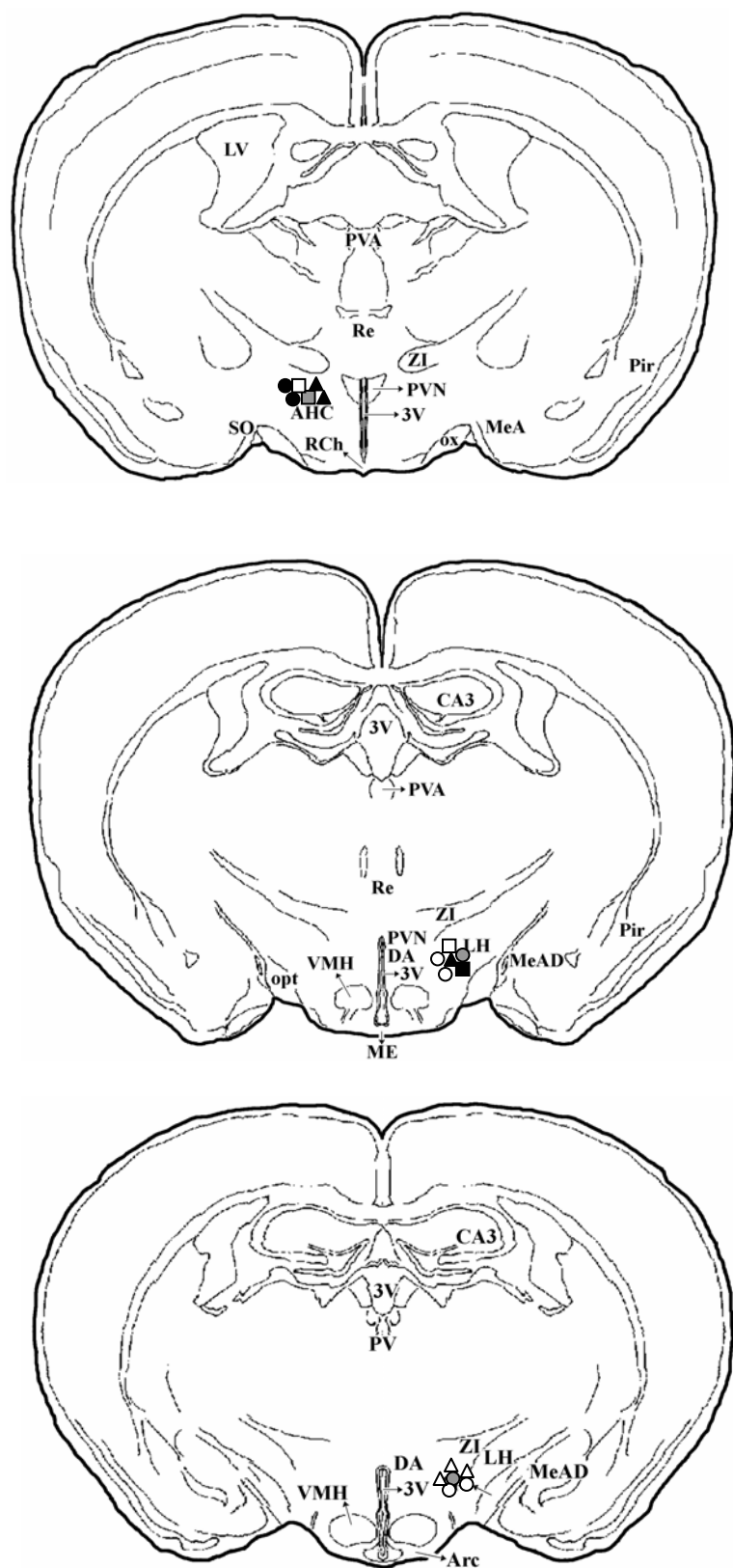
0.176nmol 0.352nmol 0.704nmol

■ 200-400% change from vehicle

● 401-600% change from vehicle

▲ 601-800% change from vehicle

Figure 2.6a



NPY into the PFA Free Wheel Wheel Revolutions

Shading represents most effective dose



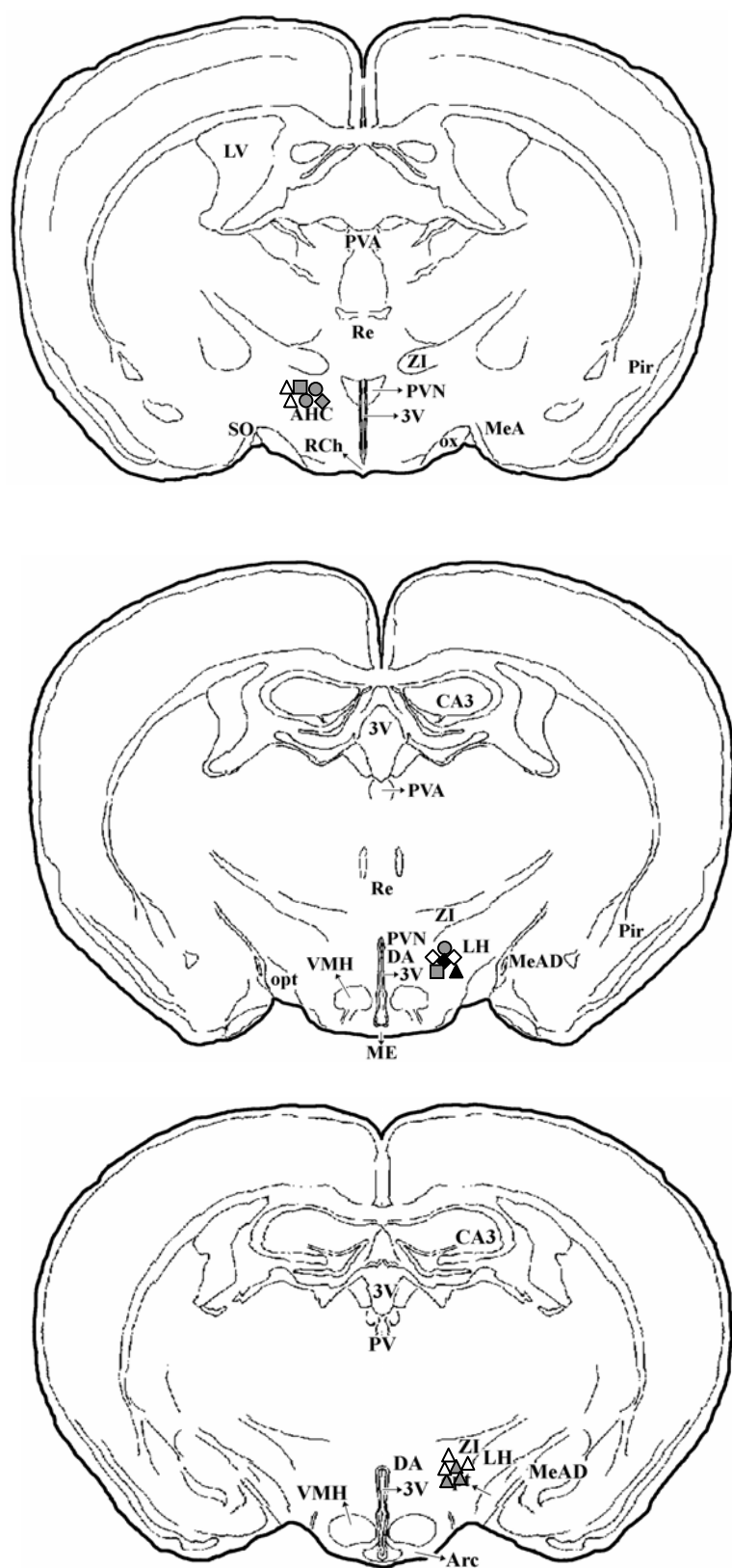
0.176nmol 0.352nmol 0.704nmol

■ 100-200% change from vehicle

● 201-400% change from vehicle

▲ 401-600% change from vehicle

Figure 2.6b



NPY into the PFA
Free Wheel
Food Hoard

Shading represents most effective dose



0.176nmol 0.352nmol 0.704nmol

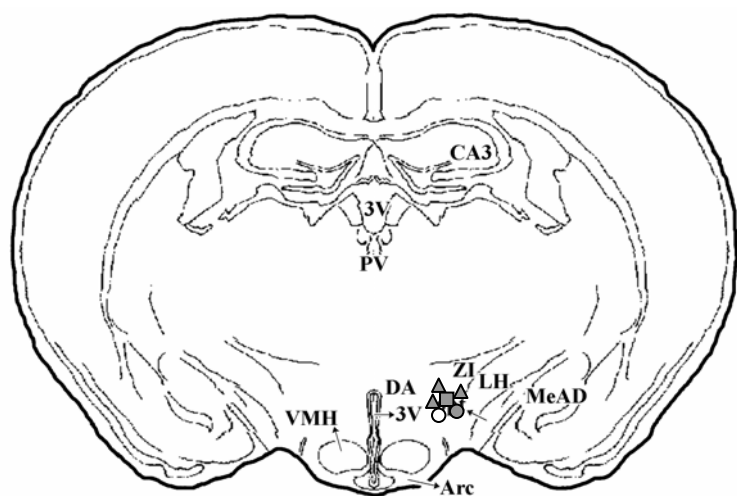
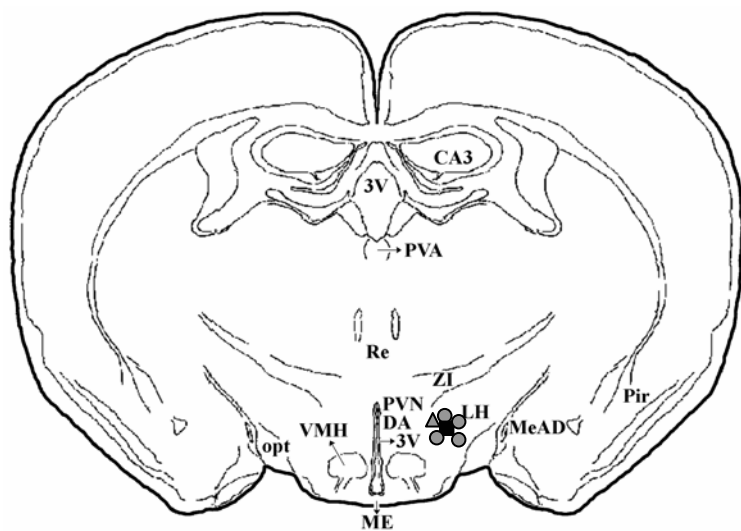
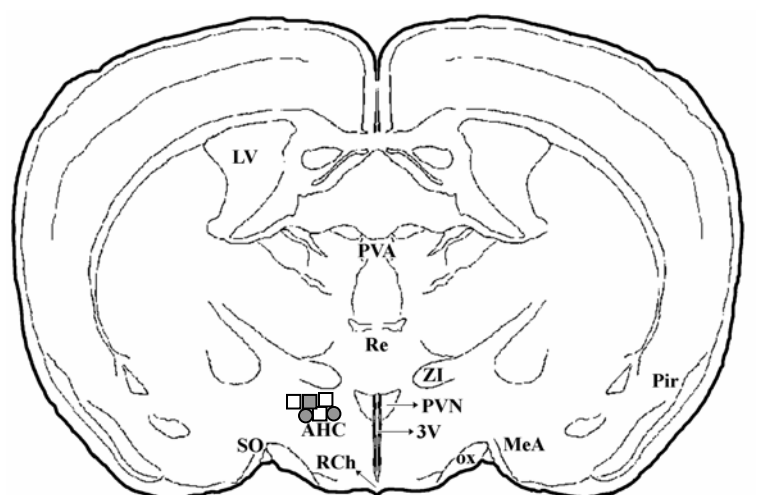
■ 200-400% change from vehicle

● 401-600% change from vehicle

▲ 601-800% change from vehicle

◆ 801-1100% change from vehicle

Figure 2.6c



NPY into the PFA Free Wheel Food Intake

Shading represents most effective dose



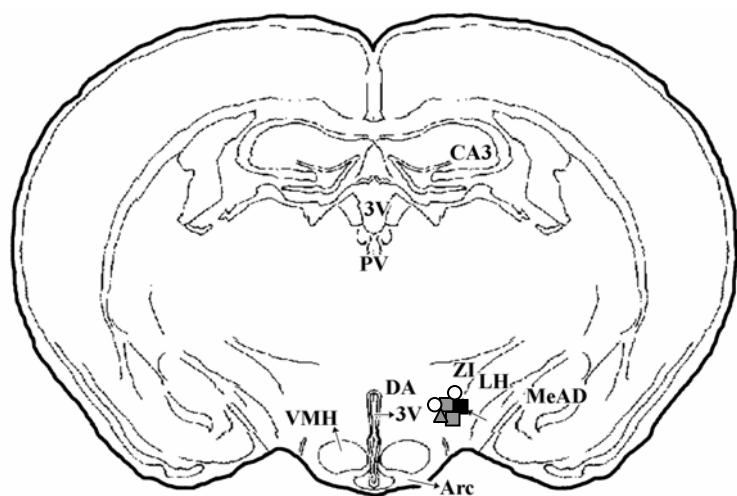
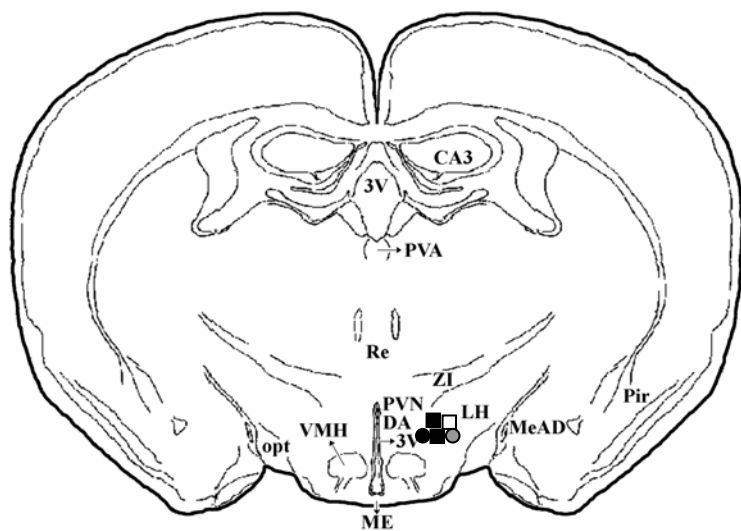
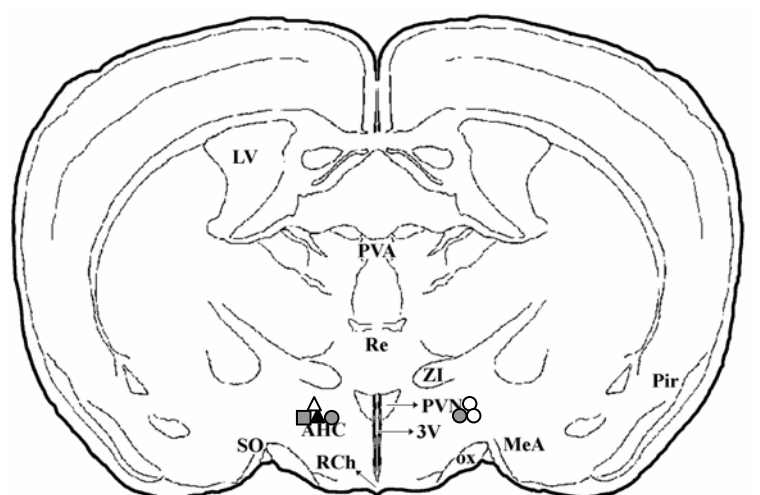
0.176nmol 0.352nmol 0.704nmol

■ 100-200% change from vehicle

● 201-300% change from vehicle

▲ 301-400% change from vehicle

Figure 2.7a



NPY into the PFA Blocked Wheel Food Hoard

Shading represents most effective dose



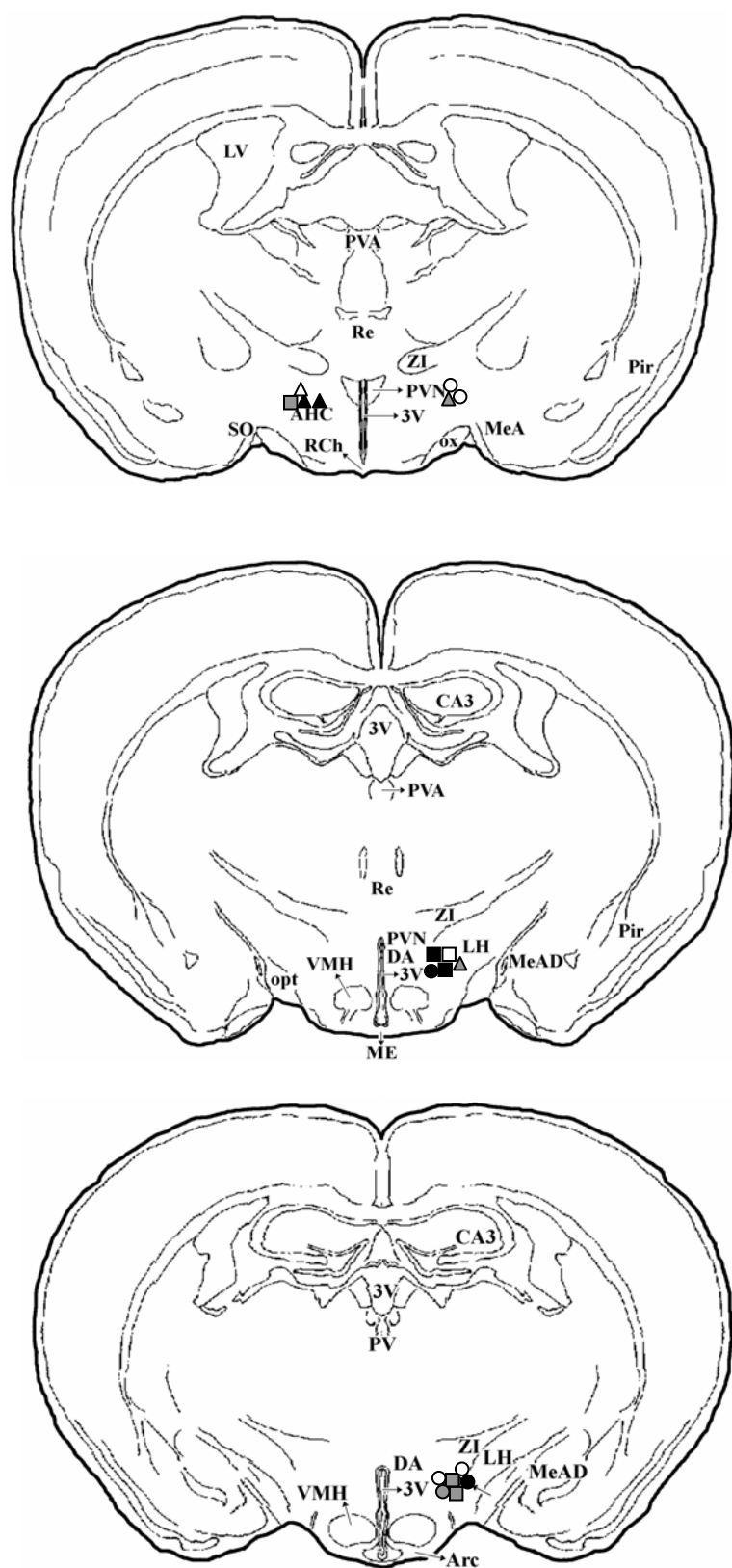
0.176nmol 0.352nmol 0.704nmol

■ 100-300% change from vehicle

● 301-500% change from vehicle

▲ 501-700% change from vehicle

Figure 2.7b



NPY into the PFA
Blocked Wheel
Food Intake

Shading represents most effective dose

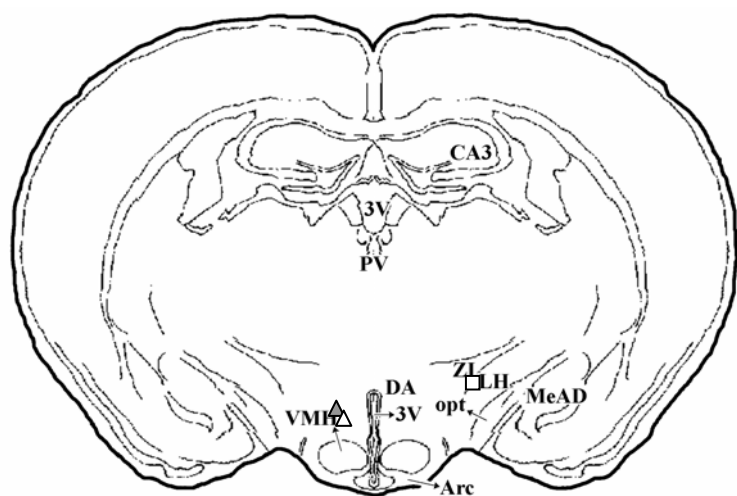
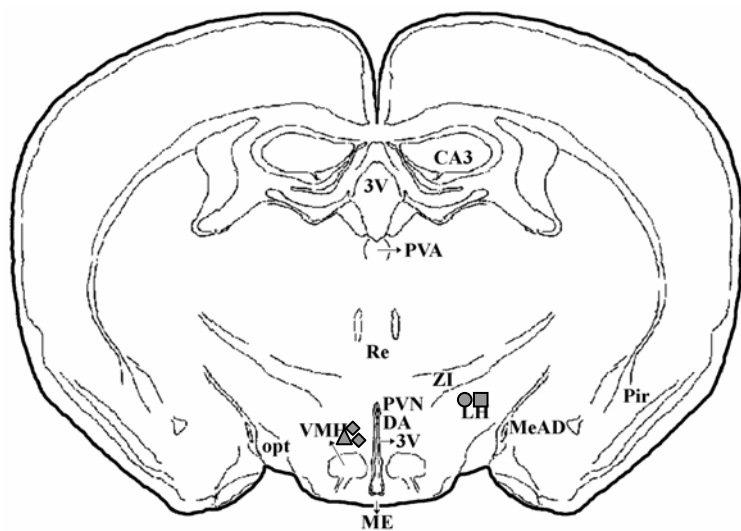
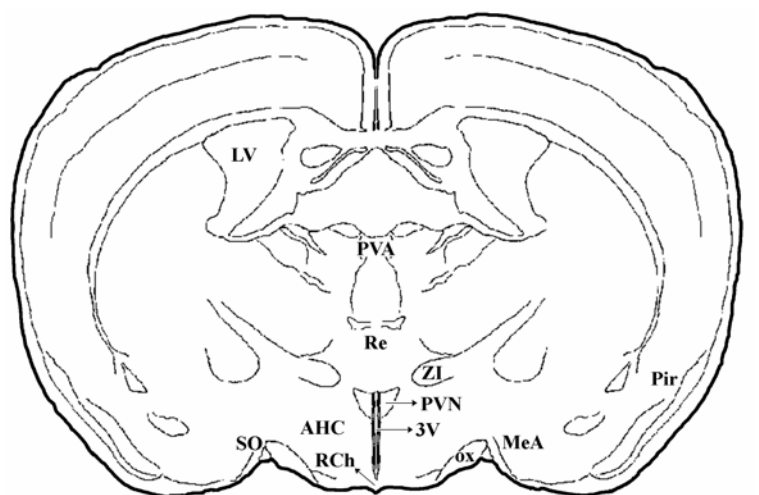
0.176nmol 0.352nmol 0.704nmol

0-200% change from vehicle

201-400% change from vehicle

401-600% change from vehicle

Figure 2.8a



NPY into the PFA
Misses
Food Hoard

Shading represents most effective dose



0.176nmol 0.352nmol 0.704nmol

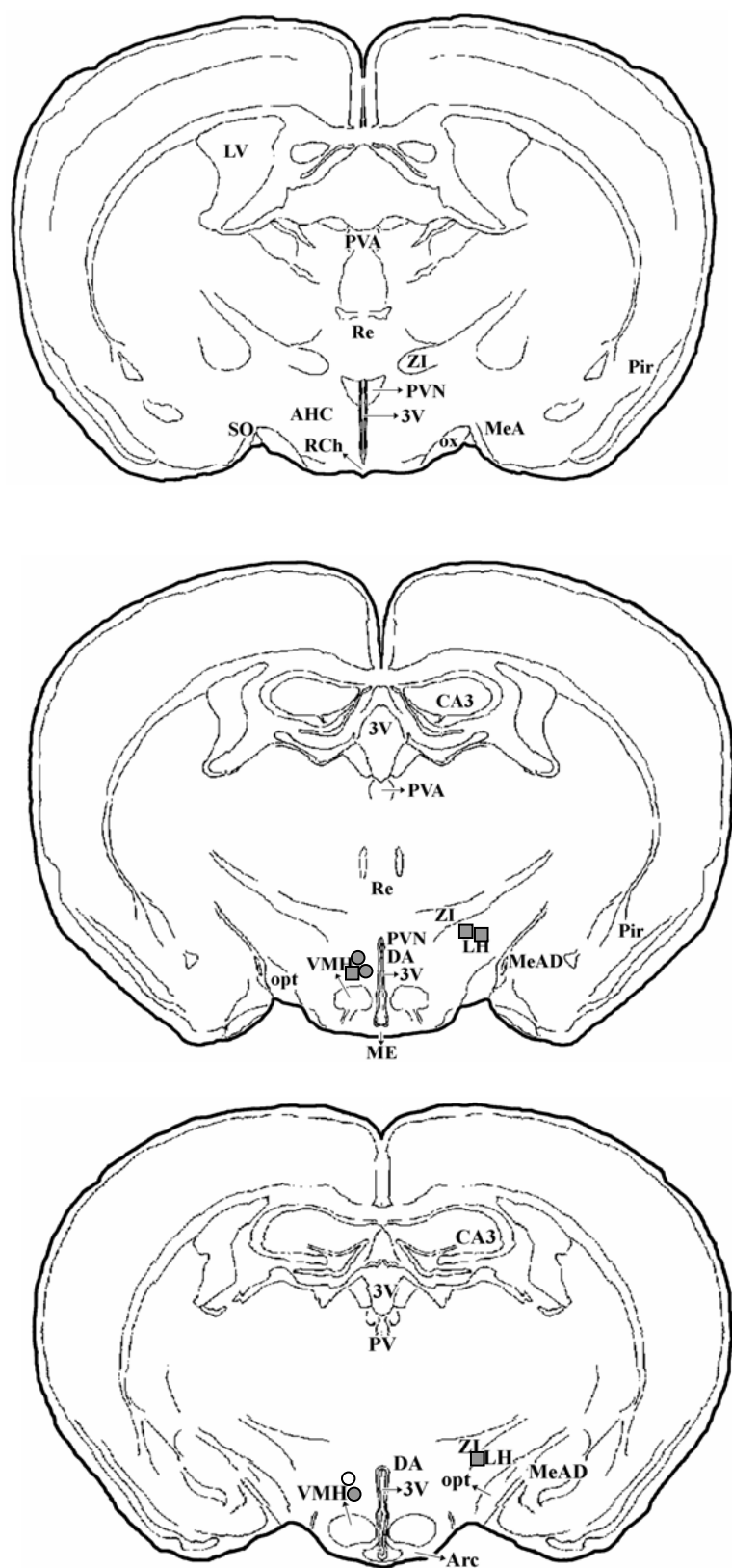
■ 0-500% change from vehicle

● 1001-2000% change from vehicle

▲ 2001-3000% change from vehicle

◆ Above 3001% change from vehicle

Figure 2.8b



NPY into the PFA
Misses
Food Intake

Shading represents most effective dose



0.176nmol 0.352nmol 0.704nmol

■ 0-500% change from vehicle

● 501-1000% change from vehicle

▲ 1001-1500% change from vehicle

Figure 2.9a

Y1 Antagonist into the PVH
10 Revolutions/pellet
Foraging



Figure 2.9b

Y1 Antagonist into the PVH
10 Revolutions/pellet
Food Hoard

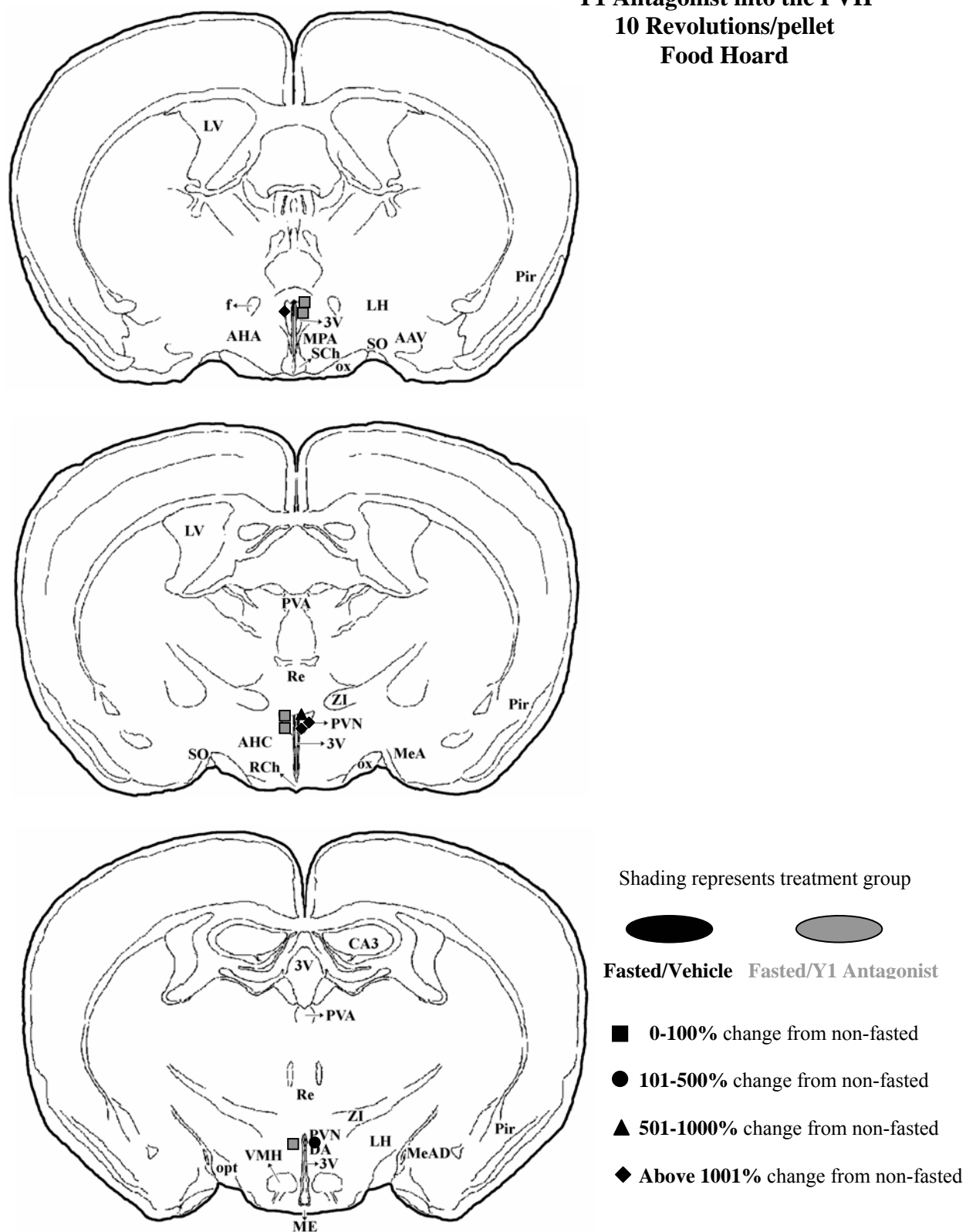


Figure 2.9c

Y1 Antagonist into the PVH
10 Revolutions/pellet
Food Intake

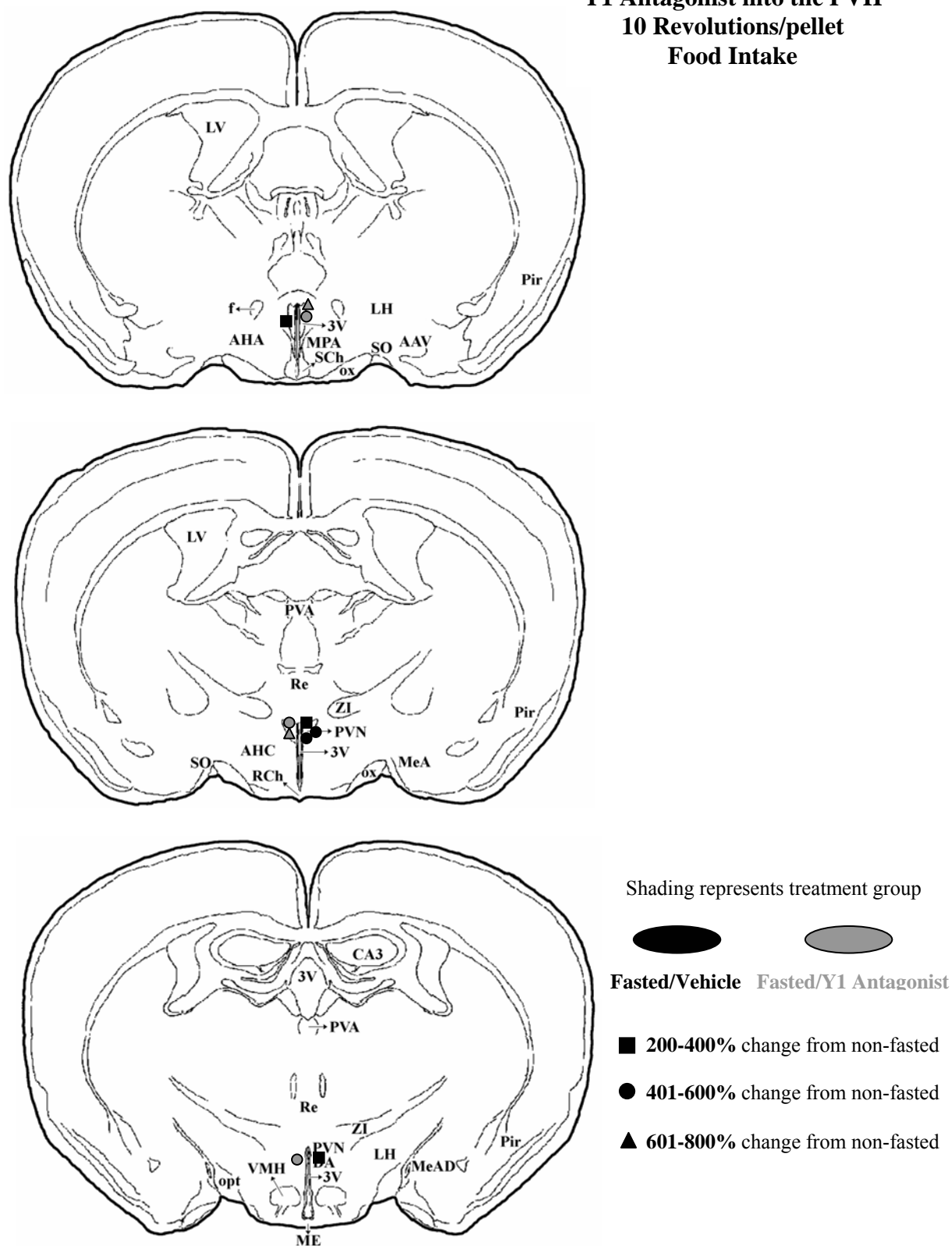


Figure 2.10a

Y1 Antagonist into the PVH
Free Wheel
Wheel Revolutions

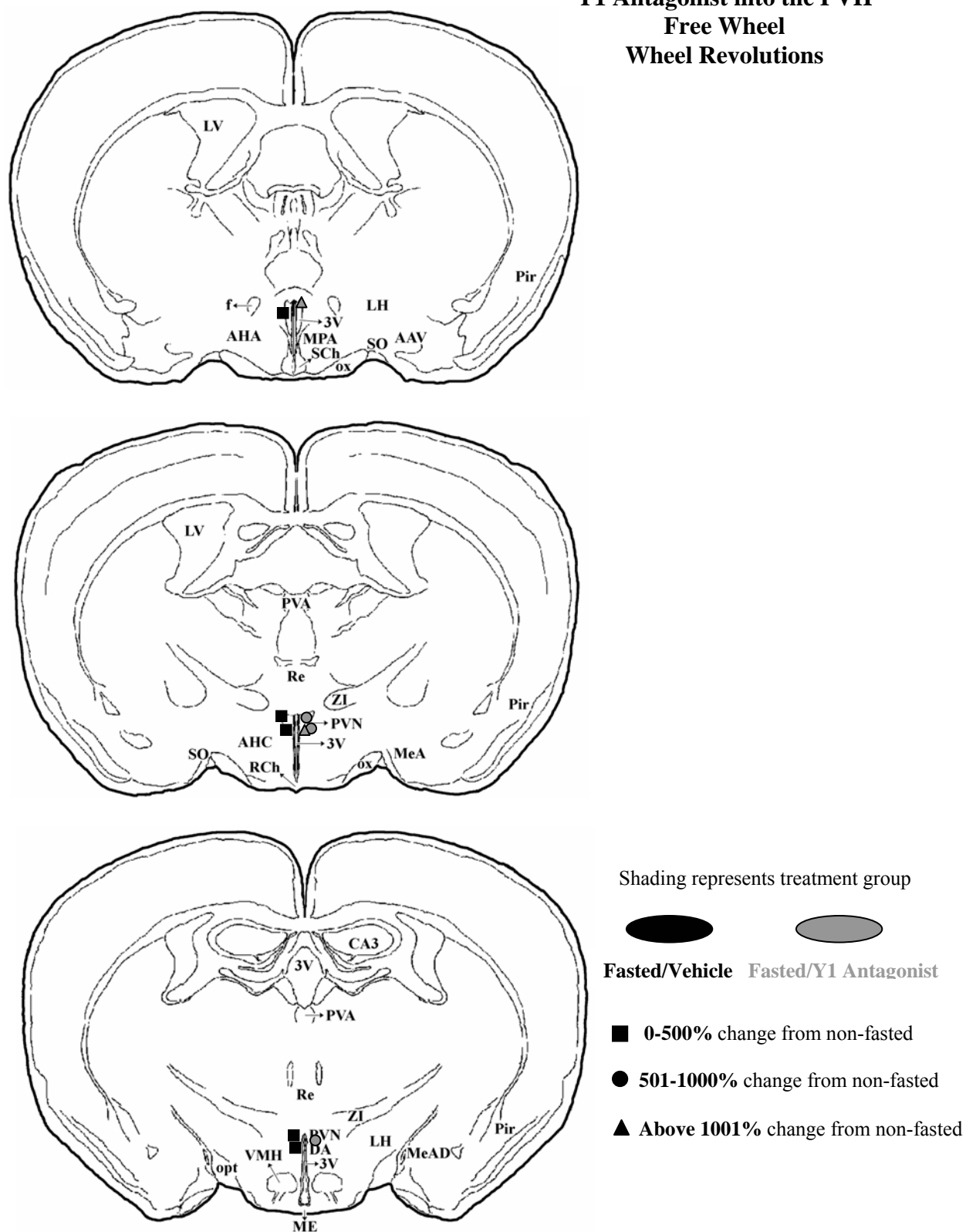


Figure 2.10b

Y1 Antagonist into the PVH
Free Wheel
Food Hoard



Figure 2.10c

Y1 Antagonist into the PVH
Free Wheel
Food Intake

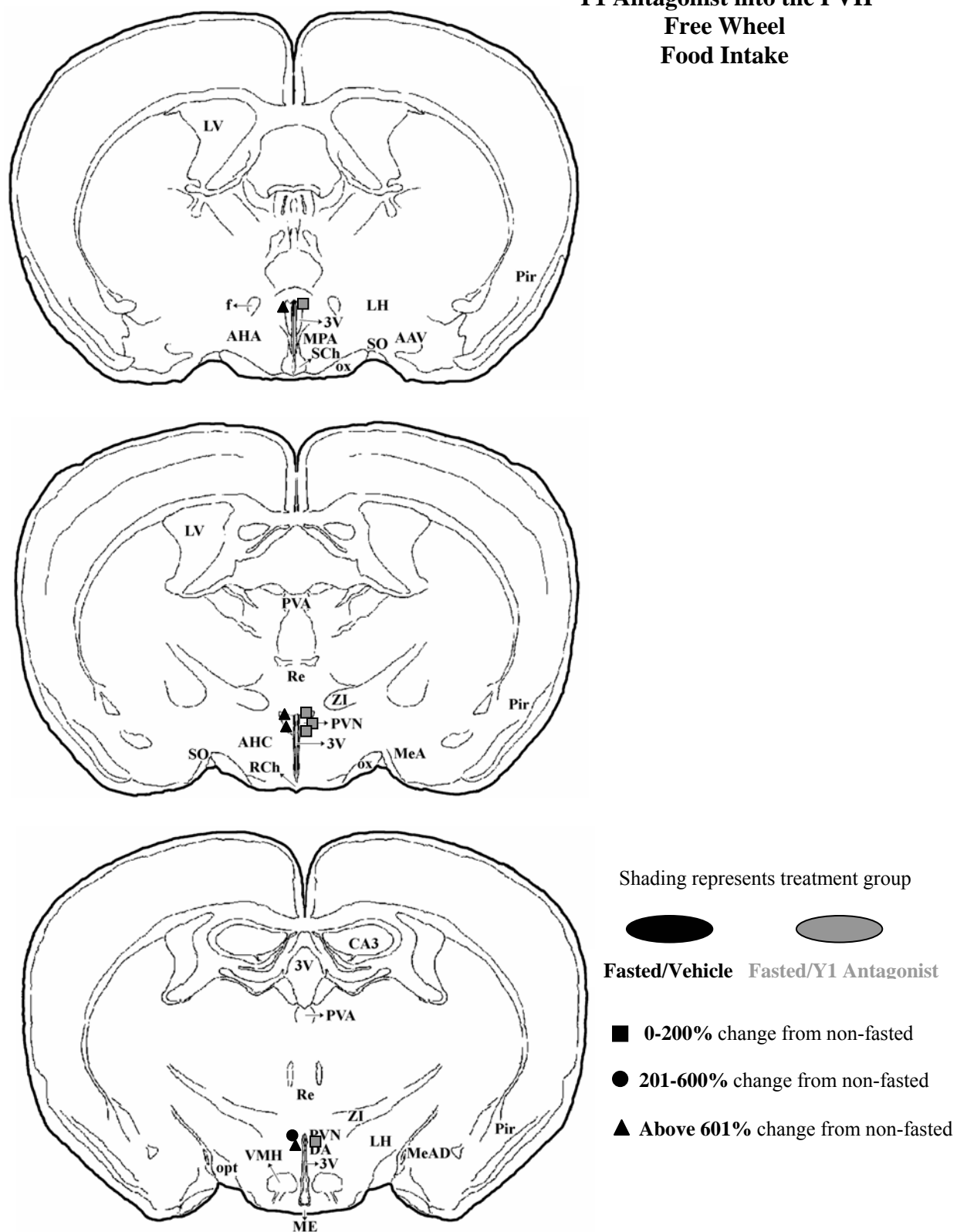


Figure 2.11a

Y1 Antagonist into the PVH
Blocked Wheel
Food Hoard

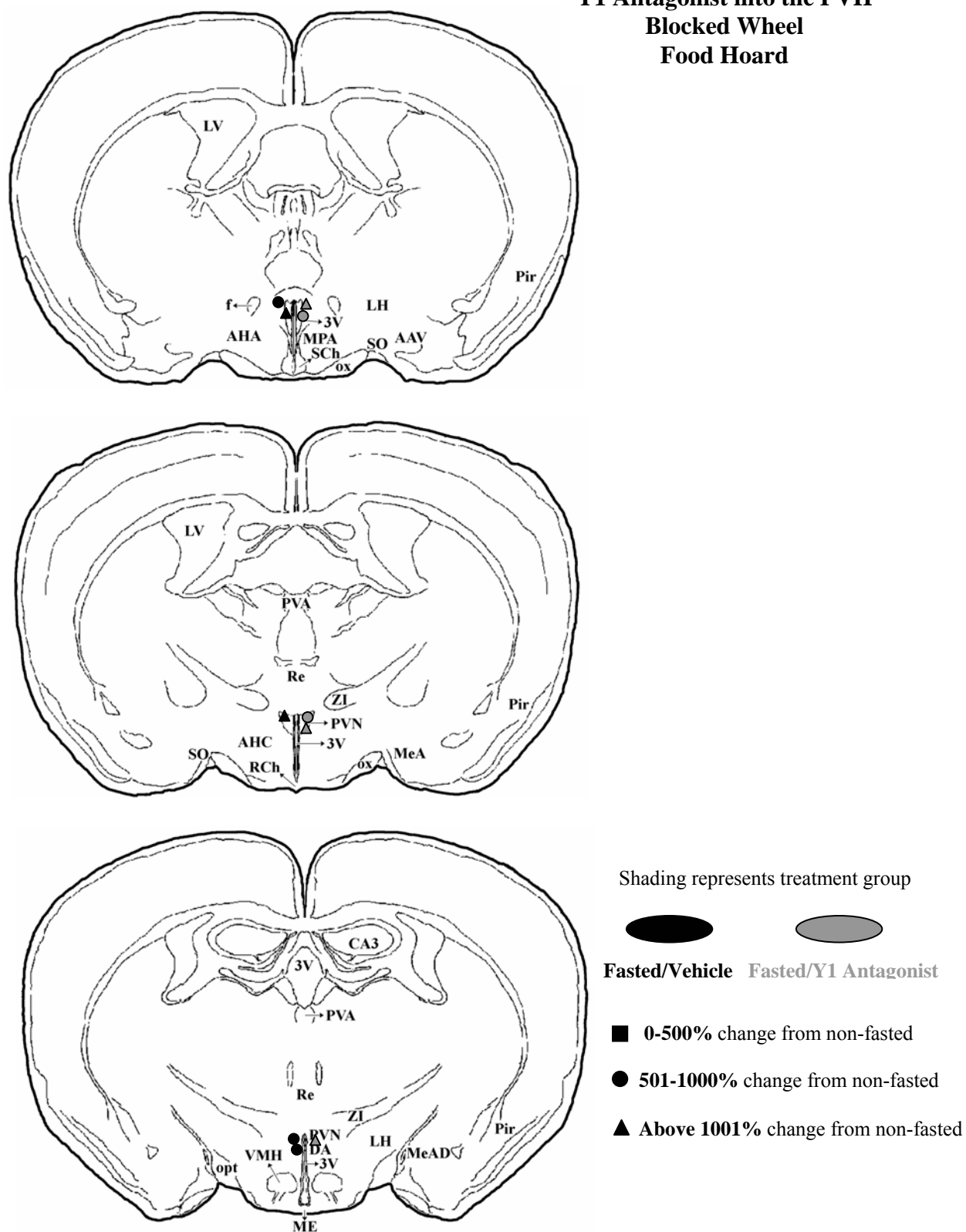


Figure 2.11b

Y1 Antagonist into the PVH
Blocked Wheel
Food Intake

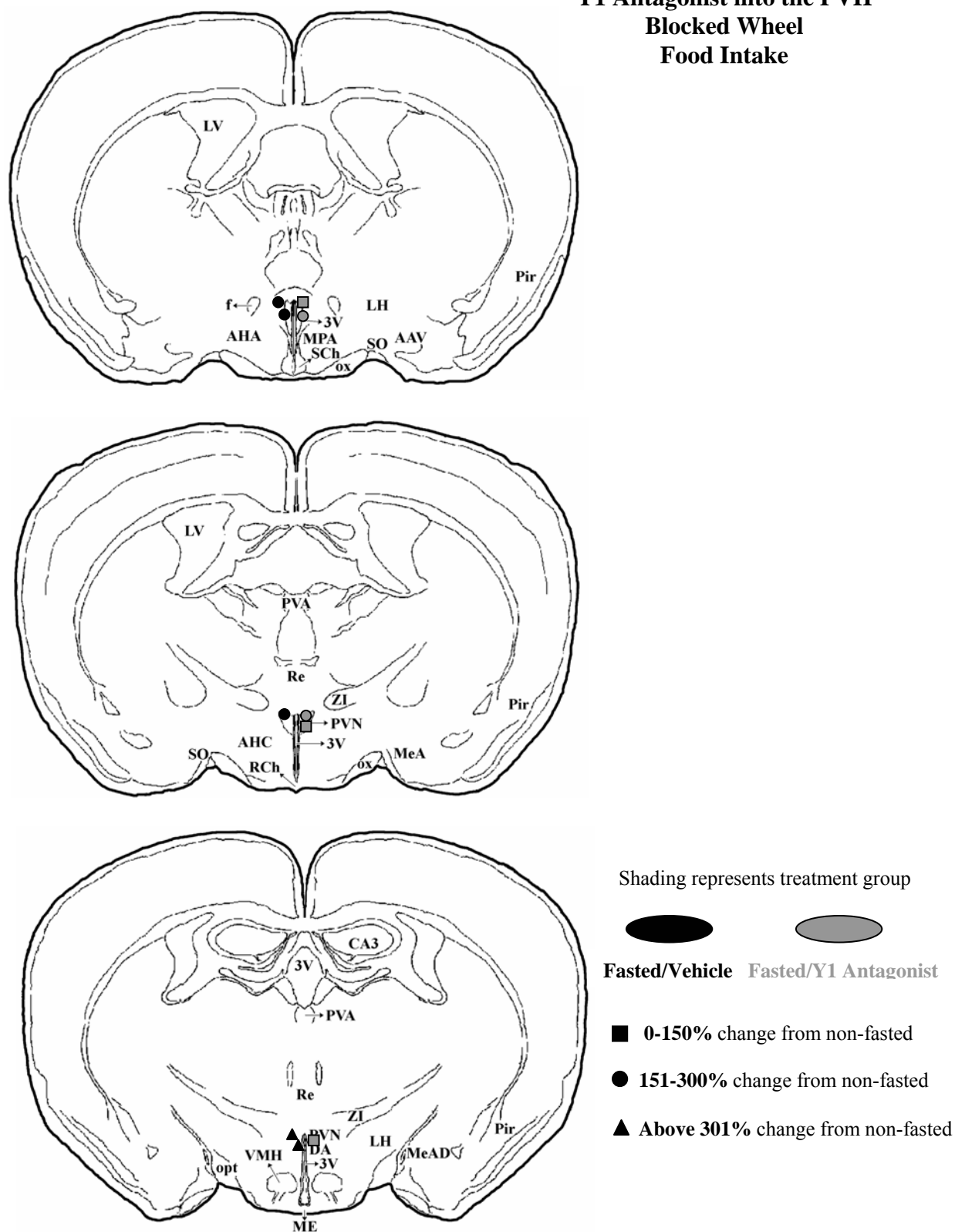
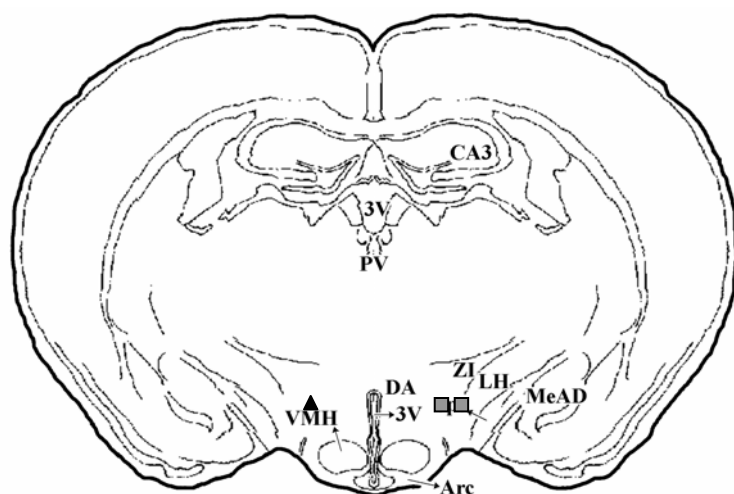
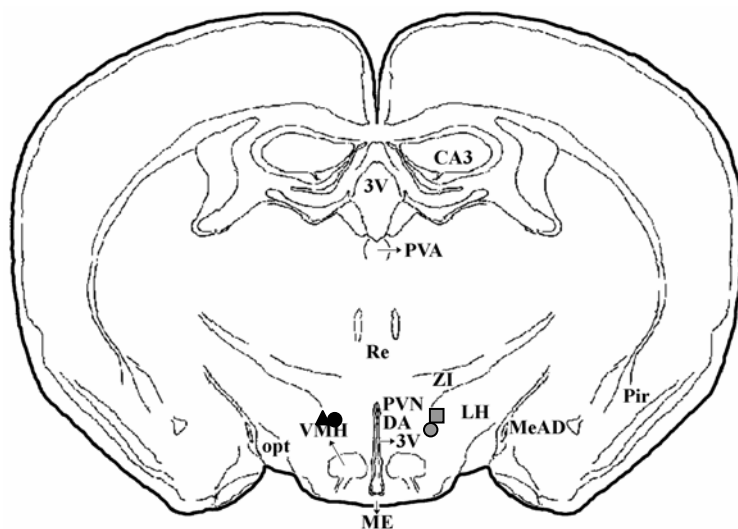
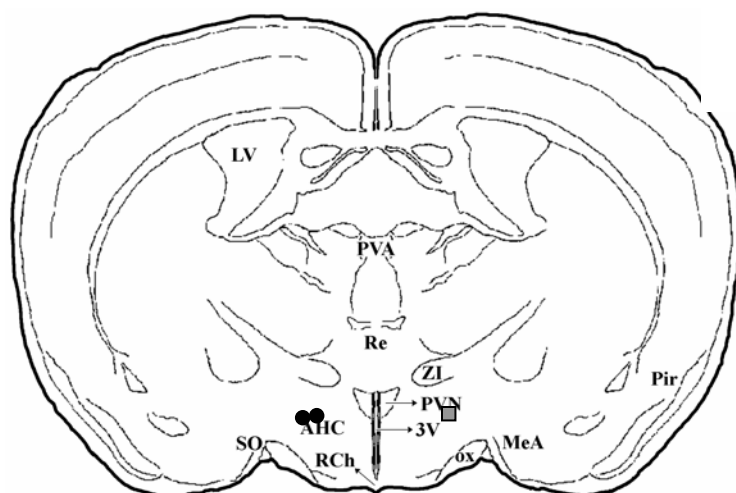


Figure 2.12a

Y1 Antagonist into the PFA
10 Revolutions/pellet
Foraging



Shading represents treatment group



Fasted/Vehicle **Fasted/Y1 Antagonist**

■ 0-1000% change from non-fasted

● 1001-2000% change from non-fasted

▲ Above 2001% change from non-fasted

Figure 2.12b

Y1 Antagonist into the PFA
10 Revolutions/pellet
Food Hoard



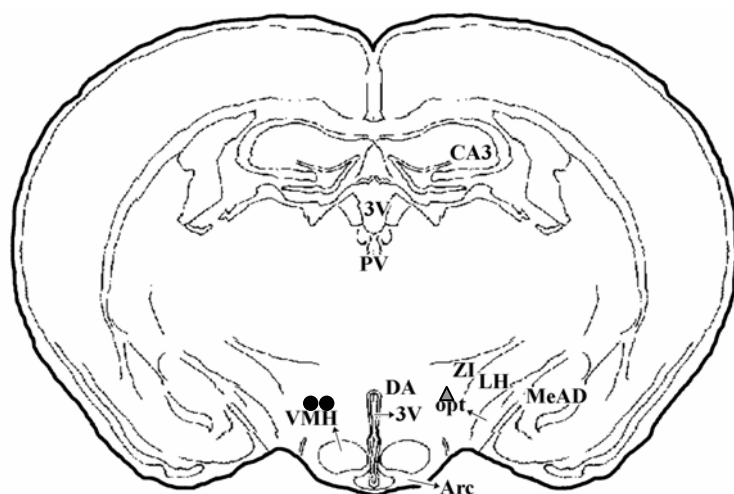
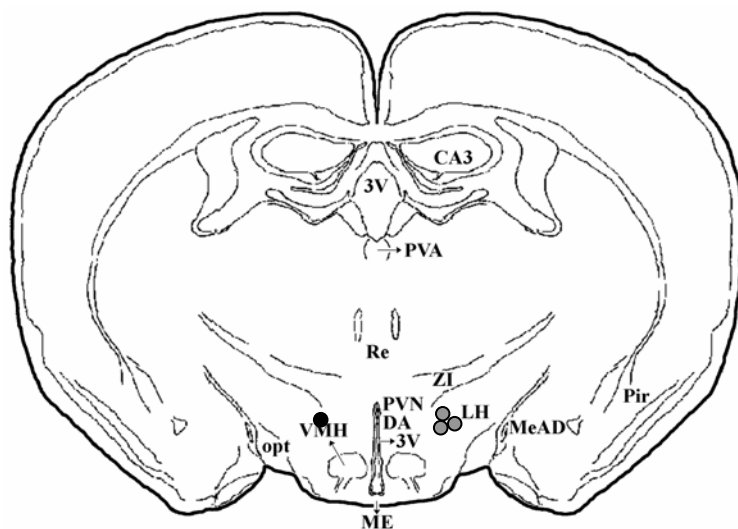
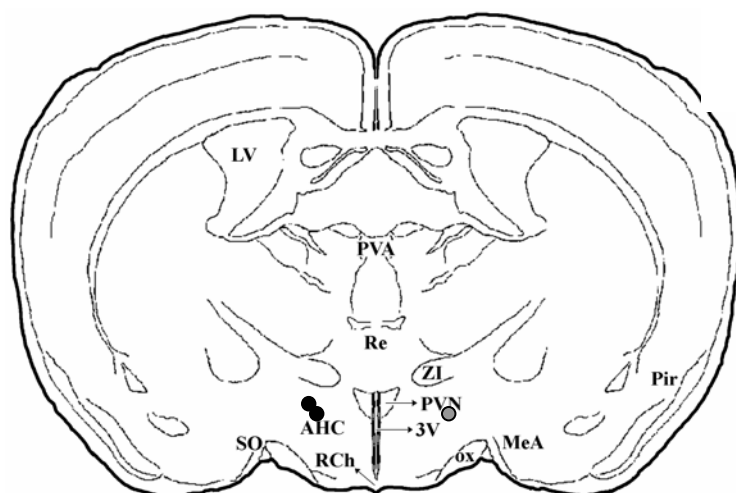
Figure 2.12c

Y1 Antagonist into the PFA
10 Revolutions/pellet
Food Intake



Figure 2.13a

Y1 Antagonist into the PFA
Free Wheel
Wheel Revolutions



Shading represents treatment group



Fasted/Vehicle **Fasted/Y1 Antagonist**

■ 0-50% change from non-fasted

● 51-100% change from non-fasted

▲ Above 101% change from non-fasted

Figure 2.13b

Y1 Antagonist into the PFA
Free Wheel
Food Hoard

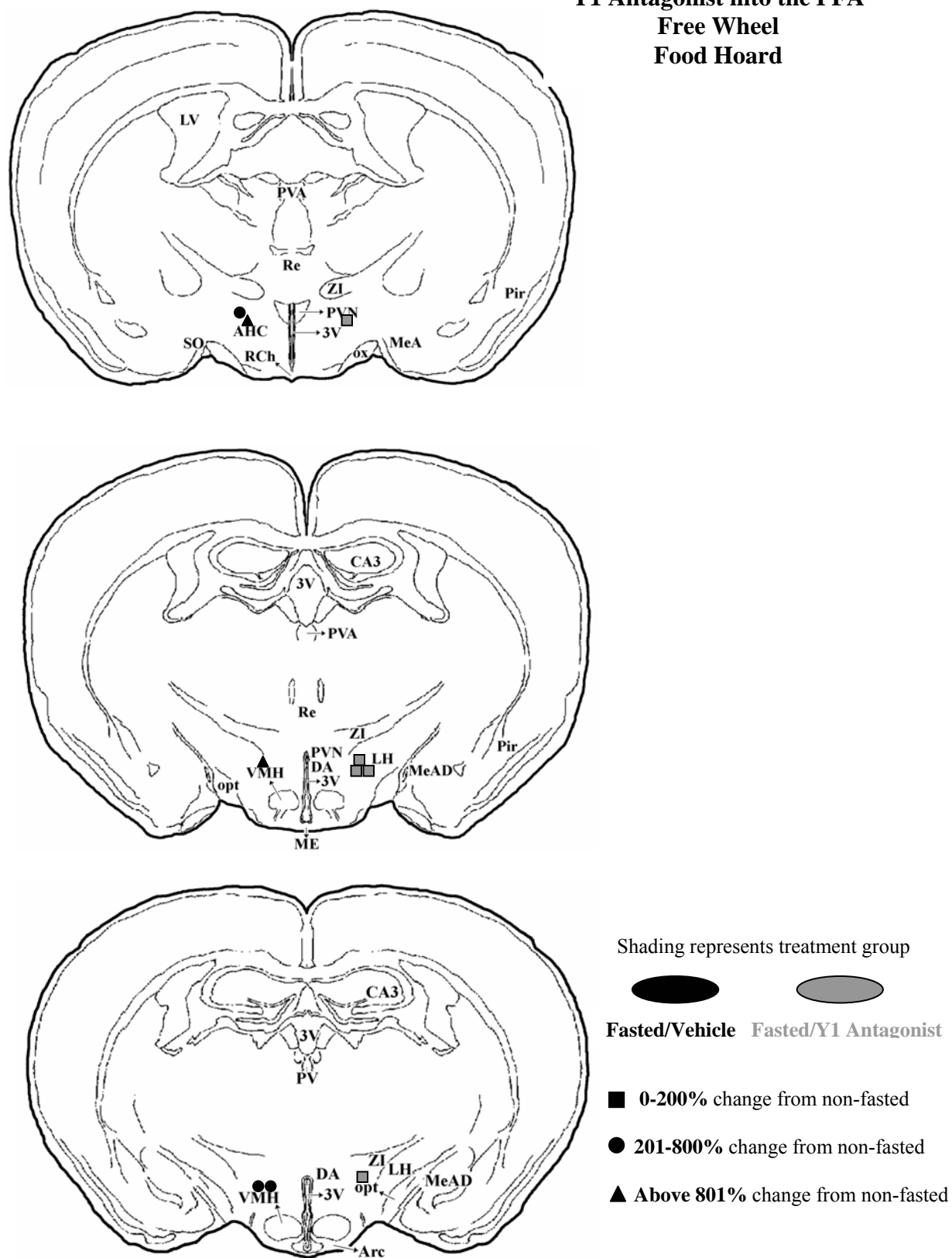


Figure 2.13c

Y1 Antagonist into the PFA
Free Wheel
Food Intake



Figure 2.14a

Y1 Antagonist into the PFA
Blocked Wheel
Food Hoard



Figure 2.14b

Y1 Antagonist into the PFA
Blocked Wheel
Food Intake

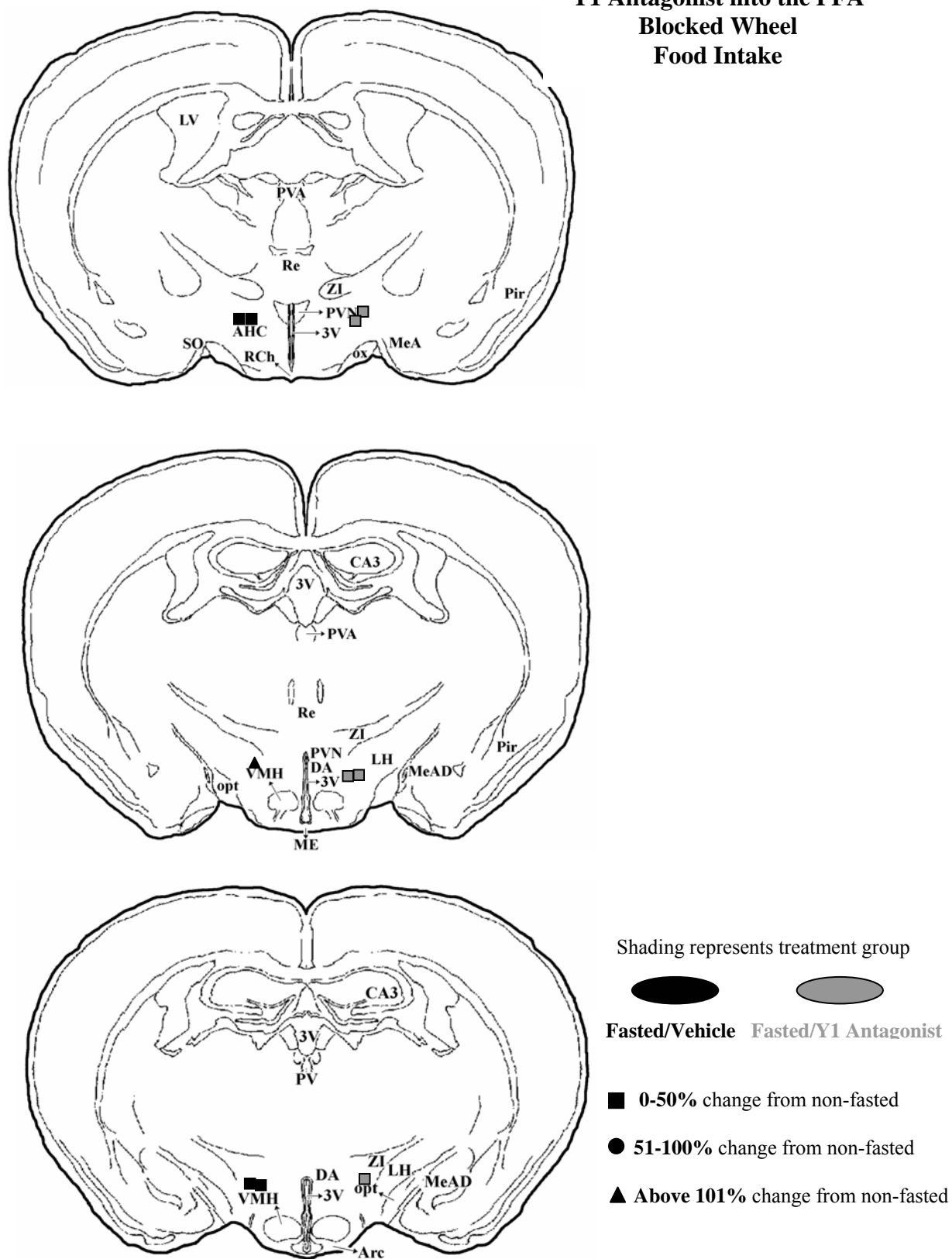


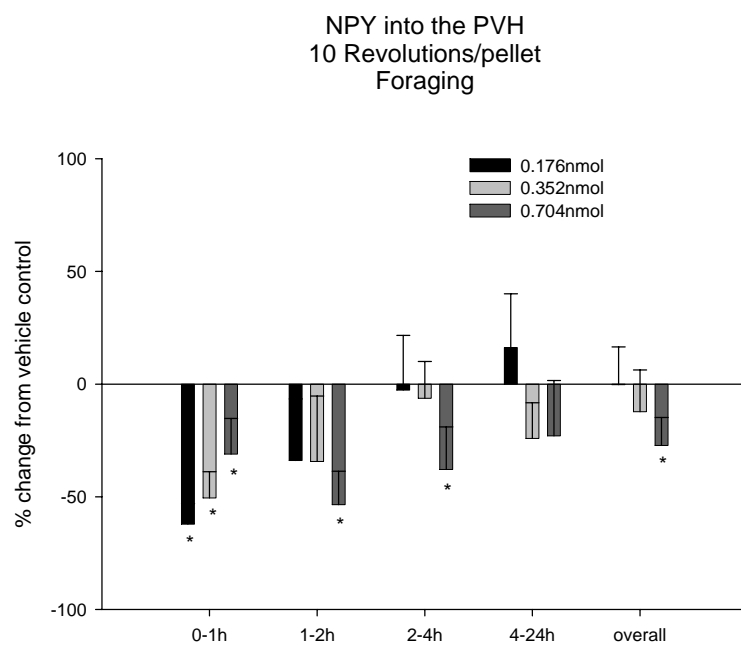
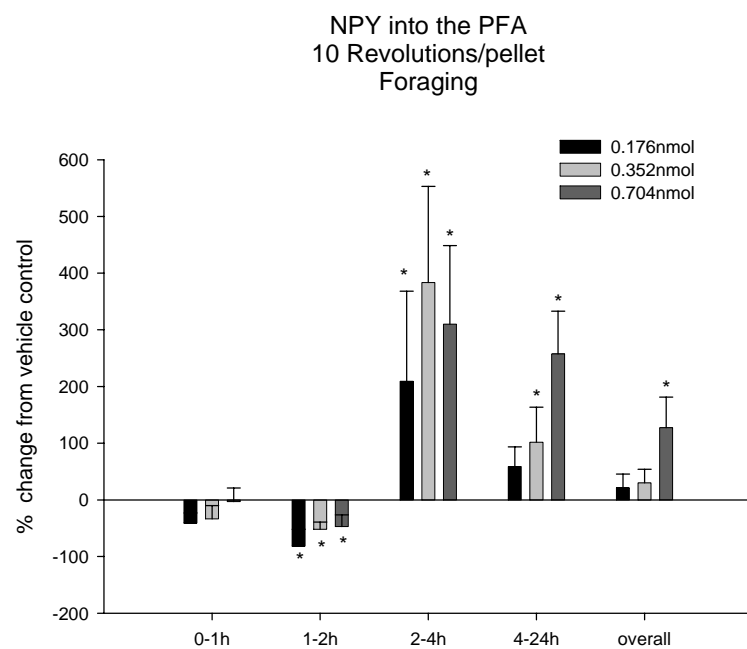
Figure 2.15a**Figure 2.15b**

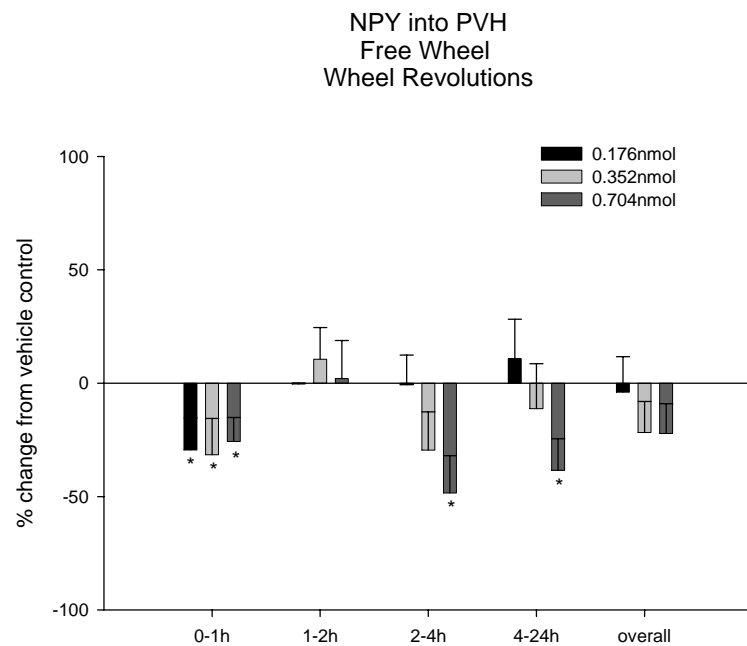
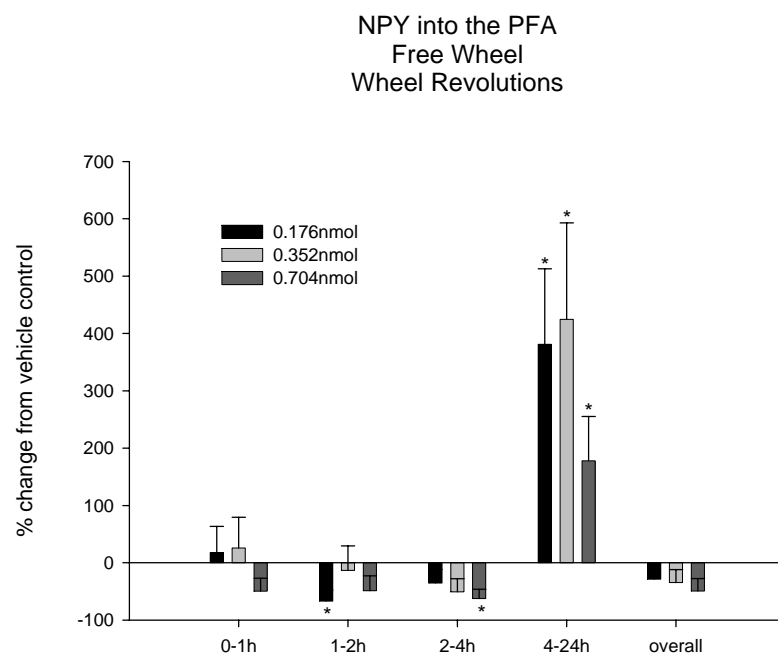
Figure 2.16a**Figure 2.16b**

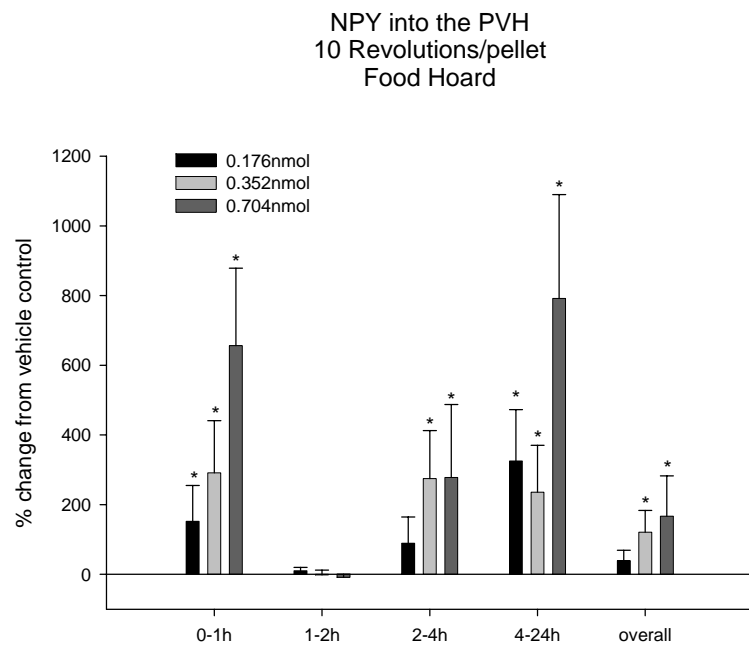
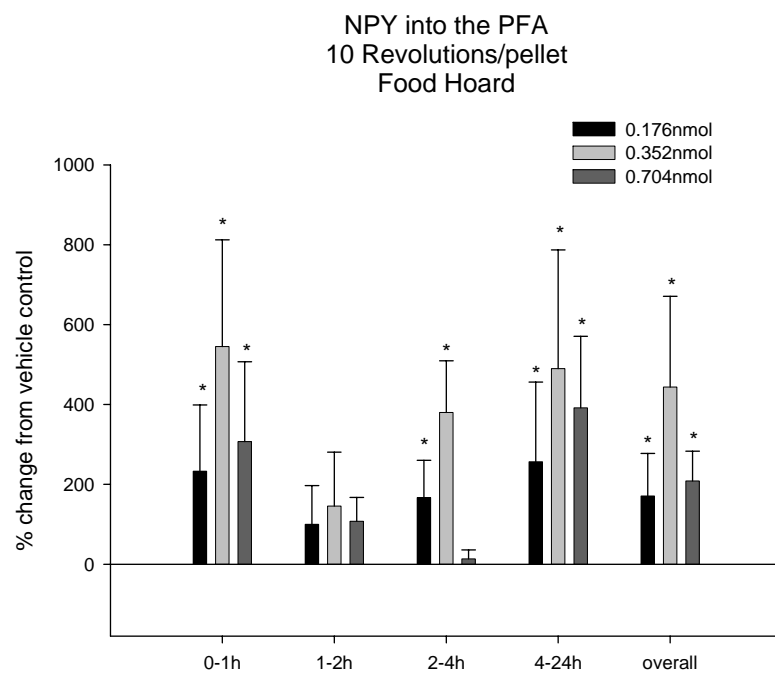
Figure 2.17a**Figure 2.17b**

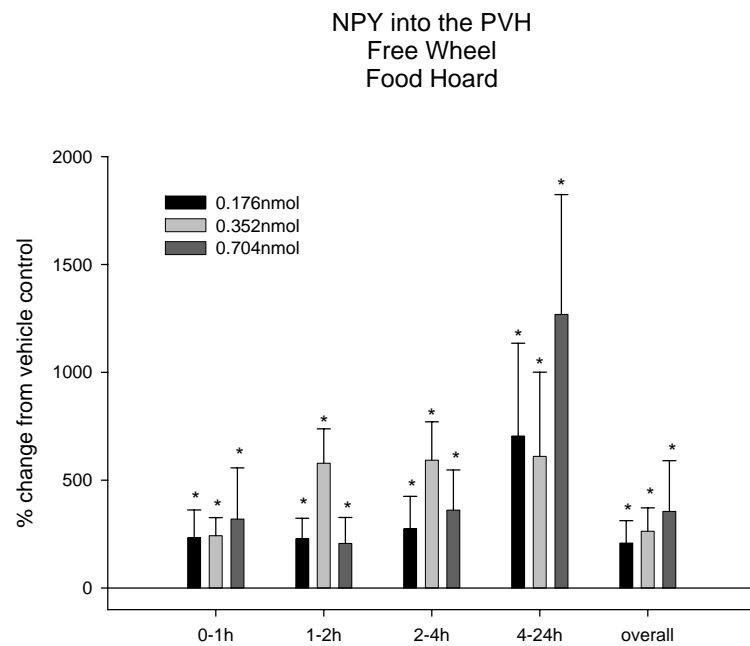
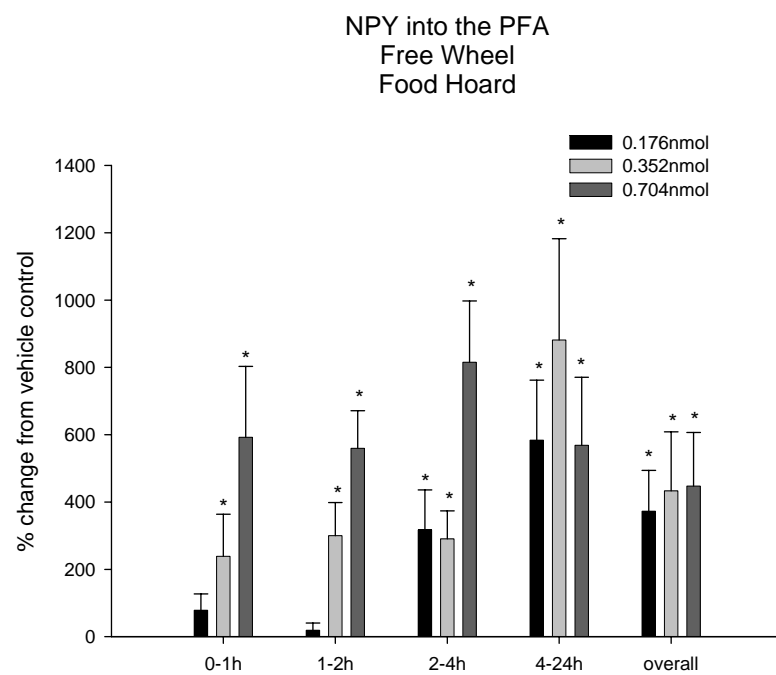
Figure 2.18a**Figure 2.18b**

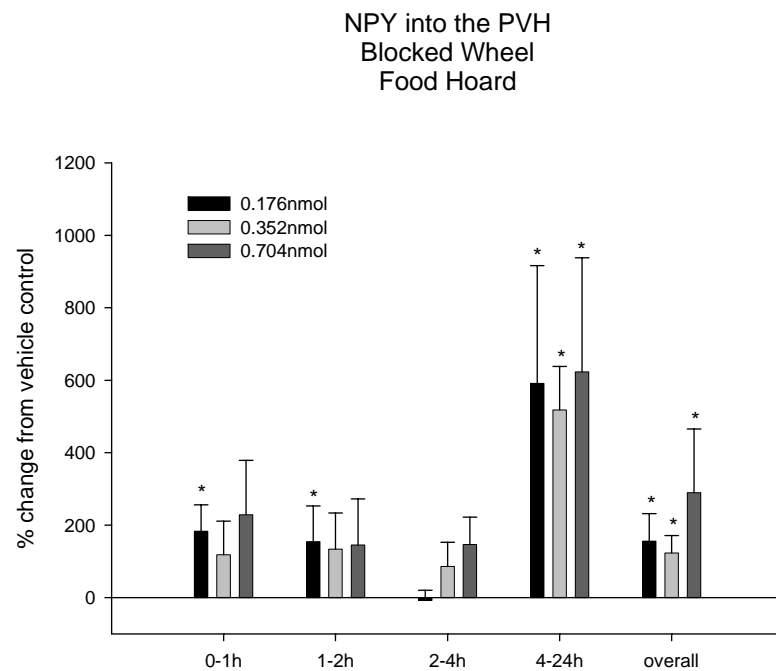
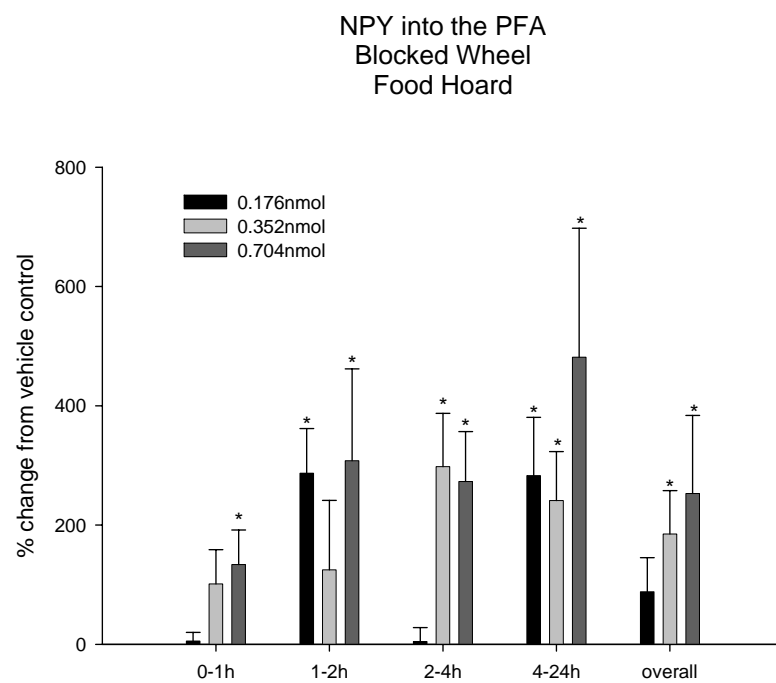
Figure 2.19a**Figure 2.19b**

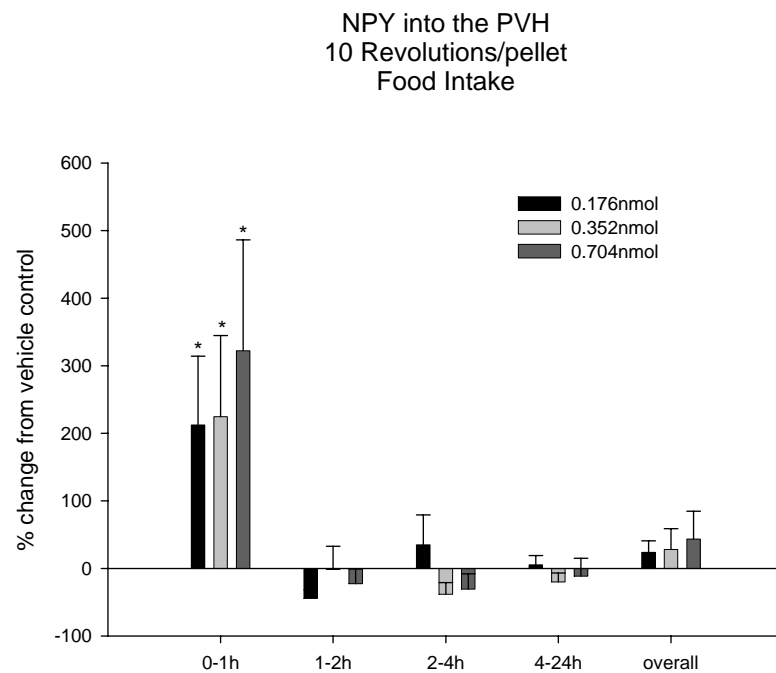
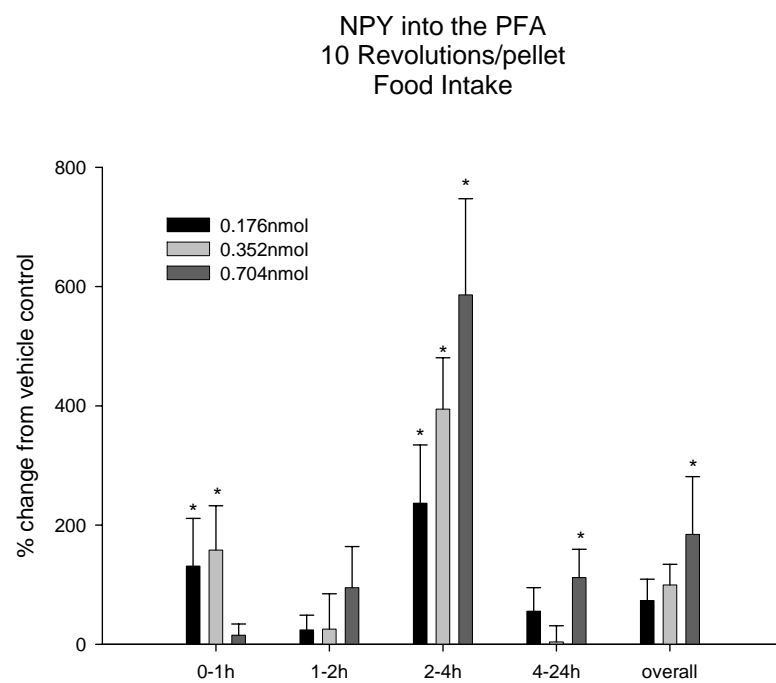
Figure 2.20a**Figure 2.20b**

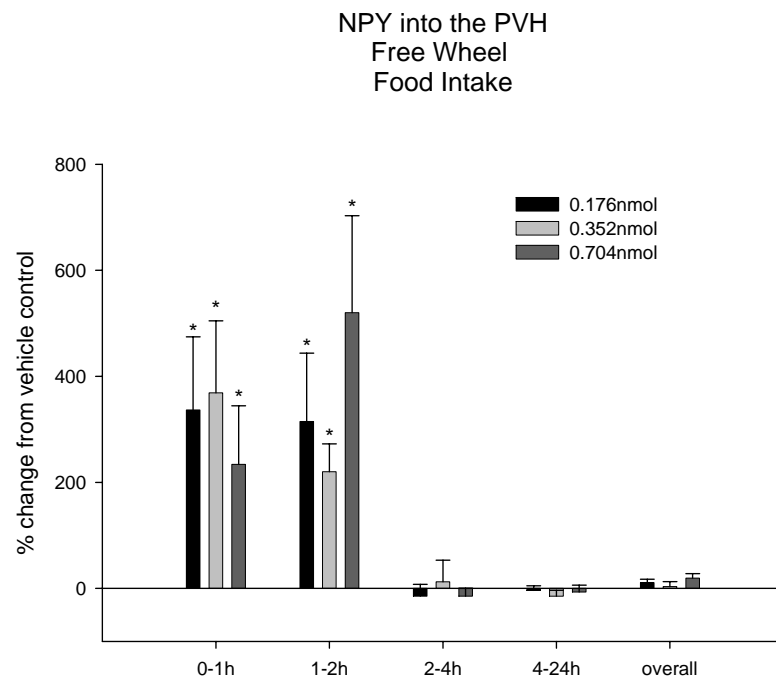
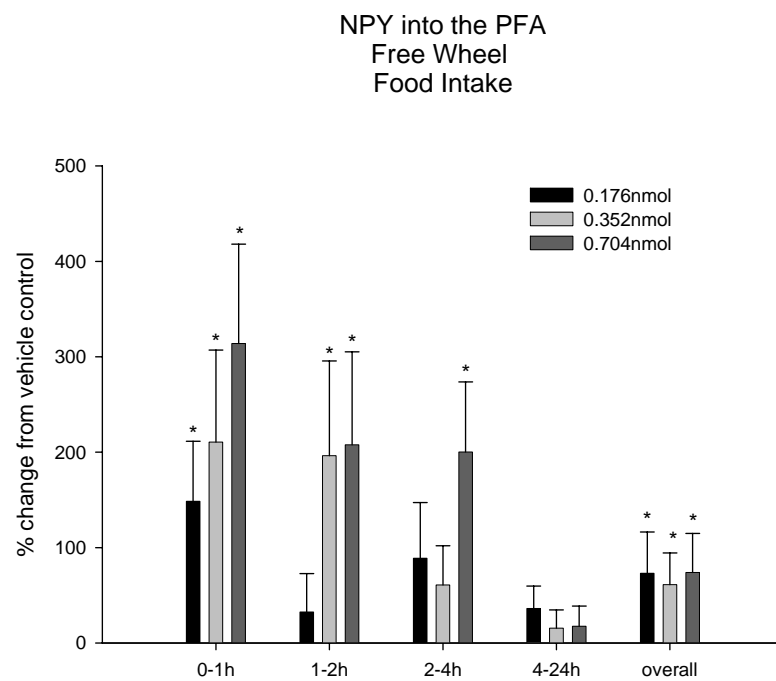
Figure 2.21a**Figure 2.21b**

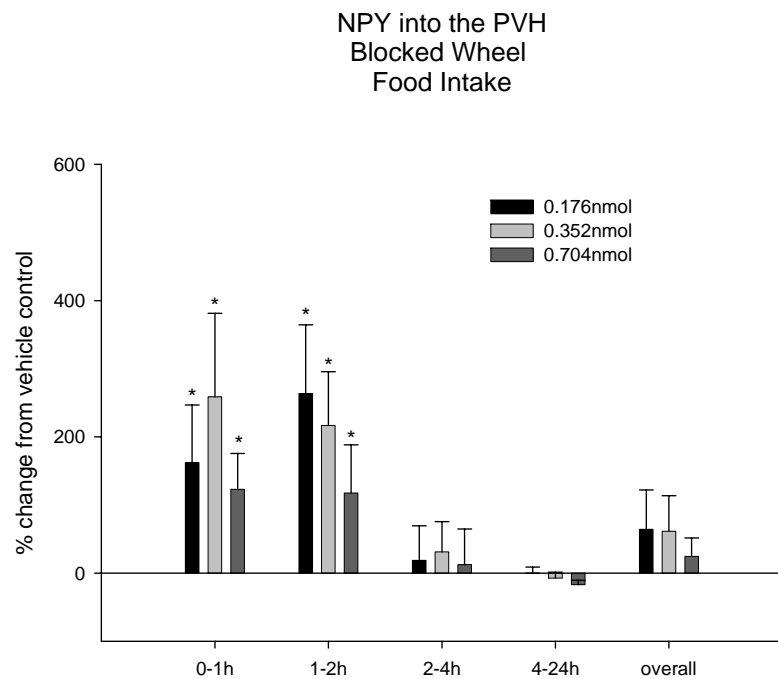
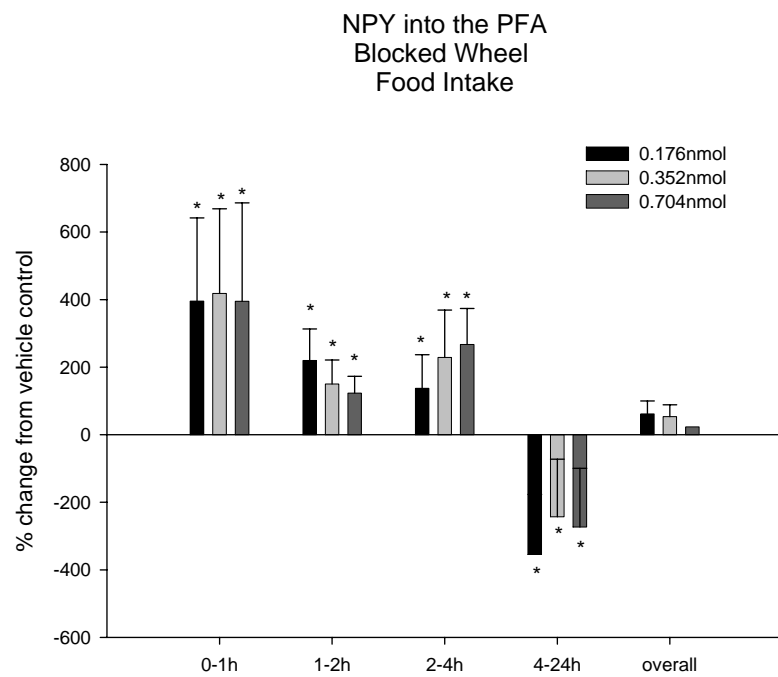
Figure 2.22a**Figure 2.22b**

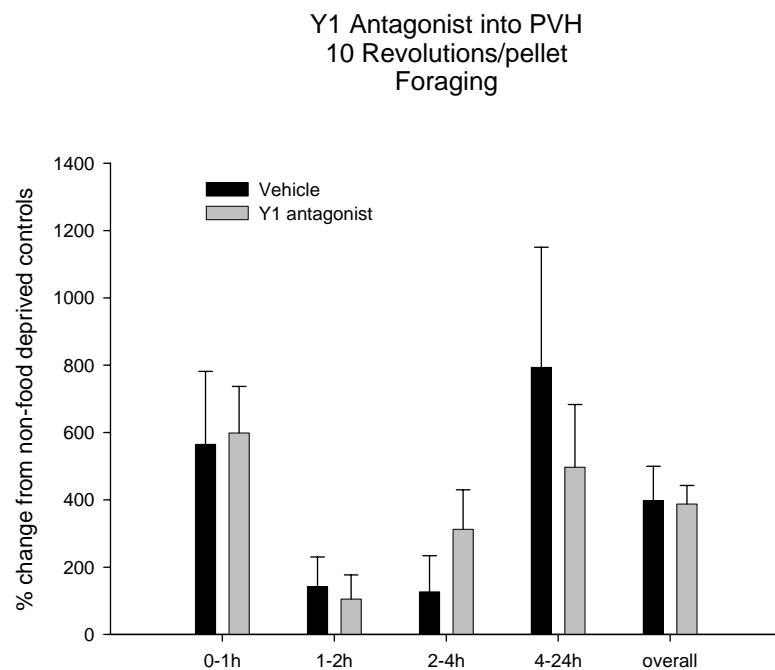
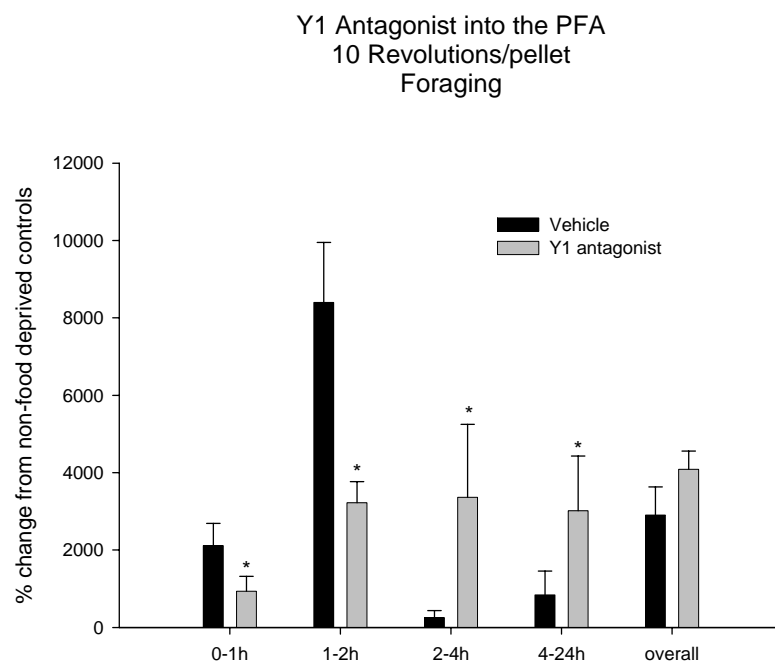
Figure 2.23a**Figure 2.23b**

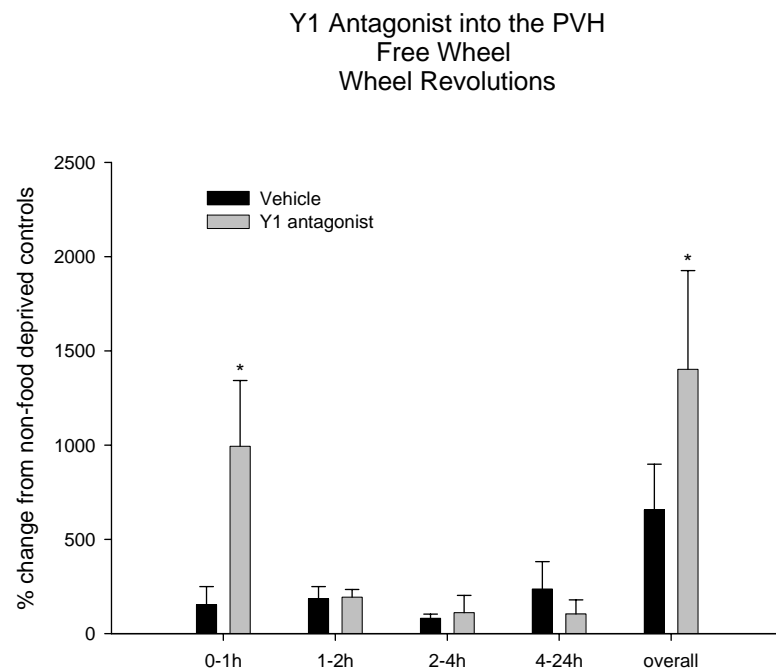
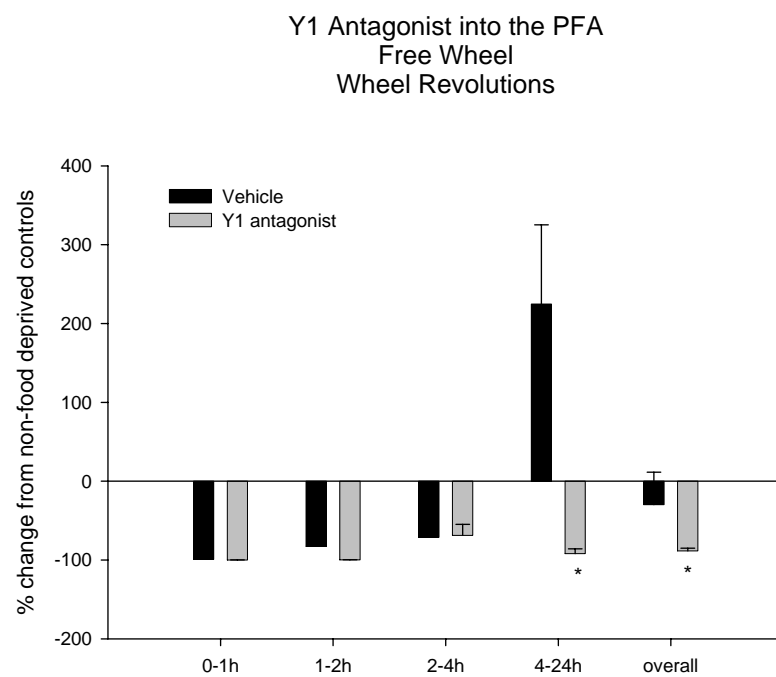
Figure 2.24a**Figure 2.24b**

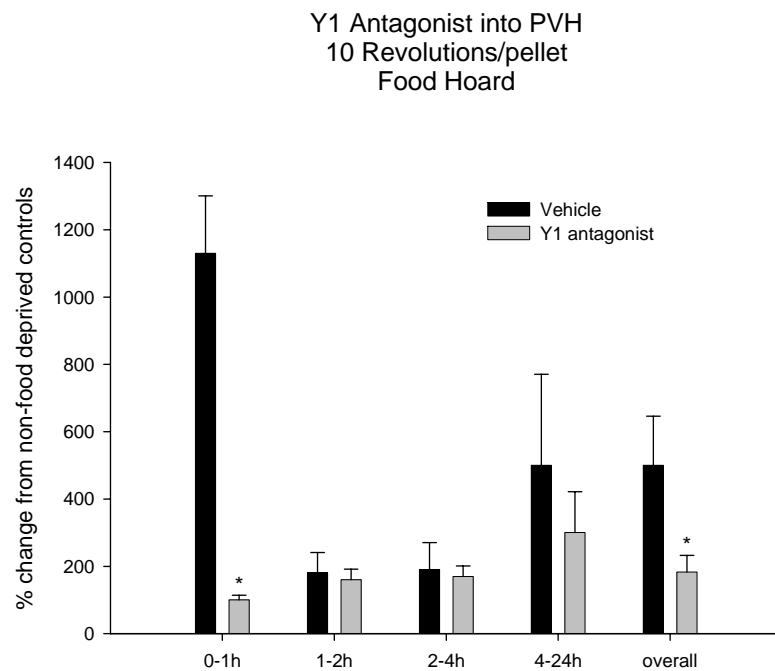
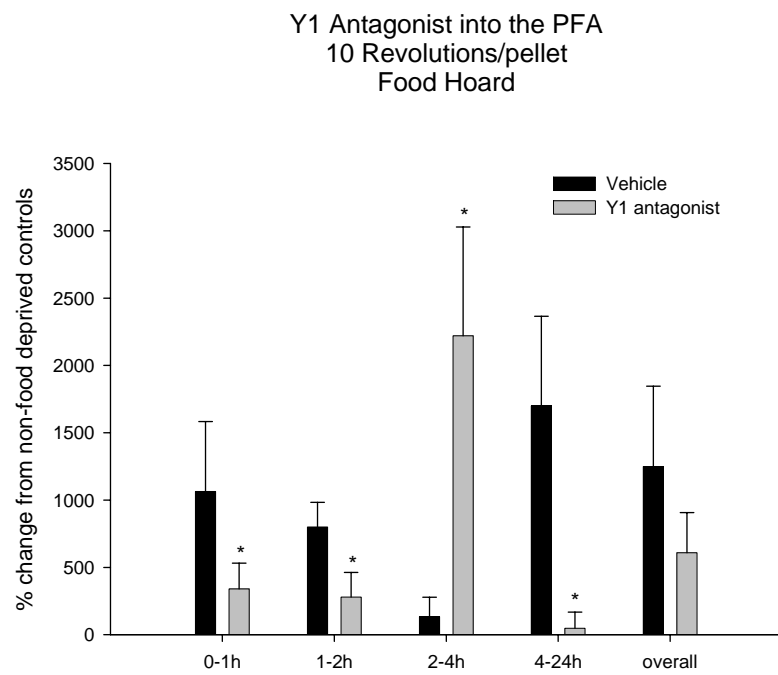
Figure 2.25a**Figure 2.25b**

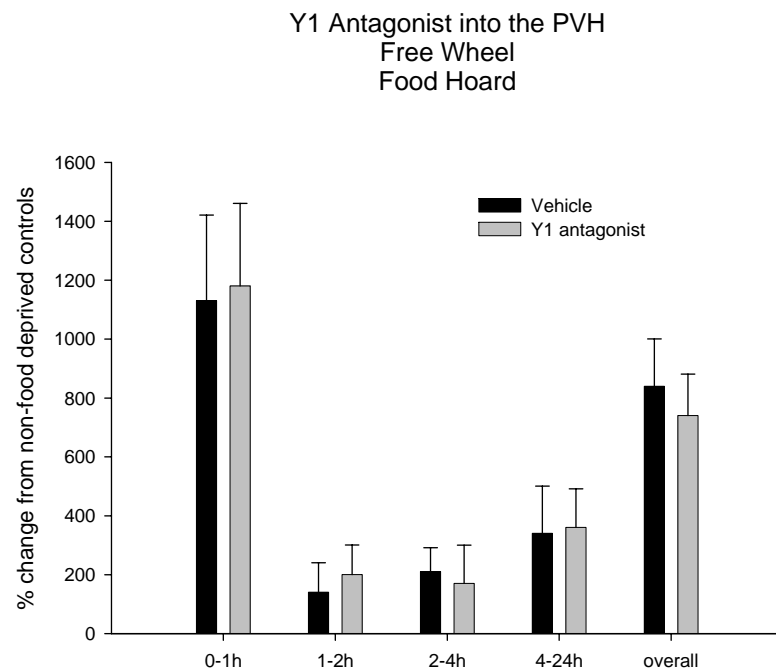
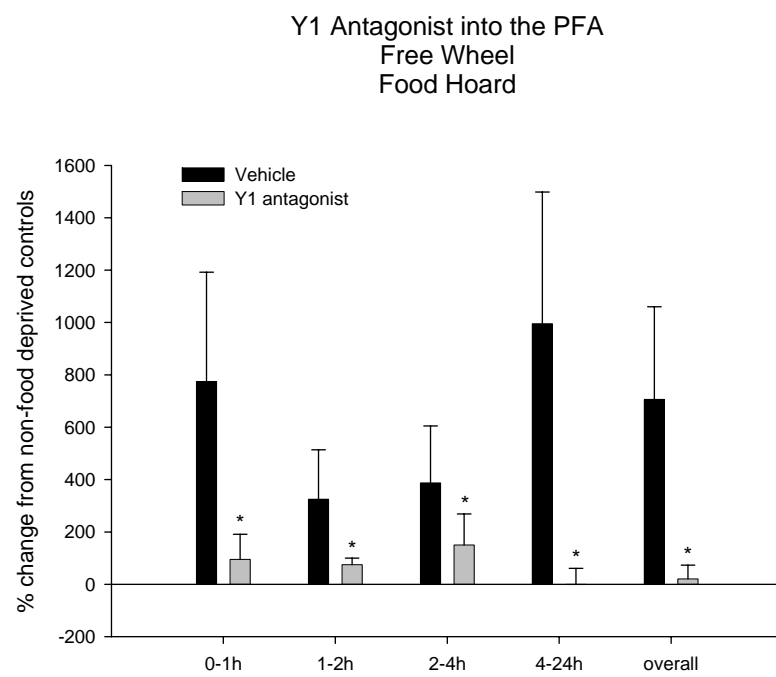
Figure 2.26a**Figure 2.26b**

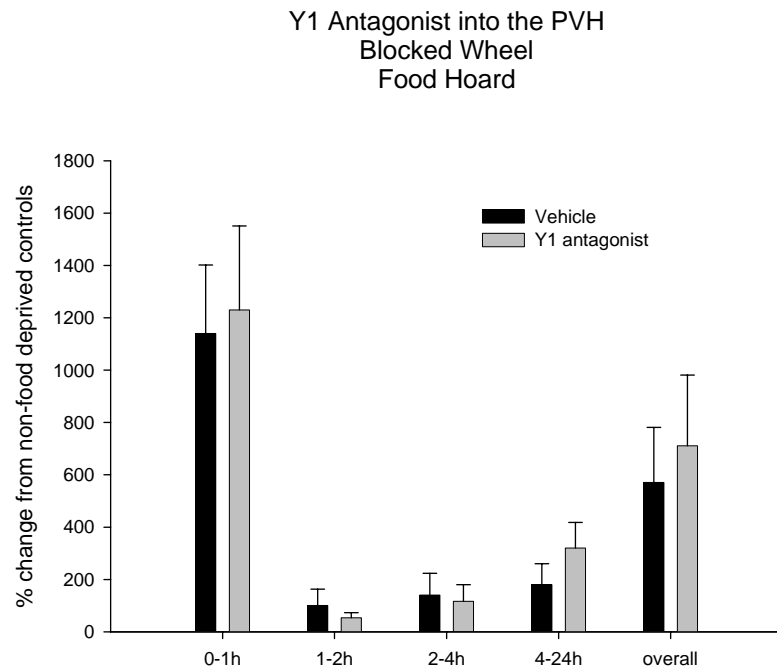
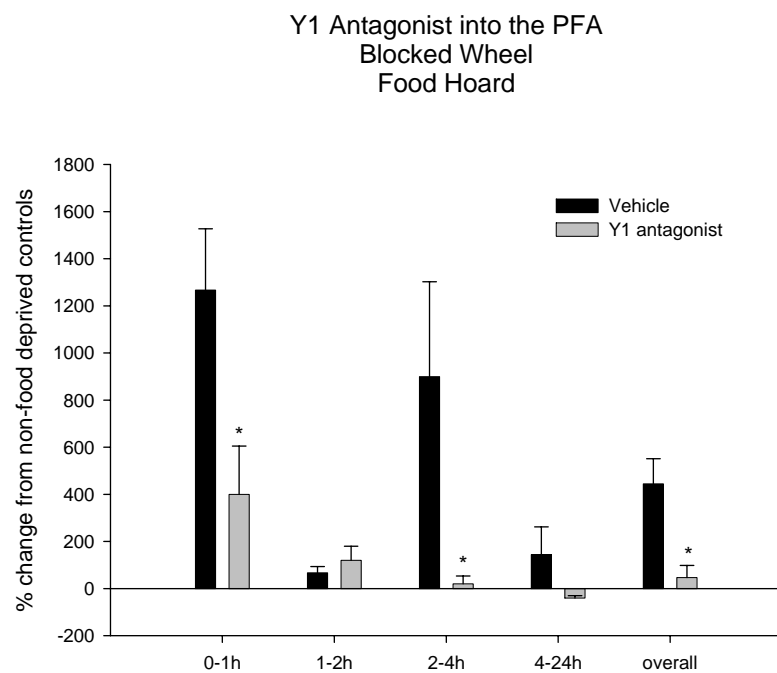
Figure 2.27a**Figure 2.27b**

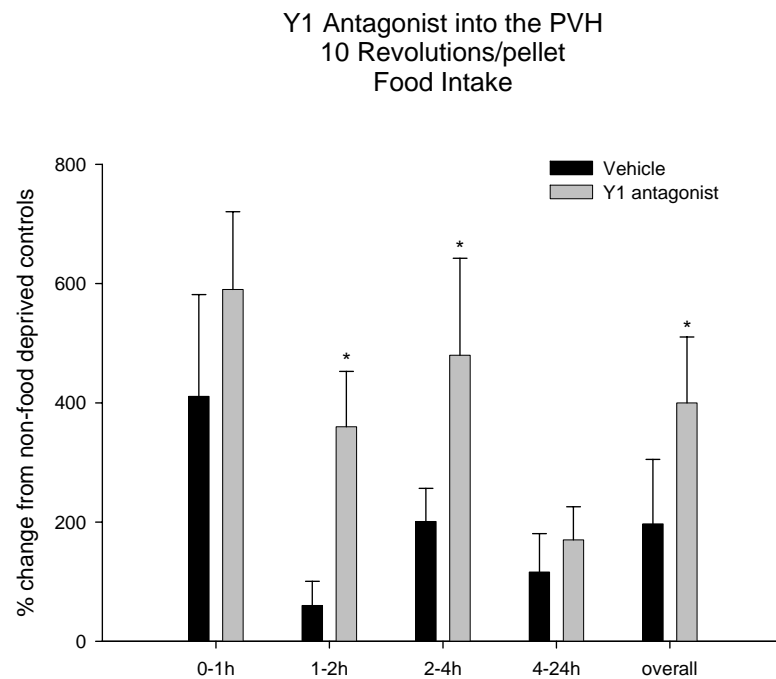
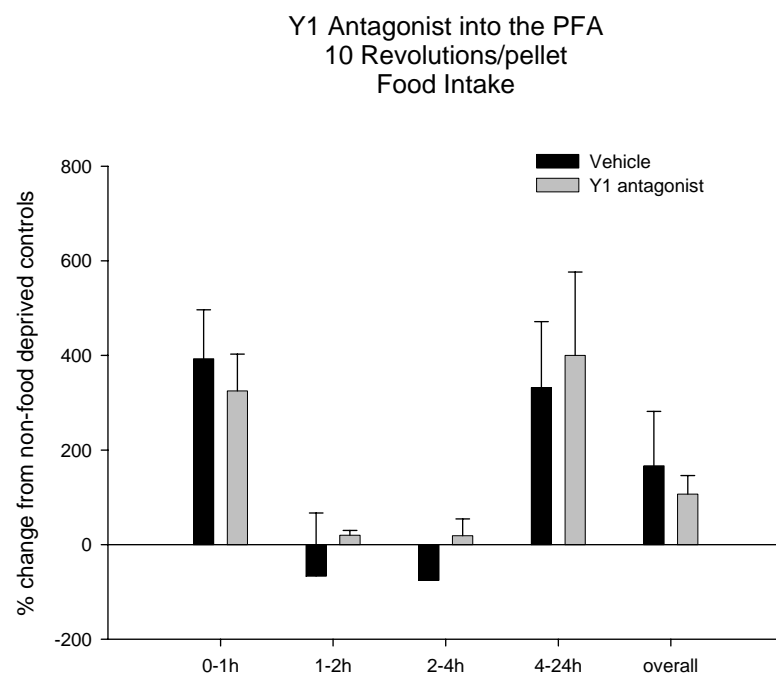
Figure 2.28a**Figure 2.28b**

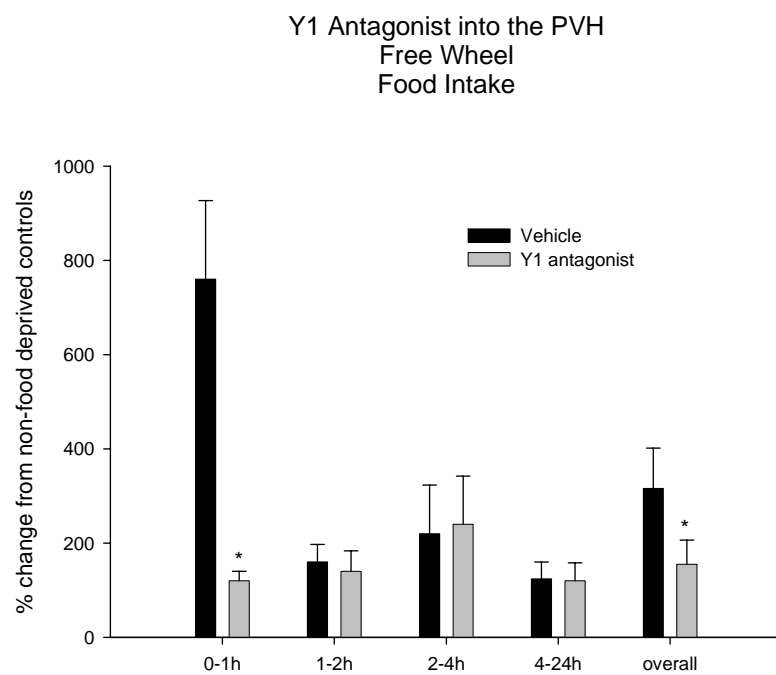
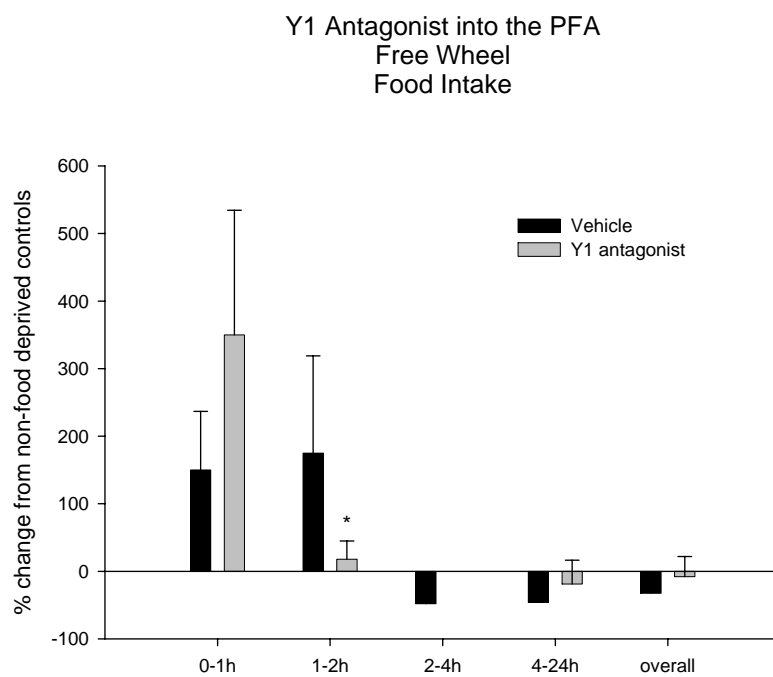
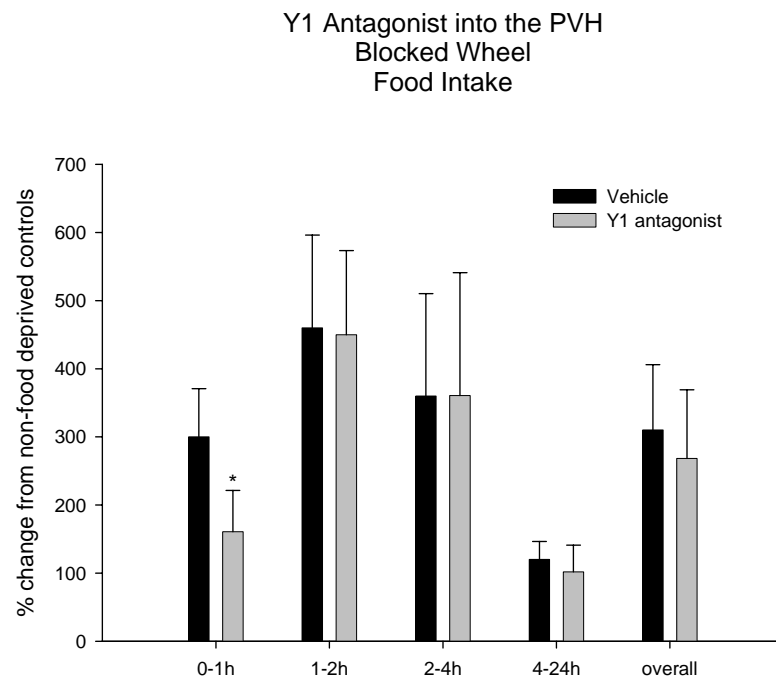
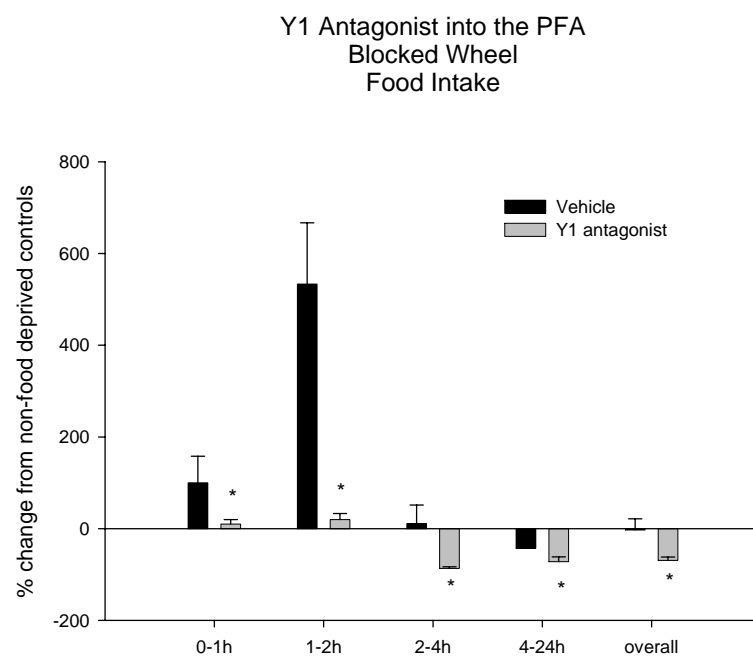
Figure 2.29a**Figure 2.29b**

Figure 2.30a**Figure 2.30b**

CHAPTER 3

DOES DESTRUCTION OF ALL NPY Y RECEPTORS IN THE PVH ABOLISH POST-FOOD DEPRIVATION INDUCED INCREASES IN FOOD HOARDING AND FORAGING IN SIBERIAN HAMSTERS?

Abstract

Obesity is an increasingly important health problem that is the result of energy intake chronically exceeding energy expenditure. It is important to understand the underlying alterations in physiology and behavior that occur when there is this deviation away from energy balance. Energy balance is normally maintained by adjustments in both consummatory ingestive behaviors (the actual consumption of food) and appetitive ingestive behaviors (foraging, transport and storage of food). Neuropeptide Y (NPY), injected directly in paraventricular nucleus of the hypothalamus (PVH), stimulates both phases of ingestion, increasing food intake in laboratory rats and food hoarding to a greater extent than food intake in Siberian hamsters. The role of the specific NPY receptor subtypes within the PVH in mediating these behavioral changes is not known. Therefore, I tested if the destruction of all Y receptor-containing neurons within the PVH using NPY conjugated to saporin (NPY-SAP) would alter baseline appetitive or consummatory ingestive behaviors and post-food deprivation induced increases in food hoarding and foraging in Siberian hamsters. The results show that NPY-SAP decreased foraging and food hoarding under baseline conditions with no change in food intake. After food deprivation, NPY-SAP inhibited post-food deprivation induced increases in foraging and food hoarding, as well as, decreased food intake. Thus, the NPY-PVH circuit may be needed for normal control of the appetitive ingestive behaviors of foraging and food hoarding and for the control of appetitive and consummatory ingestive behaviors when presented with a physiological challenge.

Introduction

Obesity is an increasingly important health problem that is the result of energy intake chronically exceeding energy expenditure. The significant increase in the rate of obesity is a cause for concern because obesity is linked with many pathological disorders, such as cardiovascular disease, stroke and type II diabetes (Vague et al., 1980; Satcher, 2001; Gasteyger and Tremblay, 2002). Combating this disease through diet and exercise alone has not been very successful. One reason for this lack of success is that current approaches to the obesity problem primarily have focused on controls of food intake alone. Food, however, almost always has to be acquired (foraging) and frequently is stored for subsequent consumption (hoarding). Even though humans do not have to expend much energy to acquire food, as do other animals, we still have to expend some energy and time to get food and alterations in how we acquire our food are seen under different physiological conditions. For example, if you go to the grocery store hungry, you will bring home more food than when you are full (Dodd et al., 1977; Beneke and Davis, 1985; Mela et al., 1996). Moreover, obese people bring home more high fat foods and more calories per person than lean people (Ransley et al., 2003e). Once food is acquired and stored in our refrigerators/freezers and pantries, we are more likely to eat this stored food than go out and acquire more food (Ransley et al., 2003d). Therefore, a more comprehensive approach of studying the underlying mechanisms in the appetitive ingestive behaviors of foraging and food storing (hoarding), in addition to the consummatory ingestive behavior of food intake, could provide an additional target for pharmaceutical or behavioral manipulations in the treatment and possibly prevention of obesity.

Although several peptides are involved in controlling energy balance, Neuropeptide Y (NPY) is one particular peptide that provides a potent orexigenic drive to alter both appetitive and consummatory ingestive behaviors in variety of species (Kalra et al., 1999; Schwartz et al., 2000; Day

et al., 2005). When animals are faced with negative energy balance, such as during food deprivation, NPY gene expression is increased within the arcuate nucleus of the hypothalamus ((ARC; (Brady et al., 1990;Hahn et al., 1998;Mizuno et al., 1999;Mercer et al., 2000b)). Central injection of NPY into the third ventricle increases food intake (consummatory ingestive behavior) in laboratory rats (Kalra and Kalra, 2000;Wirth and Giraudo, 2000), and increases food hoarding (appetitive ingestive behavior) to a greater extent than food intake in Siberian hamsters (Day et al., 2005). Thus, NPY appears to play a major role in the control of appetitive and consummatory ingestive behaviors to maintain energy balance.

NPY neurons are largely restricted to the arcuate nucleus of the hypothalamus (ARC; (Chronwall et al., 1985e;White and Kershaw, 1989)), with low levels of expression within the compact zone of the dorsomedial hypothalamus (DMH; (Li et al., 1998)) and the brainstem catecholamine cell groups A1, C1-C3 (Everitt et al., 1984f). Even though these NPY neurons have extensive efferent projections to numerous brain regions, the paraventricular nucleus of the hypothalamus (PVH) is one converging site that is considered a key regulatory element in the control of both appetitive and consummatory ingestive behaviors (de Quidt and Emson, 1986;Broberger et al., 1998;Broberger et al., 1999c). NPY administered into the PVH potently stimulates food intake in rats (Stanley and Leibowitz, 1985) and food hoarding to a greater extent than food intake in Siberian hamsters (Day et al., 2005). In addition, NPY release within the PVH has been shown to change appropriately during the pre- and postmeal periods in response to food deprivation and food restriction (Yoshihara et al., 1996a;Yoshihara et al., 1996b;Jain et al., 1998). The PVH also has neurons that express four of the five NPY receptor subtypes that have been cloned, Y1, Y2, Y4, Y5 (Inui, 1999;Parker and Herzog, 1999). Although the exact role of each of the NPY receptor subtypes is unclear, there is extensive evidence for the involvement of Y1 and Y5

receptor subtypes in the control of ingestive behaviors. Y1 receptor agonists administered into the PVH, stimulate hyperphagia in laboratory rats (O'Shea et al., 1997b), whereas antagonists significantly reduce the hyperphagia induced by centrally-administered NPY or food deprivation (O'Shea et al., 1997a; Wieland et al., 1998; Morgan et al., 1998). Y5 agonists injected into the PVH increase food intake and a Y5 receptor antagonist blocks NPY-induced feeding (Kask et al., 1998; Yokosuka et al., 2001). In Chapter 2, antagonism of Y1 receptor in the PVH blocked post-food deprivation induced increases in food hoarding with no inhibition of foraging or food intake. Thus, there is evidence for the involvement of specific Y receptors in the PVH in controlling both appetitive and consummatory ingestive behaviors.

Although studies using Y agonists/antagonists for Y1 or Y5 receptor subtypes have been useful in identifying their roles in controlling appetitive and consummatory behaviors, the lack of subtype specific ligands for Y2 or Y4 has limited the success of these studies to clarify the relative importance of these Y receptors within the PVH. In addition, the ability of specific NPY receptor subtype agonists to increase food hoarding or food intake does not mimic the increases in both behaviors after NPY injection alone in Siberian hamsters (Day et al., 2005). This leads us to believe that other Y receptor subtypes may contribute to controlling these behaviors. Therefore, we tested the effect of destroying all Y receptor-containing neurons within the PVH using a ribosomal inactivating toxin, saporin, conjugated to NPY (NPY-SAP). Saporin is a type 1 ribosomal inactivating protein (Ferraras et al., 1993) that can be targeted to destroy specific populations of neurons by conjugation with antibodies that are selectively internalized by the targeted cell population (Wiley and Kline IV, 2000). Using NPY-SAP would allow us to determine the role of NPY in the PVH on both appetitive and consummatory ingestive behaviors. This was accomplished by injecting NPY-SAP or a Blank-SAP control into the PVH of Siberian hamsters and measuring

foraging, food hoarding, and food intake under baseline conditions and after an energetic challenge of food deprivation.

Methods

Animals and Housing

Adult male Siberian hamsters ~3 months old and weighing 35-46 g were obtained from our breeding colony. The colony was established in 1988 and its genealogy was described recently (Day and Bartness, 2001). Hamsters were group-housed and reared from birth in a 16:8 h light-dark cycle (lights-on at 2030). Room temperature was maintained at 21 ± 2 °C and relative humidity was 50 ± 10 %. All procedures were approved by the Georgia State University Institutional Animals Care and Use Committee and were in accordance with the Public Health Service and United States Department of Agriculture guidelines.

Animals for Experiments 1 and 2 were transferred from group-housing and acclimated for one wk in our hoarding/foraging apparatus as previously shown and described (Day and Bartness, 2001). Briefly, two cages were connected with a convoluted polyvinyl-chloride tubing system (38.1 mm inner diameter and ~1.52 m long), with corner and straightways for both horizontal and vertical climbs. The top or “food cage” was 456 x 234 x 200 mm (length x width x height) equipped with a water bottle and running wheel. The bottom or “burrow cage” was 290 x 180 x 130 mm and was covered to simulate the darkness of a natural burrow. The burrow cage contained Alpha-Dri (Specialty Papers, Kalamazoo, MI) bedding and cotton nesting material. The animals were fed 75 mg pellets (Purified Rodent Diet; Research Diets, New Brunswick, NJ) and tap water were available *ad libitum* during this period.

Measurement of Foraging, Food Hoarding, and Food Intake

Foraging (pellets earned) was defined as the number of pellets delivered upon completion of the requisite wheel revolutions. Food hoarding (pellets earned) was defined as the number of pellets found in the bottom “burrow” cage in addition to those removed from the cheek pouches. For the 10 rev group, food intake (pellets eaten) was defined as pellets earned – surplus pellets – hoarded pellets = food intake. For the free and blocked wheel groups, food intake (pellets eaten) was defined as pellets given – pellets left in the top cage – hoarded pellets = food intake. The electronic balance used to weigh the food pellets was set to “parts” measurement rather than obtaining fractions of a pellet in milligrams; thus, the smallest unit measured was one 75-mg food pellet and equal to 1.

Training and Baseline Measures

At the end of the acclimation period, 64 animals were trained to forage for their food based on procedures previously published (Day and Bartness, 2001). In brief, hamsters were given free access to food for 2 d while they adapted to the running wheel. In addition to the free food, a 75 mg food pellet was dispensed upon completion of every 10 wheel revolutions. Wheel revolutions were counted using a magnetic detection system and monitored by a computer-based hardware/software system (Med Associated, Lancaster, NH). On the third day, the free food condition was replaced by a response-contingent condition in which only every 10 wheel revolutions triggered the delivery of a pellet. This condition was in effect for the remaining 5 d of the 1 wk-long training period. The hamsters were then separated into 12 groups: 2 drug groups (NPY-SAP or B-SAP), 2 feeding conditions (Food deprived or Non-Food deprived), and 3 foraging groups that were matched for percent change in body mass and average hoard size.

The three foraging groups were 10 revolutions/pellet (10 Rev), Free Wheel/Free Food (FW; food was available non-contingently [not earned]), but the running wheel was active [locomotor activity control group]) or Blocked Wheel/Free Food (BW; food was available non-contingently [not earned], but the running wheel was blocked [sedentary control group]).

Intracranial injections

For PVH administration of NPY-SAP and the blank saporin control solution (B-SAP; Advanced Targeting Systems, San Diego, CA), hamsters were anesthetized with isoflurane and fur at the top of the head was removed to expose the area to be incised. A hole was trephined at the stereotaxic coordinates above the PVH and the injector was lowered into the PVH (AP -0.3 mm, ML-0.3 mm, DV-6.0 mm). Injections of 48 ng in 100 nl per side of NPY-SAP or B-SAP were delivered bilaterally (100 nl/side) into the PVH. The dose of NPY-SAP was chosen based on previously published data showing it to be effective at producing significant destruction of Y receptor-containing neurons (Bugarith et al., 2005g). Injections were made through an internal cannula (26-gauge stainless steel; Plastics One, Roanoke, VA) connected to a 0.5 μ l microsyringe with polyethylene tubing. The solution was delivered slowly over a 5-min period. Fresh apple slices were given to facilitate fluid and caloric intake for the first 2-3 d post surgery. The animals also received subcutaneous buprenorphine (0.05 mg/kg, s.c.) injections for 2 d after surgery. Animals then recovered for 7 d in single-shoebox cages with food and water available *ad libitum*. After recovery, hamsters were returned to their respective hoarding/foraging cages maintaining the same group membership.

Experimental protocol

The time course adopted to initiate and terminate behavioral testing was chosen based on previously published data of NPY-SAP effects on NPY terminals in the hypothalamus (Bugarith et al., 2005f). In that study, a significant reduction of terminal was present by the second week after injection. Therefore, feeding studies were conducted to begin approximately at this time. From Day 8 until Day 21 after injection of NPY-SAP or B-SAP, daily measurements of foraging, food hoarding and food intake were taken just before lights out. On Day 22 after injection, a subset of animals in each group was food deprived for 56 h. Two hours before the onset of the dark phase before refeeding, food was removed from the hamsters' pouches of the non-food deprived group and all animals were placed in clean burrow cages with access to the tubes blocked before refeeding. At the onset of the dark phase, access to the tubes and top cage was restored. Foraging, food hoarding, and food intake were measured 1 h, 2 h, 4 h, and 24 h after refeeding.

Tissue preparation

After the 24 h data was collected, animals were deeply anesthetized with pentobarbital sodium (80 mg/kg ip) and perfused transcardially with 100 ml of 0.15 M saline followed by 150 ml of 4 % paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were extracted and post-fixed for 48 h at 4 °C, followed by a submersion in 30 % sucrose for at least 24 h at 4 °C. Brains were then frozen and sectioned on a microtome into 30 µm section through the hypothalamus and distributed serially into five sets. The first set was mounted immediately onto gelatinized glass slides and counter-stained with cresyl violet. The remaining sections were stored in cryoprotectant for storage at -20 °C until immunohistochemical processing.

Lesion verification using immunohistochemistry

Free-floating sections were removed from the cryoprotectant and washed with PBS. All washes and incubations occurred at room temperature. Sections were then incubated in 0.03 % H_2O_2 (Fisher Scientific, Houston, TX) and 0.3 % Triton X-100 (Sigma, St. Louis, MO) in PBS for 15 min. Sections were then transferred to wells containing primary antisera, either rabbit anti-Y1 receptor (1:1000; ImmunoStar Inc., Hudson, WI), sheep anti-NPY (1:10,000; Chemicon, Temecula, CA), rabbit anti-tyrosine hydroxalase (1:10,000; Chemicon, Temecula, CA), or rabbit anti-alphaMSH (1:10,000; ImmunoStar, Hudson, WI), diluted in 3 % normal donkey serum and 0.3 % Triton X-100 in PBS for 48 h. Sections were then washed with PBS and incubated in secondary antisera for 2 h using either donkey anti-goat or donkey anti-sheep (1:500; Jackson ImmunoResearch, West Grove, PA). Secondary antibodies were diluted in 3 % normal donkey serum corresponding to the host species of the secondary antibody and 0.3 % Triton X-100 in PBS. After washing with PBS, sections were incubated in a 1:222 solution of the avidin-biotin-peroxidase complex (Vector Elite kit; Vector Laboratories, Burlingame, CA) in 0.3 % Triton X-100 in PBS for 1 h at RT. Sections were then washed with PBS, followed by visualization by reacting the sections with 3,3'-diaminobenzidine (0.2 mg/ml; Sigma, St. Louis, MO) and 0.025 % H_2O_2 in PBS for 5-10 min. The reaction was terminated with PBS washes. Sections were mounted on gelatinized slides and dehydrated with increasing concentration of alcohol followed by Xylenes (Fisher Scientific, Fair Lawn, NJ) and then coverslipped with Permount (Fisher Scientific, Fair Lawn, NJ).

Statistical analysis

Behavioral measures were analyzed using a three-way mixed model ANOVA with repeated measures (3 x 4 x 4; foraging group x drug x time) using Number Crunching Statistical Software v 2000 (Kaysville, UT). Duncan's new multiple range tests were used for post hoc tests when appropriate. Differences among groups were considered statistically significant if $P_s < 0.05$. Exact probabilities and test values were omitted for simplicity and clarity of the presentation.

Results

Destruction of PVH. NPY-SAP-treated animals had decreased cellularity in the PVH compared with B-SAP-treated animals as assessed by cresyl violet staining sections and a decrease in Y1 receptor, OT and AVP immunoreactive cells ($P_s < 0.05$; Fig. 3.0). B-SAP did not cause comparable destruction to PVH cells.

Wheel Revolutions. Under baseline conditions, NPY-SAP significantly decreased wheel running by the FW group compared with the B-SAP animals ($P_s < 0.05$; Fig. 3.1b). After food deprivation, NPY-SAP treated hamsters significantly increased wheel running at 0-1 h compared with B-SAP/food deprived animals ($P_s < 0.05$; Fig. 3.4). This was followed by a significant decrease in wheel running by the NPY-SAP/food deprived animals compared with all other groups at 2-4 h, 4-24 h and cumulative for the 24 h test ($P_s < 0.05$; Fig. 3.4).

Foraging. Under baseline conditions, NPY-SAP significantly decreased foraging in the 10 Rev group compared with B-SAP animals ($P_s < 0.05$; Fig. 3.1a). After food deprivation, NPY-SAP significantly inhibited the post-food deprivation increase in foraging compared with B-

SAP/fasted animals at 0-1 h, 2-4 h, 4-24 h and cumulatively across the 24 h test ($P_s < 0.05$; Fig. 3.4a). In addition, NPY-SAP significantly decreased foraging when NPY-SAP food deprived and non-food deprived animals were compared with B-SAP food deprived and non-food deprived animals at 2-4 h, 4-24 h and cumulative for the 24 h test ($P_s < 0.05$; Fig. 3.4a).

Food Hoarding. Under baseline conditions, NPY-SAP treated hamsters had significantly decreased food hoarding compared with B-SAP animals in the 10 Rev and FW groups ($P_s < 0.05$; Figs. 3.2a and 3.2b). In the BW group, NPY-SAP significantly increased food hoarding compared with B-SAP treatment ($P_s < 0.05$; Fig. 3.2c). After food deprivation, NPY-SAP inhibited the post-food deprivation induced increase in food hoarding compared with B-SAP in all foraging conditions across time ($P_s < 0.05$; Fig. 3.5). In the 10 Rev group, NPY-SAP inhibited post-food deprivation increases in hoarding at 1-2 h, 2-4 h, 4-24 h and cumulatively across the 24 h test ($P_s < 0.05$; Fig. 3.5). In the FW group, NPY-SAP inhibited the post-food deprivation increase in hoarding at 0-1 h, 1-2 h, 2-4 h and cumulatively across the 24 h test ($P_s < 0.05$; Fig. 3.5). In the BW group, NPY-SAP inhibited the post-food deprivation increase in hoarding at 0-1 h, 4-24 h and cumulatively across the 24 h test ($P_s < 0.05$; Fig. 3.5).

Food Intake. Under baseline conditions, NPY-SAP had no effect on food intake compared to B-SAP treated hamsters in the 10 Rev and FW groups (Figs. 3.3a, 3.3b). In the BW group, NPY-SAP significantly increased food intake compared with B-SAP treated hamsters ($P_s < 0.05$; Fig. 3.3c). After food deprivation, NPY-SAP/food deprived and non-food deprived animals significantly decreased food intake compared with B-SAP/food deprived and non-food deprived animals in the 10 Rev group at 2-4 h and cumulatively across the 24 h test ($P_s < 0.05$; Fig. 3.6).

In the FW group, NPY-SAP/food deprived animals significantly increased food intake compared with B-SAP/fasted animals at 0-1 h, 1-2 h, 2-4 h and cumulatively across the 24 h test ($P_s < 0.05$; Fig. 3.6). In the BW group, NPY-SAP had no effect on food intake after food deprivation (Fig. 3.6).

Discussion

The results of the present study show that the destruction of Y receptor-containing neurons in the PVH by NPY-SAP decreased foraging and food hoarding under baseline conditions with no change in food intake. After food deprivation, NPY-SAP inhibited post-food deprivation induced increases in foraging and food hoarding, as well as, decreased food intake (there was not a post fast-induced increase in food intake). In Chapter 2, Y1 receptor alone inhibited post-food deprivation induced increases in food hoarding, but not foraging. In this study, blocking the effect of all NPY receptors inhibited post-food deprivation increases in both foraging and food hoarding. Thus, by subtractive reasoning, receptor subtypes other than PVH NPY Y1 receptors are likely involved in controlling foraging within the PVH.

Studies using various animal models have shown the potent effect of NPY and specific Y receptor subtypes at stimulating both appetitive (see Chapter 2) and consummatory ingestive behaviors in the PVH (Stanley and Leibowitz, 1984; Stanley and Leibowitz, 1985; Kalra et al., 1991a). There have been studies, however, that have suggested that NPY and its receptors only mediate changes in food intake in laboratory rats after exogenous NPY administration or food deprivation, but do not control food intake under normal conditions (Kalra et al., 1991b; Bugarith et al., 2005e). In support of the notion that NPY may not mediate changes in food intake under normal conditions are studies of NPY and Y receptor knockout models. Specifically, there is no change in

food intake in NPY null mice (Hollopeter et al., 1998) and Y1 (Kushi et al., 1998) or Y5 (Marsh et al., 1998) receptor null mice under normal conditions. In the present study, NPY-SAP treatment did not affect food intake under baseline conditions. It was only after food deprivation that food intake was significantly decreased by NPY-SAP treatment. This suggests that NPY and the Y receptors in the PVH are needed under such challenging physiological conditions, but this circuit may not be needed for normal feeding. This is the first study to suggest that Y receptor bearing PVH neurons are needed for both foraging and food hoarding under baseline as well as energetically challenging conditions.

In this study, we used a foraging/hoarding paradigm that incorporates a simulated burrow housing system to study food hoarding and a wheel running-based model to study foraging (Perrigo and Bronson, 1985; Bartness and Clein, 1994). Using this housing system not only allows for the study of both appetitive and consummatory ingestive behaviors, but also yields two important characteristics of foraging and hoarding in a natural setting - effort and distance. The results of this study show the importance of including both of these characteristics in a model system designed to study the physiological mechanisms involved in overall energy balance because the effect of NPY-SAP varied based on the foraging requirement. That is, under baseline conditions, both 10 Rev and FW animals were equally affected by NPY-SAP with decreases in foraging and food hoarding and no change in food intake. In the NPY-SAP BW group, however, food hoarding and food intake were increased under baseline conditions compared with the B-SAP controls. This suggests that the functioning of the NPY-PVH circuitry underlying food hoarding and intake is altered by increases in energy expenditure as undoubtedly occurred in the 10 Rev hamsters required to wheel run for their food as well as the FW hamster that expended more energy through voluntary wheel running than did the sedentary control hamsters of the BW group. After food deprivation, however, NPY-SAP

inhibited the post-food deprivation increase in food hoarding in all groups regardless of whether they were wheel running or sedentary. Thus the energetic challenge of the 56 h food deprivation overcame any dysfunction of the NPY-PVH circuitry either through toxin surviving Y receptor bearing neurons and their efferents and/or via non-NPY mediated mechanisms. Because the NPY-SAP inhibited this response in all three groups, it suggests that the NPY-PVH circuit is necessary for this response. By contrast, food deprivation-triggered changes in food intake were differentially affected by NPY-SAP treatment. That is, NPY-SAP decreased food intake after food deprivation only by the 10 Rev group, whereas food intake remained unchanged by the FW and BW groups and was similar to that of B-SAP food deprived controls. Regardless, as is usually seen in this (Bartness and Clein, 1994; Day and Bartness, 2003) and other hamster species (Lea and Tarpy, 1986; Schneider and Buckley, 2001) food deprivation did not increase food intake.

The PVH serves as an integrator and link between the neuroendocrine and autonomic nervous systems where NPY may be able to affect numerous circuits involved in energy homeostasis and generate appropriate responses to modulate ingestive behaviors. NPY Y receptors are found throughout the PVH of rodents and are colocalized with many other neuropeptides or hormones that are important regulators of energy balance and endocrine axes (Parker and Herzog, 1999). A subpopulation of corticotropin-releasing hormone (CRH) neurons express the Y5 receptor, but not the Y1 receptor (Campbell, 2000; Li, 2000). Y1 positive nerve terminals, however, are in close proximity to CRH neurons and may suggest that NPY has both pre-and post-synaptic actions on these neurons (Li, 2000). Y1 receptors are expressed on thyrotropin-releasing hormone (TRH) neurons within the PVH (Broberger et al., 1999c). In addition, the Y5 receptor is expressed on both oxytocinergic (OT) and arginine vasopressin (AVP) neurons within the PVH that are important for autonomic control of energy expenditure (Watts, 2000; Campbell, 2001). These data provide

morphological evidence for the role of NPY in the PVH influencing energy metabolism through actions affecting downstream hypothalamic-pituitary axes and the autonomic nervous system through Y receptor-mediated mechanisms.

Collectively, the results of the present study show that the NPY-PVH circuit may be needed for normal control of the appetitive ingestive behaviors of foraging and food hoarding and for the control of appetitive and consummatory ingestive behaviors when presented with a physiological challenge. In addition, this study presents a model that could be used to investigate the role of NPY in the PVH in mediating effects on downstream endocrine or autonomic systems.

Figure Captions

Figure 3.0. Representative coronal sections of the PVH showing effects of Blank-Saporin (B-SAP; *left*) or NPY-Saporin (NPY-SAP; *right*) on cresyl violet (a), Y1 receptor (b), arginine vasopressin (AVP; c) and oxytocin (OT; d).

Figure 3.1. Mean \pm SEM baseline foraging for the 10 Revolutions/pellet group (10 Rev; a) and wheel revolutions for the Free Wheel group (FW; b) 8-21 d after injection of Blank-Saporin (B-SAP) or NPY-Saporin (NPY-SAP). * $P_s < 0.05$ compared with B-SAP animals.

Figure 3.2. Mean \pm SEM baseline food hoarding for 10 Revolutions/pellet group (10 Rev; a), Free Wheel group (FW; b) and Blocked Wheel group (BW; c) 8-21 d after injection of Blank-Saporin (B-SAP) or NPY-Saporin (NPY-SAP). * $P_s < 0.05$ compared with B-SAP animals.

Figure 3.3. Mean \pm SEM baseline food intake for 10 Revolutions/pellet group (10 Rev; a), Free Wheel group (FW; b) and Blocked Wheel group (BW; c) 8-21 d after injection of Blank-Saporin (B-SAP) or NPY-Saporin (NPY-SAP). * $P_s < 0.05$ compared with B-SAP animals.

Figure 3.4. Mean \pm SEM foraging for the 10 Revolutions/pellet group (10 Rev; a) and wheel revolutions for the Free Wheel group (FW; b) after food deprivation for Blank-Saporin (B-SAP) and NPY-Saporin (NPY-SAP) animals. * $P_s < 0.05$ compared with B-SAP/Food deprived animals.

$P_s < 0.05$ compared with B-SAP animals.

Figure 3.5. Mean \pm SEM food hoarding for the 10 Revolutions/pellet group (10 Rev; a), Free Wheel group (FW; b) and Blocked Wheel group (BW; c) after food deprivation for Blank-Saporin (B-SAP) and NPY-Saporin (NPY-SAP) animals. *Ps<0.05 compared with B-SAP/Food deprived animals. #Ps<0.05 compared with B-SAP animals.

Figure 3.6. Mean \pm SEM food intake for the 10 Revolutions/pellet group (10 Rev; a), Free Wheel group (FW; b) and Blocked Wheel group (BW; c) after food deprivation for Blank-Saporin (B-SAP) and NPY-Saporin (NPY-SAP) animals. *Ps<0.05 compared with B-SAP/Food deprived animals. #Ps<0.05 compared with B-SAP animals.

Figure 3.0a

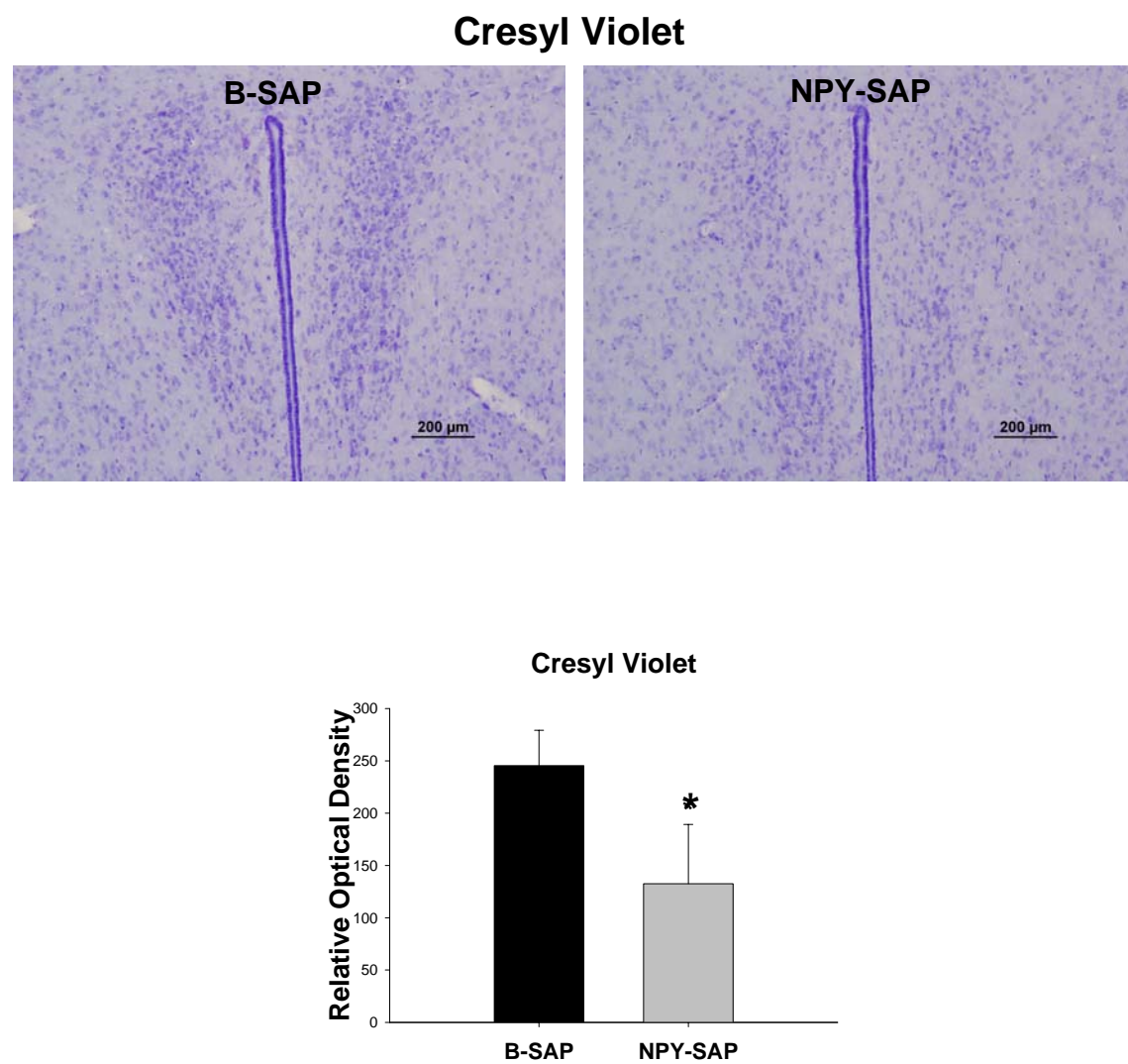


Figure 3.0b

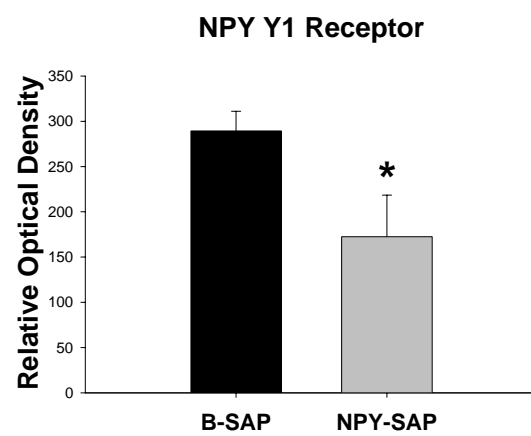
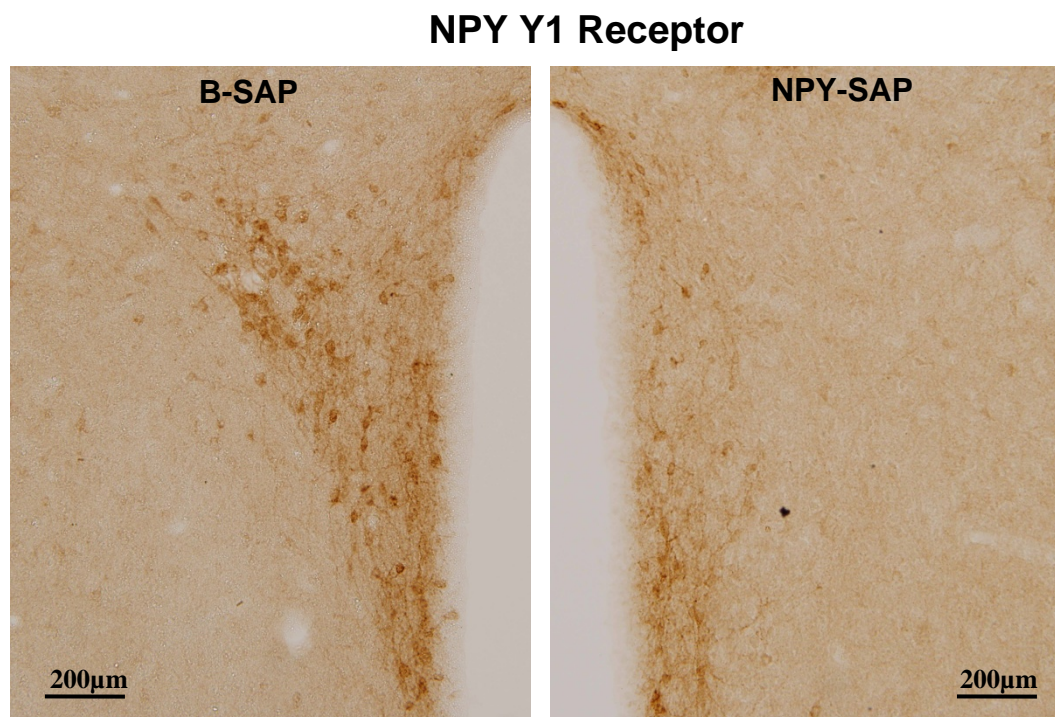


Figure 3.0c

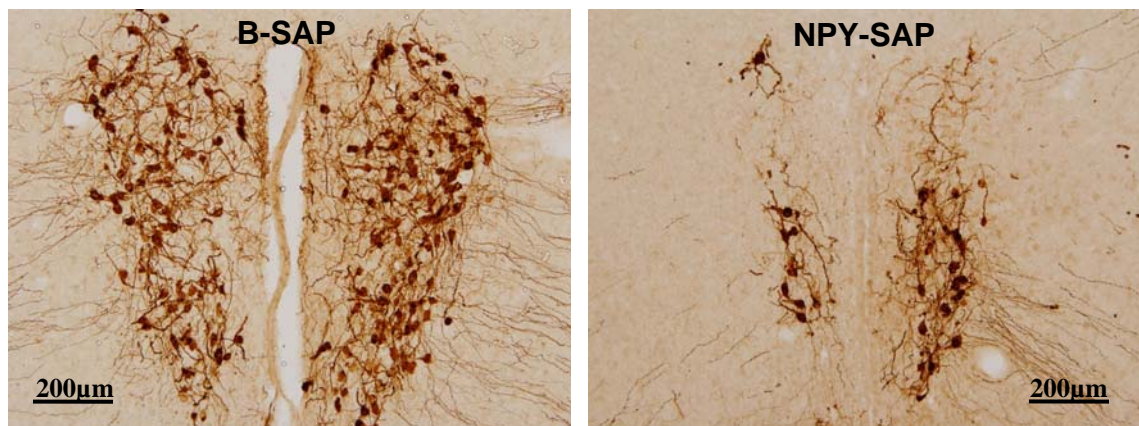
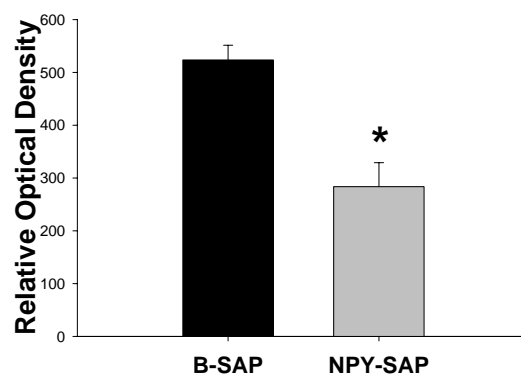
Arginine Vasopressin**Arginine Vasopressin**

Figure 3.0c

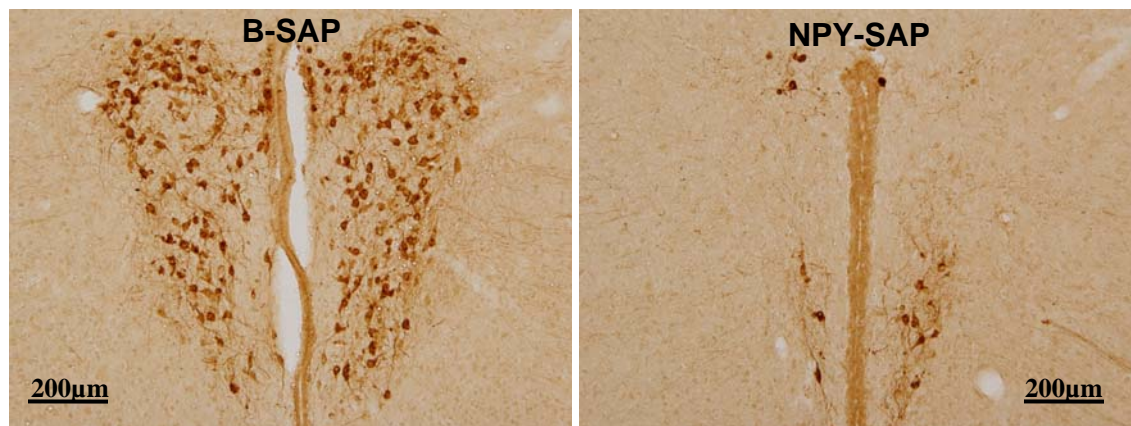
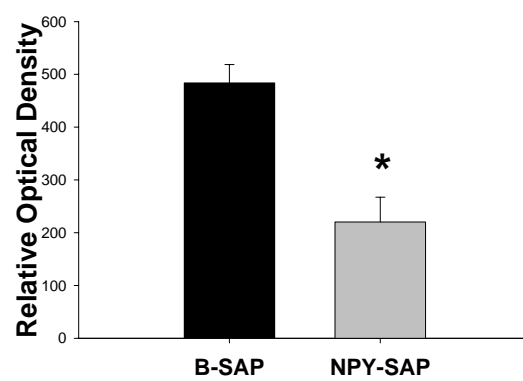
Oxytocin**Oxytocin**

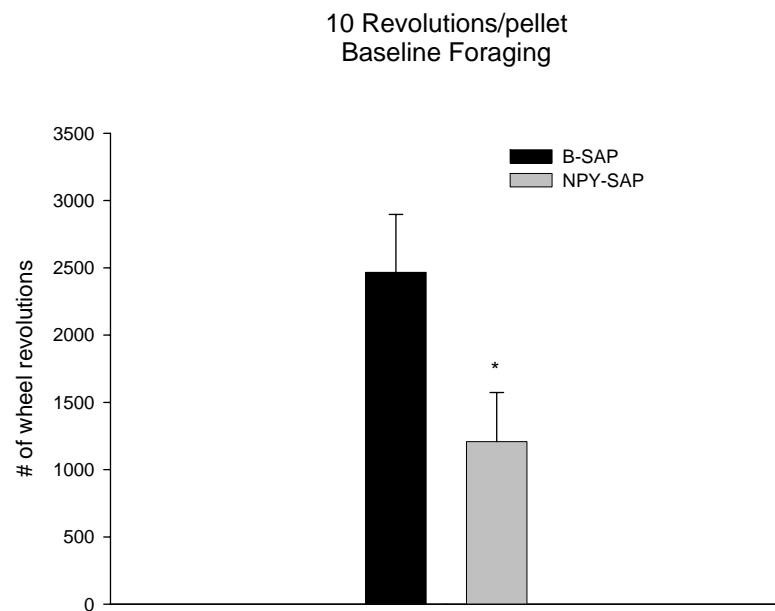
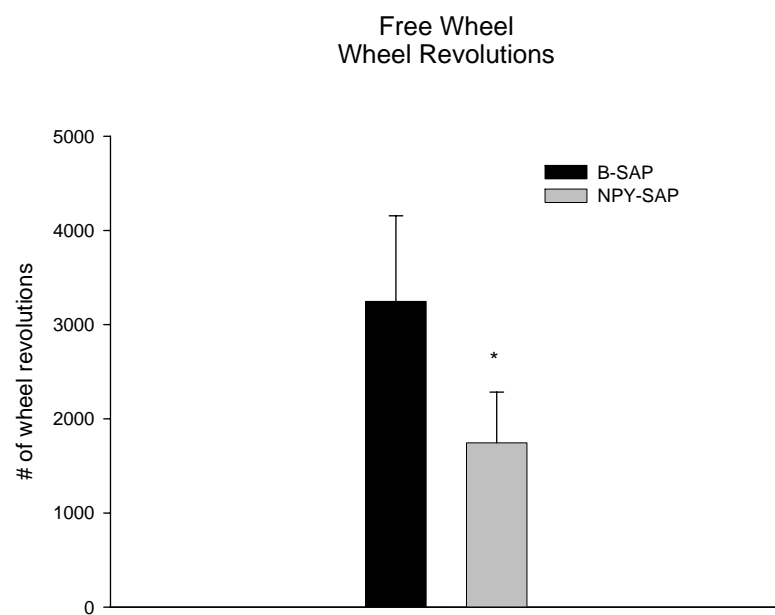
Figure 3.1a**Figure 3.1b**

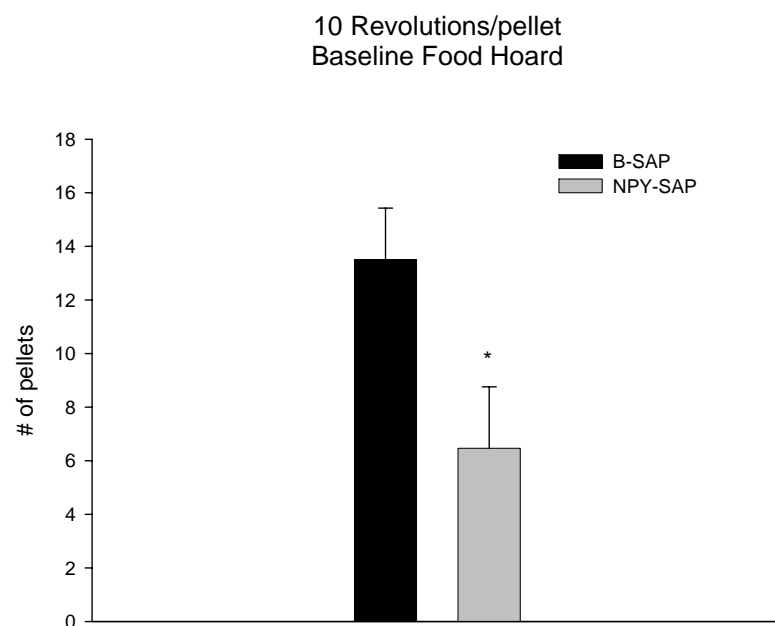
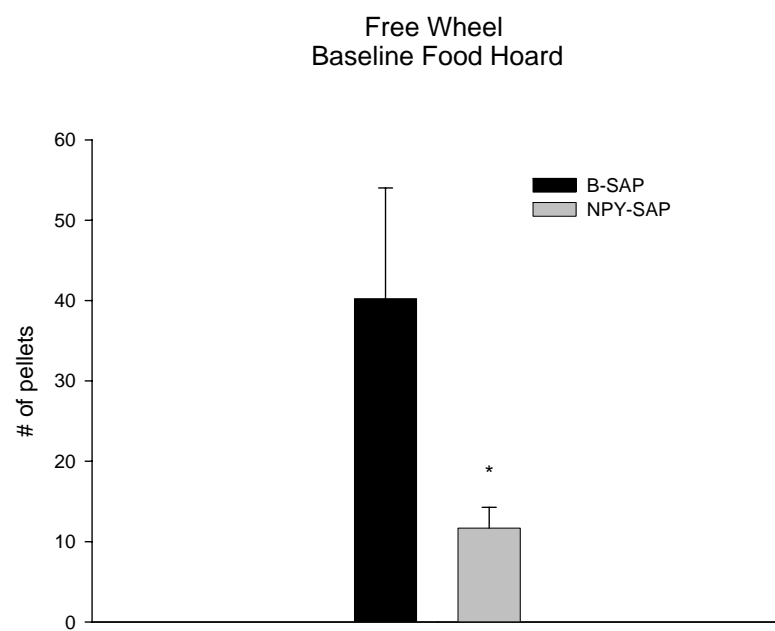
Figure 3.2a**Figure 3.2b**

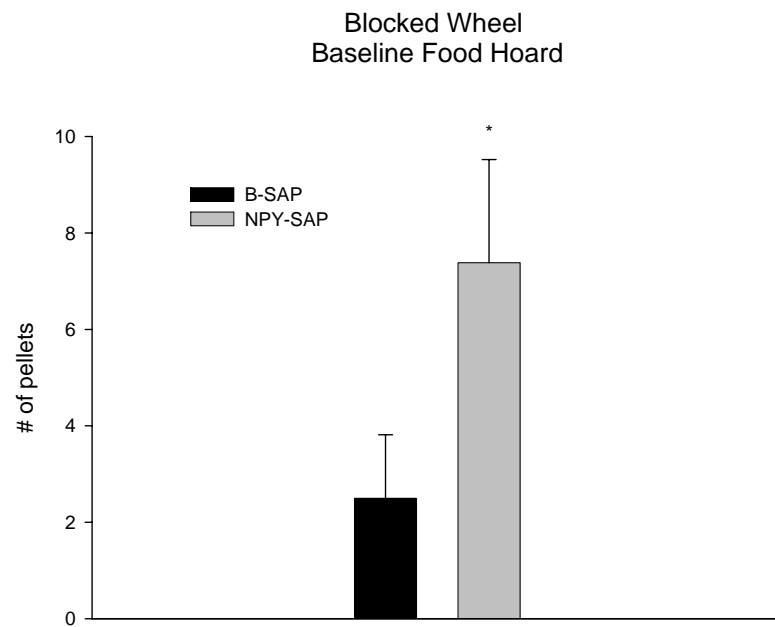
Figure 3.2c

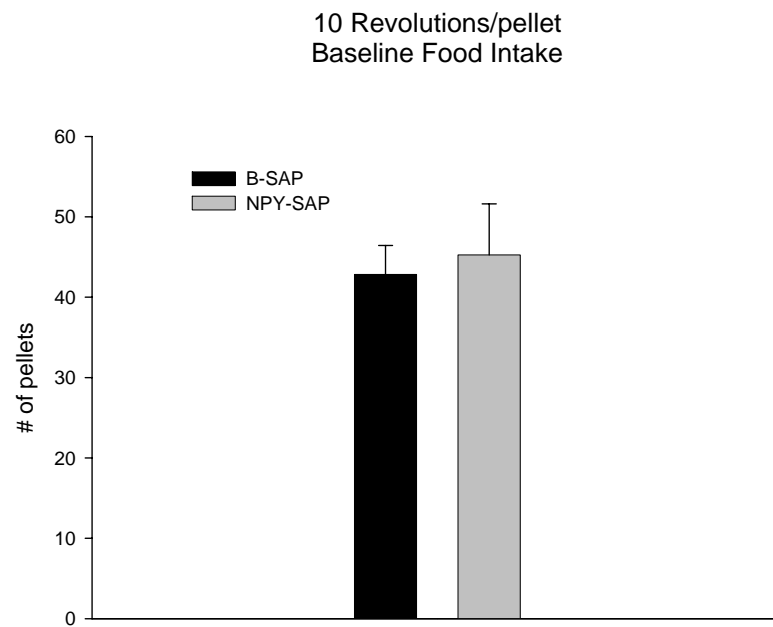
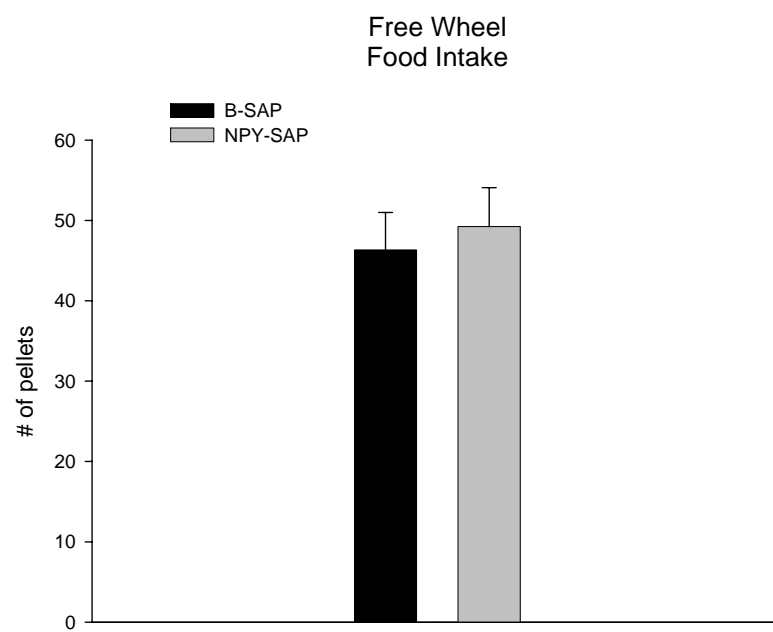
Figure 3.3a**Figure 3.3b**

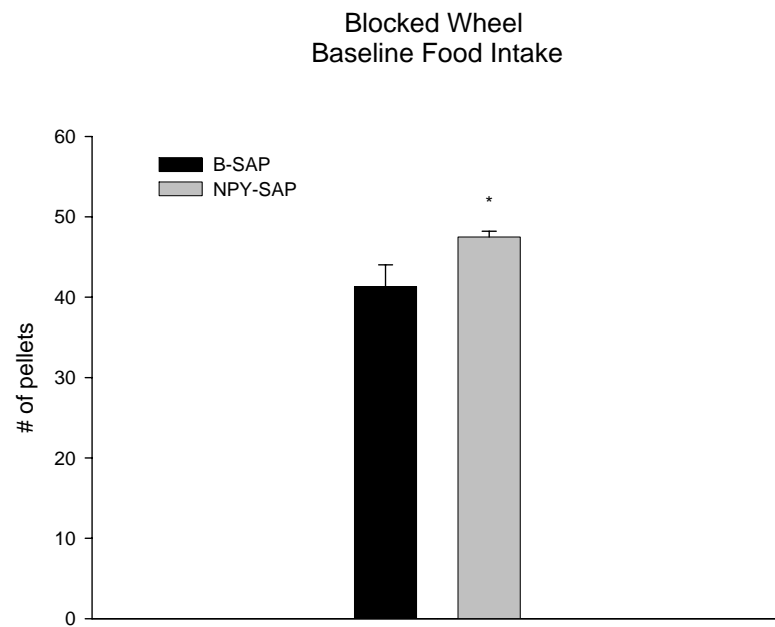
Figure 3.3c

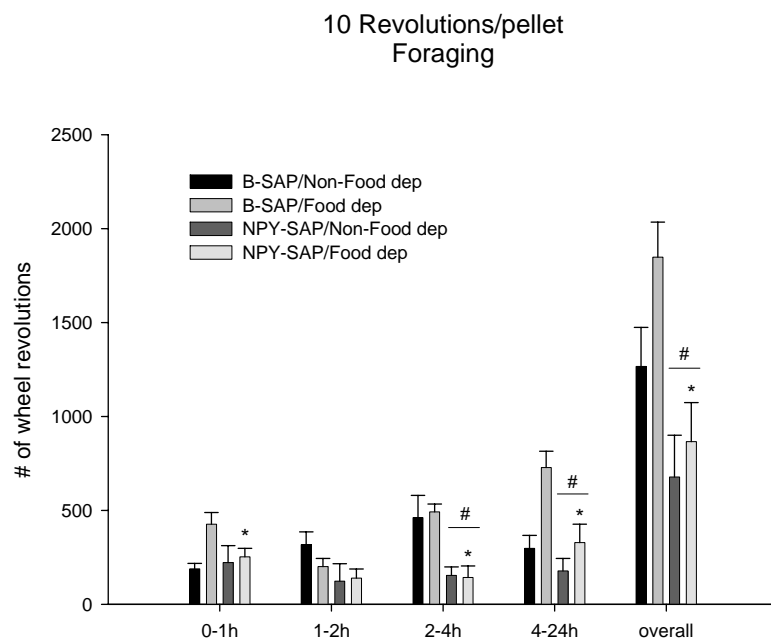
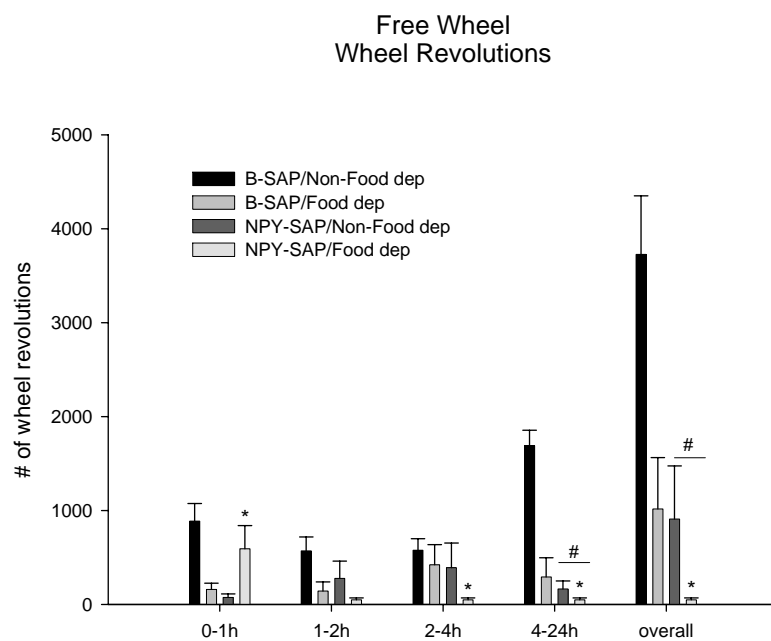
Figure 3.4a**Figure 3.4b**

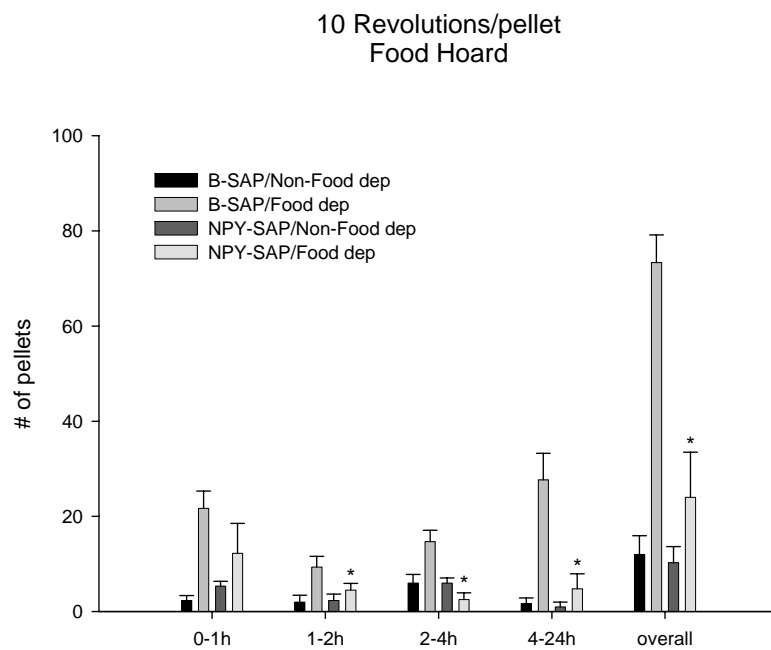
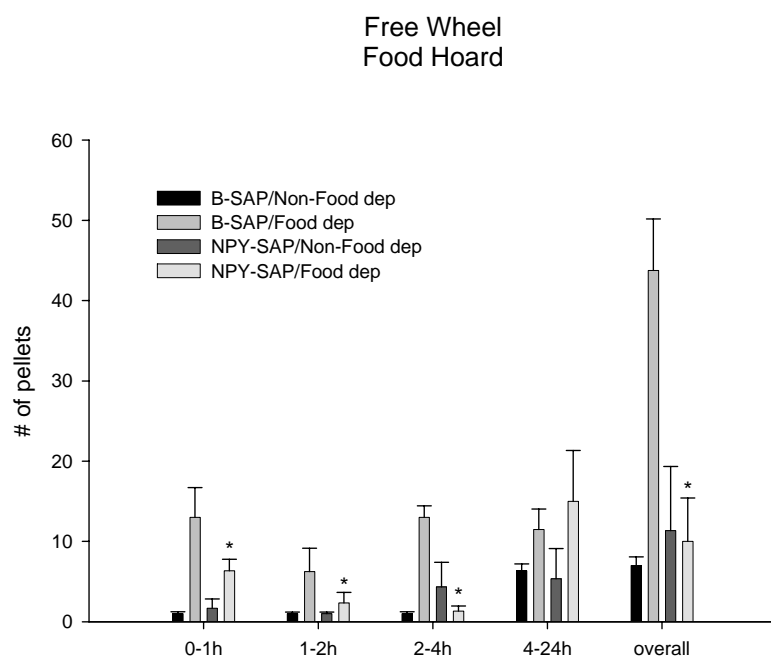
Figure 3.5a**Figure 3.5b**

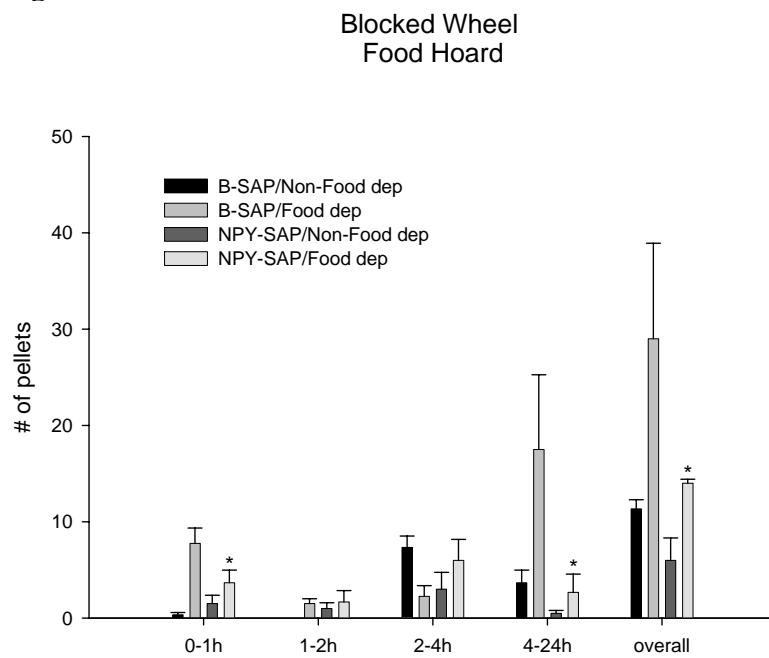
Figure 3.5c

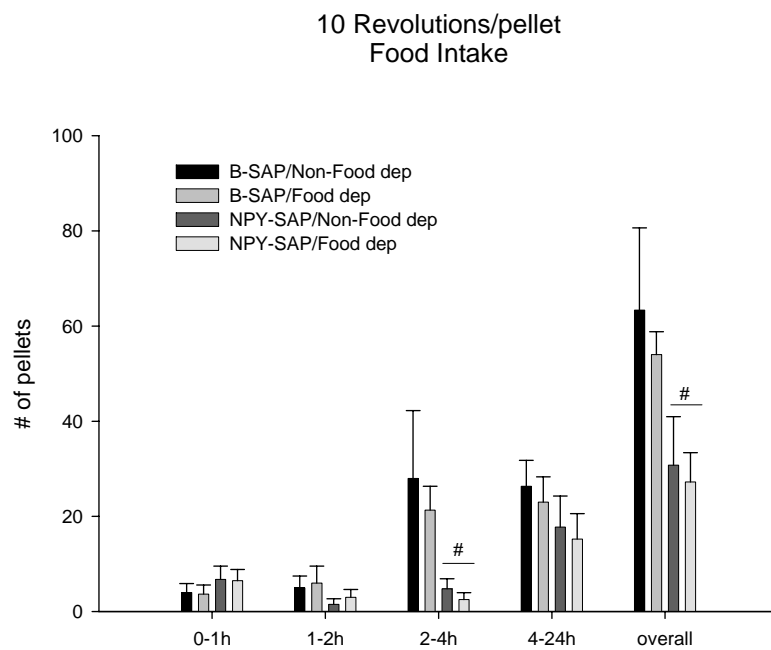
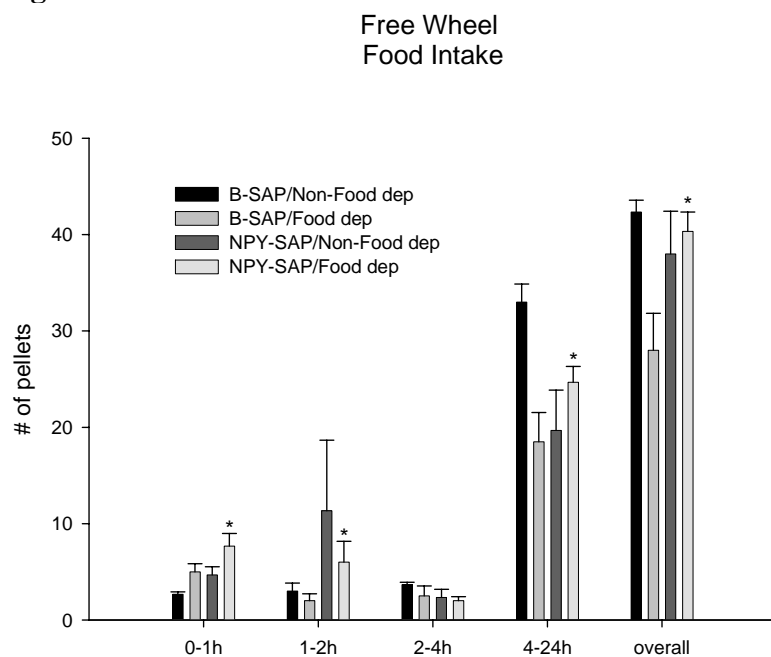
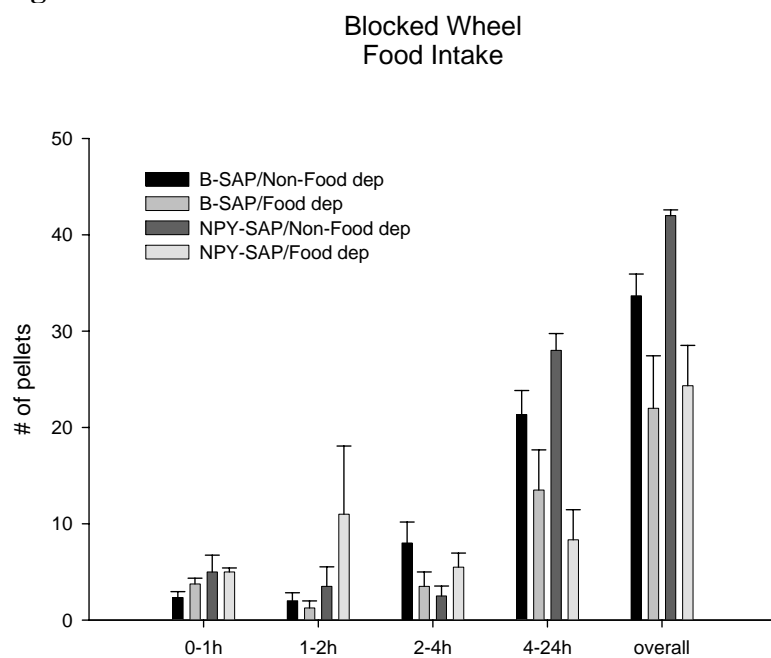
Figure 3.6a**Figure 3.6b**

Figure 3.6c

CHAPTER 4

DOES THE DESTRUCTION OF ARC-NPY ALTER APPETITIVE OR CONSUMMATORY INGESTIVE BEHAVIORS IN SIBERIAN HAMSTERS?

Abstract

Despite the increased consumption of fast food, greater than 80 % of all food eaten occurs at home. In addition, hungry humans and fasted hamsters hoard more food than their non-hungry counterparts. Given the increasing prevalence of obesity, understanding the mechanisms underlying appetitive ingestive behaviors (foraging, food hoarding) may provide a new target for intervention beyond the more well-studied consummatory ingestive behaviors (food intake). Neuropeptide Y plays a role in both phases of ingestion in a variety of species. Central injections of NPY increase food intake in laboratory rats and food hoarding in Siberian hamsters. The likely source of NPY subserving these responses endogenously is NPY synthesizing arcuate (ARC) neurons. Because food deprivation increases ARC-NPY synthesis in rats and Siberian hamsters, and because food deprivation increases food hoarding in hamsters, we tested whether destruction of ARC-NPY neurons blocks food deprivation-induced increases in food hoarding in Siberian hamsters. This was accomplished in two separate experiments using either the immunotoxin NPY conjugated to saporin (NPY-SAP) or neonatal treatment with monosodium glutamate (MSG) to produce lesions of the ARC. Both methods produced a decreased cellularity in the ARC as assessed by cresyl violet staining and a decrease in ARC Y1 receptor, TH and α -MSH immunoreactive cells. Surprisingly, however, food hoarding in animals with lesions of ARC lesions in both experiments was increased 100 % more than controls with refeeding after 56 h food deprivation, with the greatest increase occurring during the 1 h post food deprivation. The underlying cause of this increase could be an upregulation of Y1 receptors due to the denervation of the ARC to PVH NPY projections produced

by NPY-SAP or MSG. Even though NPY-SAP and MSG-treated animals displayed a loss of cellularity in the ARC, there was a substantial amount of NPY-immunoreactive fibers in the PVH and PFA, two sites known to play a role in ingestive behaviors. The converging evidence from both experiments suggests that NPY-producing sites other than the ARC may contribute to compensatory increases in food hoarding post food deprivation.

Introduction

One cannot turn on the television without being bombarded by advertisements from companies trying to sell their latest weight loss pills, fast food restaurants publicizing their new “low-calorie” combinations and exercise facilities trying to help one achieve “a whole new you.” These examples are an indicator of the growing significance of obesity. The fact that obesity leads to many secondary pathological disorders such as cardiovascular disease, stroke, and diabetes is of major concern (Vague et al., 1980; Satcher, 2001; Gasteyger and Tremblay, 2002). The main precursor to obesity is energy intake exceeding energy expenditure. Both appetitive and consummatory ingestive behaviors contribute to this increase in energy intake. Appetitive ingestive behaviors motivate animals to obtain (forage) and store food (hoard), whereas consummatory ingestive behavior is the actual consumption of the acquired food (Craig, 1918). The mechanisms underlying the consummatory ingestive behaviors have been extensively studied, whereas those controlling appetitive ingestive behaviors have been largely ignored. Because humans show differences in foraging and food hoarding based on their physiological conditions, such as whether they are hungry or sated (Dodd et al., 1977; Beneke and Davis, 1985; Mela et al., 1996) and increases in these behaviors lead to increases in food intake (Ransley

et al., 2003c), it is important to investigate the underlying mechanisms of both phases of ingestion in order to better target treatments for obesity.

Although several neuronal populations within the brain contribute to energy balance, Neuropeptide Y (NPY) is a peptide that provides a potent orexigenic drive to alter both appetitive (Day et al., 2005) and consummatory ingestive behaviors in variety of species (Kalra et al., 1999; Schwartz et al., 2000; Day et al., 2005). NPY neurons are largely restricted to the arcuate nucleus of the hypothalamus (ARC; (Chronwall et al., 1985d; White and Kershaw, 1989)), with low levels of expression within the compact zone of the dorsomedial hypothalamus (DMH; (Li et al., 1998)) and the brainstem catecholamine cell groups A1, C1-C3 (Everitt et al., 1984e). Out of these neuronal populations that produce NPY, only the ARC has a reduced blood brain barrier so that ARC-NPY neurons are positioned to sense peripheral metabolic and hormonal signals (*ie.* leptin, ghrelin, insulin, and glucose) and to relay this information to other brain regions to drive changes in ingestive behaviors (Sawchenko, 1998; Watts, 2000). When animals are faced with negative energy balance, such as in food deprivation, ARC-NPY gene expression is increased (Brady et al., 1990; Hahn et al., 1998; Mizuno et al., 1999; Mercer et al., 2000b) and NPY is released (Kalra et al., 1991b). Central injection of NPY into the third ventricle increases food intake in laboratory rats (Clark et al., 1984), and it increases food hoarding to a greater extent than food intake in Siberian hamsters (Day et al., 2005). Despite the evidence of the role of NPY increasing both appetitive and consummatory ingestive behaviors, destruction of NPY-producing neurons and other neurons within the ARC by neonatal treatment of MSG produces a negligible decrease in food intake in rats (Bunyan et al., 1976; Nikolettseas, 1977; Stricker-Krongrad et al., 1992). Therefore, ARC-NPY neurons may be more important for controlling appetitive ingestive behaviors. Thus, the purpose of this study was to test the effect of ARC destruction on appetitive ingestive behaviors, as well as

consummatory, in Siberian hamsters under baseline conditions. In addition, because food deprivation increases ARC-NPY synthesis in rats (Brady et al., 1990; Hahn et al., 1998; Mizuno et al., 1999) and Siberian hamsters (Mercer et al., 1995) and because food deprivation increases food hoarding in hamsters (Day and Bartness, 2003), we tested whether destruction of ARC cells, including ARC-NPY producing neurons, blocks food deprivation-induced increases in food hoarding in Siberian hamsters. This was accomplished using two separate methods. In Experiment 1, we injected NPY conjugated to saporin (NPY-SAP), a ribosomal inactivating toxin, to selectively destroy the Y receptor-containing cells of the ARC, which include the NPY-expressing neurons. In Experiment 2, we neonatally-treated hamsters with MSG to produce ARC lesions. MSG treatment is a well established method known to produce substantial destruction of the arcuate nucleus in rodents (Olney, 1969; Ebling et al., 1998; Meister, Ceccatelli, 1989; Kerkerian, Pelletier, 1986). We then collected baseline measures from adult hamsters for foraging, food hoarding, and food intake and then subsequently food deprived a subset of animals for 56 h and measured the same behaviors 1, 2, 4, and 24 h after refeeding.

Methods

Experiment 1: Does destruction of ARC-NPY neurons by injection of NPY-SAP into the ARC

block food deprivation-induced increases in food hoarding in Siberian hamsters?

Animals and Housing

Adult male Siberian hamsters ~3 months old and weighing 35-46 g were obtained from our breeding colony. The colony was established in 1988 and its genealogy was described recently (Day and Bartness, 2001). Hamsters were group-housed and reared from birth in a 16:8 h light-dark cycle (lights-on at 2030). Room temperature was maintained at 21 ± 2 °C and

relative humidity was 50 ± 10 %. All procedures were approved by the Georgia State University Institutional Animals Care and Use Committee and were in accordance with the Public Health Service and United States Department of Agriculture guidelines.

Acclimation and Baseline Measures

64 animals were transferred from group-housing and acclimated and trained for one wk in our hoarding/foraging apparatus as previously shown and described (Day and Bartness, 2001). Briefly, two cages were connected with a convoluted polyvinyl-chloride tubing system (38.1 mm inner diameter and ~ 1.52 m long), with corner and straightways for both horizontal and vertical climbs. The top or “food cage” was 456 x 234 x 200 mm (length x width x height) equipped with a water bottle and running wheel. The bottom or “burrow cage” was 290 x 180 x 130 mm and was covered to simulate the darkness of a natural burrow. The burrow cage contained Alpha-Dri (Specialty Papers, Kalamazoo, MI) bedding and cotton nesting material. The animals were fed 75 mg pellets (Purified Rodent Diet; Research Diets, New Brunswick, NJ) throughout the experiment. During this period, animals were trained to forage for their food based on procedures previously published (Day and Bartness, 2001). In brief, hamsters were given free access to food for 2 d while they adapted to the running wheel. In addition to the free food, a 75 mg food pellet was dispensed upon completion of every 10 wheel revolutions. Wheel revolutions were counted using a magnetic detection system and monitored by a computer-based hardware/software system (Med Associated, Lancaster, NH). On the third day, the free food condition was replaced by a response-contingent condition in which only every 10 wheel revolutions triggered the delivery of a pellet. This condition was in effect for the remaining 5 d of the 1 wk-long training period. The hamsters were then separated into 12 groups: 2 drug

groups (NPY-SAP or B-SAP), 2 feeding conditions (Fasted or Non-Fasted), and 3 foraging groups that were matched for percent change in body mass and average hoard size. The three foraging groups were 10 revolutions/pellet (10 Rev), Free Wheel/Free Food (FW; food was available non-contingently [not earned]), but the running wheel was active [locomotor activity control group]) or Blocked Wheel/Free Food (BW; food was available non-contingently [not earned], but the running wheel was blocked [sedentary control group]).

Measurement of Foraging, Food Hoarding, and Food Intake

Foraging (pellets earned) was defined as the number of pellets delivered upon completion of the requisite wheel revolutions. Food hoarding (pellets earned) was defined as the number of pellets found in the bottom “burrow” cage in addition to those removed from the cheek pouches. For the 10 Rev group, food intake (pellets eaten) was defined as pellets earned – surplus pellets – hoarded pellets = food intake. For the free and blocked wheel groups, food intake (pellets eaten) was defined as pellets given – pellets left in the top cage – hoarded pellets = food intake. The electronic balance used to weigh the food pellets was set to “parts” measurement rather than obtaining fractions of a pellet in milligrams; thus, the smallest unit measured was one 75-mg food pellet and equal to 1.

Intracranial injections

For ARC administration of NPY-SAP and the blank saporin control solution (B-SAP; Advanced Targeting Systems, San Diego, CA), hamsters were anesthetized with isoflurane and fur at the top of the head was removed to expose the area to be incised. A hole was trephined at the stereotaxic coordinates above the ARC and the injector was lowered into the ARC (AP -0.14

cm, ML -0.03 cm, DV -0.7 cm). Injections of 48 ng in 100 nl per side of NPY-SAP or B-SAP (Advanced Targeting Systems, San Diego, CA) were delivered bilaterally (100 nl/side) to the ARC. The dose of NPY-SAP was chosen based on previously published data showing it to be effective at producing significant destruction of Y receptor-containing neurons within the ARC (Bugarith et al., 2005d). Injections were made through an internal cannula (26-gauge stainless steel; Plastics One, Roanoke, VA) connected to a 0.5 μ l microsyringe with polyethylene tubing. The solution was delivered slowly over a 5 min period. Fresh apple slices were given to facilitate fluid and caloric intake for the first 2-3 d post surgery. The animals also received subcutaneous buprenorphine (0.05 mg/kg, s.c.) injections for 2 d after surgery. Animals then recovered for 7 d in single-shoebox cages with food and water available *ad libitum*. After recovery, hamsters were returned to their respective hoarding/foraging cages maintaining the same group membership.

Experimental protocol

The time course adopted to initiate and terminate behavioral testing was chosen based on previously published data of NPY-SAP effects on NPY terminals in the hypothalamus (Bugarith et al., 2005c). In that study, a significant reduction of NPY terminals was present by the second week after injection. Therefore, feeding studies were conducted beginning at approximately this time. From Day 8 until Day 21 after injection of NPY-SAP or B-SAP, daily measurements of foraging, food hoarding and food intake were taken just before lights out. On Day 22 after injection, a subset of animals in each group was food deprived for 56 h. Two hours before the onset of the dark phase and refeeding, food was removed from the hamsters' pouches of the non-food deprived group and all animals were placed in clean burrow cages with access to the tubes

blocked before refeeding. At the onset of the dark phase, access to the tubes and top cage was restored. Foraging, food hoarding, and food intake were measured 1 h, 2 h, 4 h, and 24 h after refeeding.

Experiment 2: Does destruction of ARC-NPY neurons by neonatal injections of MSG block food deprivation-induced increases in food hoarding in Siberian hamsters?

Animals and housing

Animals were obtained from our breeding colony as described in Experiment 1. We adopted a previously published protocol to treat the animals neonatally with MSG (Ebling et al., 1998). Briefly, pups were injected subcutaneously in the dorsum using a 30 gauge needle with vehicle (0.1 M phosphate buffer, pH 7.4) or MSG (*L*-monosodium glutamate; Sigma, St. Louis, MO, USA) at a dose of 4 mg/g of body mass. MSG was diluted to a concentration of 160 mg/ml such that a 1g pup would receive an injection volume of 25 μ l to deliver 4 mg MSG. Injections occurred once daily for 5 consecutive days from post partum Day 4-8. All pups within a litter received a single treatment with no mixing of treatments within a litter. The pups were then reared as described in Experiment 1 in group housing with littermates of the same treatment group. At ~3 m of age, 56 males were transferred from group-housing and acclimated for 1 wk in our hoarding/foraging apparatus (see below).

Acclimation and Baseline Measures

At ~3 m of age, 56 males were transferred from group-housing and acclimated for one wk in our hoarding/foraging apparatus. Because of reports that MSG treatment decreases spontaneous motor activity (*e.g.*, (Nakagawa et al., 2000)), we reasoned that foraging for food

would be too challenging for these animals and they would not earn enough food to survive. Therefore, all animals were kept in the Free Wheel condition and wheel revolutions were monitored. Data was collected as described in Experiment 1. The hamsters were then separated into Food deprived and Non-food deprived groups that were matched for percent change in body mass and average hoard size.

Experimental protocol

A subset of animals in each of the MSG or PBS treated groups was food deprived for 56 h. Two hours before the onset of the dark phase and refeeding, food was removed from the hamsters' pouches of the non-food deprived group and all animals were placed in clean burrow cages with access to the tubes blocked before refeeding. At the onset of the dark phase, access to the tubes and top cage was restored. Foraging, food hoarding, and food intake were measured 1 h, 2 h, 4 h and 24 h after refeeding.

Experiment 1 and Experiment 2:

Tissue preparation

After the 24 h data was collected, animals were deeply anesthetized with pentobarbital sodium (80 mg/kg ip) and perfused transcardially with 100 ml of 0.15 M saline followed by 150 ml of 4 % paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were extracted and post-fixed for 48 h at 4 °C, followed by a submersion in 30 % sucrose for at least 24 h at 4 °C. Brains were then frozen and sectioned on a microtome into 30 µm section through the hypothalamus and distributed serially into five sets. The first set was mounted immediately onto

gelatinized glass slides and counter-stained with cresyl violet. The remaining sections were stored in cryoprotectant for storage at -20 °C until immunohistochemical processing.

Lesion verification using immunohistochemistry

Free-floating sections were removed from the cryoprotectant and washed with PBS. All washes and incubations occurred at room temperature. Sections were then incubated in 0.03 % H₂O₂ (Fisher Scientific, Houston, TX) and 0.25 % Triton X-100 (Sigma, St. Louis, MO) in PBS for 15 min. Sections were then transferred to wells containing primary antisera, either rabbit anti-Y1 receptor (1:1000; ImmunoStar Inc., Hudson, WI), sheep anti-NPY (1:10,000, Chemicon, Temecula, CA), rabbit anti-tyrosine hydroxalase (1:10,000; Chemicon, Temecula, CA), or rabbit anti- α MSH (1:10,000; ImmunoStar, Hudson, WI), diluted in 3 % normal donkey serum and 0.25 % Triton X-100 in PBS for 48 h. Sections were then washed with PBS and incubated in secondary antisera for 2 h using either donkey anti-goat or donkey anti-sheep (1:500; Jackson ImmunoResearch, West Grove, PA). Secondary antibodies were diluted in 3 % normal donkey serum corresponding to the host species of the secondary antibody and 0.25 % Triton X-100 in PBS. After washing with PBS, sections were incubated in a 1:222 solution of the avidin-biotin-peroxidase complex (Vector Elite kit; Vector laboratories, Burlingame, CA) in 0.3 % Triton X-100 in PBS for 1 h at RT. Sections were then washed with PBS, followed by visualization by reacting the sections with 3,3'-diaminobenzidine (0.2 mg/ml; Sigma, St. Louis, MO) and 0.025 % H₂O₂ in PBS for 5-10 min. The reaction was terminated with PBS washes. Sections were mounted on gelatinized slides and dehydrated with increasing concentration of alcohol followed by Xylenes (Fisher Scientific, Fair Lawn, NJ) and then coverslipped with Permount (Fisher Scientific, Fair Lawn, NJ).

Statistical analysis

Behavioral measures were analyzed using a three-way mixed model ANOVA with repeated measures (3 x 4 x 4; foraging group x drug x time) using Number Crunching Statistical Software v 2000 (Kaysville, UT). Duncan's new multiple range tests were used for post hoc tests when appropriate. Differences among groups were considered statistically significant if $P_s < 0.05$. Exact probabilities and test values were omitted for simplicity and clarity of the presentation.

Results

Experiment 1: Does destruction of ARC-NPY neurons by injection of NPY-SAP into the ARC block food deprivation-induced increases in food hoarding in Siberian hamsters?

Destruction of ARC. NPY-SAP-treated animals had decreased cellularity in the ARC compared with B-SAP-treated animals as assessed by cresyl violet staining and a decrease in ARC Y1 receptor, TH and α -MSH immunoreactive cells (Fig. 4.0). There also was a significant reduction in NPY-immunoreactive fibers after NPY-SAP treatment in two sites known to be innervated by ARC-NPY cells -- the PVH and PFA (Fig. 4.0). B-SAP did not cause comparable destruction to either ARC cells or NPY fibers.

Wheel Revolutions. Under baseline conditions, NPY-SAP had no effect on the number of revolutions in the FW group compared with the B-SAP animals (Fig. 4.1b). After food deprivation, NPY-SAP inhibited the post food deprivation-induced increase in the number of revolutions at 2-4 h, 4-24 h and the cumulative total ($P_s < 0.05$; Fig. 4.4b). In addition, food deprived and non-food deprived NPY-SAP treated animals ran significantly less than did the B-SAP treated animals at 1-2 h, 2-4 h, and cumulatively ($P_s < 0.05$; Fig. 4.4b).

Foraging. Under baseline conditions, NPY-SAP had no effect on foraging in the 10 Rev group compared with the B-SAP animals (Fig. 4.1a). After food deprivation, NPY-SAP had no effect on foraging when NPY-SAP/food deprived and NPY-SAP/non-food deprived animals were compared to their B-SAP counterparts (Fig. 4.4a).

Food Hoarding. Under baseline conditions, NPY-SAP had no effect on food hoarding compared with B-SAP animals in any group (Fig. 4.2). After food deprivation, NPY-SAP significantly exaggerated the post food deprivation-induced increase in hoarding compared with B-SAP animals in the 10 Rev group at 0-1 h, 2-4 h and cumulatively ($P < 0.05$; Fig. 4.5a). In the FW and BW groups, there was no difference between the NPY-SAP/food deprived animals compared to the B-SAP/food deprived animals (Figs. 4.5b, 4.5c).

Food Intake. Under baseline conditions, NPY-SAP significantly decreased food intake compared with B-SAP animals in the 10 Rev and BW groups ($P < 0.05$; Figs. 4.3a, 4.3c). After food deprivation, NPY-SAP had no overall effect on food intake in the 10 Rev or FW groups (Figs. 4.6a, 4.6b). In the BW group, NPY-SAP significantly decreased food intake after food deprivation compared with B-SAP/food deprived animals at 2-4 h and cumulatively ($P < 0.05$; Fig. 4.6c).

Experiment 2: Does destruction of ARC neurons by neonatal injections of MSG block food deprivation-induced increases in food hoarding in Siberian hamsters?

Destruction of ARC neurons. MSG-treated animals had significantly decreased cellularity in the ARC compared with PBS-treated animals as assessed by cresyl violet staining and a significant decrease in ARC Y1 receptor, TH and alpha-MSH immunoreactive cells (Fig. 4.7). There also

was a significant reduction in NPY-immunoreactive fibers after MSG treatment in two sites known to be innervated by ARC-NPY cells -- the PVH and PFA (Fig. 4.7). PBS did not cause comparable destruction to the ARC cells or the NPY fibers.

Wheel Revolutions. Under baseline conditions, MSG had no effect on the number of revolutions in the FW group compared with the PBS-treated animals ($P_s < 0.05$; Fig. 4.8). After food deprivation, MSG significantly decreased the number of revolutions compared with PBS-treated animals at 0-1 h and cumulatively ($P_s < 0.05$; Fig. 4.11).

Food Hoarding. Under baseline conditions, MSG had no effect on food hoarding compared with PBS-treated animals ($P_s < 0.05$; Fig. 4.9). After food deprivation, MSG significantly exaggerated the post food deprivation-induced increase in hoarding compared with PBS animals at 0-1 h, 4-24 h and cumulatively ($P_s < 0.05$; Fig. 4.12).

Food Intake. Under baseline conditions, MSG had no effect on food intake compared to treated animals (Fig. 4.10). After food deprivation, MSG had no effect on food intake at any time point or overall (Fig. 4.13).

Discussion

The present experiments tested the importance of the ARC in the control of appetitive and consummatory ingestive behaviors in Siberian hamsters under baseline conditions and after food deprivation. The major findings of this study were that: a) ARC cells are needed for controlling baseline food intake b) ARC cells are not needed for post food deprivation-induced

increases in food hoarding and c) destruction of ARC cells produces an exaggerated increase in food hoarding after food deprivation. Therefore, ARC cells, including ARC-NPY producing neurons, may be needed to mediate changes in endogenous control of food intake, but are not necessary to control foraging, hoarding or food intake after the energetic challenge of food deprivation.

Our results show that ARC cell bodies were destroyed by both the NPY-SAP lesion and MSG treatment. Both treatments induced a significant decrease in the number of cells in the ARC with no damage to adjacent hypothalamic nuclei, which is in concordance with previous studies (Ebling et al., 1998;Bugarith et al., 2005b). Some of these ARC cells destroyed by both methods were undoubtedly NPY neurons and even though the ARC-NPY neurons contribute a major source of NPY innervation of hypothalamic nuclei (Everitt et al., 1984d;Chronwall et al., 1985c;White and Kershaw, 1989;Beck et al., 1990;Brady et al., 1990), many areas including the PVH and PFA still exhibited substantial NPY immunoreactive fibers. The substantial NPY-immunoreactive fibers that remain after the destruction of likely ARC-NPY neurons supports the view that hypothalamic nuclei receive NPY inputs from several sources (Bai et al., 1985;Sawchenko et al., 1985). That is, a proportion of hypothalamic NPY innervation originates from brainstem catecholamine neurons A1, C1-C3, as well (Everitt et al., 1984c). Although brainstem NPY usually makes-up only a partial innervation of the hypothalamus (Broberger and Hokfelt, 2001), there may be sprouting of these fibers to fill the void structurally and functionally left by the loss of ARC-NPY neurons, as suggested previously (Bugarith et al., 2005a). We do not know, of course, whether the remaining NPY fibers in the PVH and PFA derive from unharmed ARC-NPY neurons or from these brainstem NPY sources.

In the present study, baseline food intake was decreased after ARC destruction by NPY-SAP with no change in baseline foraging or food hoarding. Because some of these ARC cells destroyed by NPY-SAP were undoubtedly NPY neurons and NPY stimulates food intake in Siberian hamsters (Day et al., 2005), it would be likely that decreases in ARC-NPY could contribute to the decreases in baseline food intake after NPY-SAP treatment. In Chapter 3, however, we showed that blocking the effect of NPY in the PVH, one target site of ARC-NPY fibers (Broberger et al., 1999c), by destroying all Y receptor-containing neurons in the PVH using NPY-SAP does not produce changes in baseline food intake. This suggests that ARC-NPY may be mediating the effect on food intake through other brain sites. NPY injected into the PFA in rats produces a more robust increase in food intake than NPY in the PVH (Stanley et al., 1993). In Chapter 2, we also found that NPY injected into the PFA of Siberian hamsters produces significant increases in food intake. Given the results of these findings and that there are extensive ARC-NPY projections to the PFA (Bai et al., 1985; Broberger et al., 1999c), the ARC-NPY to PFA circuit may be a prominent mediator of normal food intake. This is in contrast to the primary NPY circuit that may be controlling foraging and food hoarding. That is, because ARC destruction did not alter baseline foraging or food hoarding in the present study, but destruction of Y receptors in the PVH decreased baseline foraging and food hoarding in Chapter 3, NPY projections from other sources to the PVH may be more important than ARC-NPY to PVH projections for controlling baseline appetitive ingestive behaviors. Thus, the extensive NPY projections throughout the brain appear to play distinctive roles in regulating appetitive and consummatory ingestive behaviors.

The exaggerated ingestive behavior responses to food deprivation after NPY-SAP or MSG treatment is consistent with several other reports using the same or other models of obesity

(Stricker-Krongrad et al., 1996b; Beck et al., 2001; Stricker-Krongrad and Beck, 2004f). For example, there were exaggerated increases in food intake after intraventricular injections of NPY in MSG treated laboratory rats (Stricker-Krongrad and Beck, 2004e). In addition, a compensatory mechanism to NPY denervation of the PVH/PFA by the likely destruction of ARC-NPY neurons in the present study, is the well-known phenomenon of denervation supersensitivity (Stricker-Krongrad and Beck, 2004d). Indeed, in MSG-treated laboratory rats, PVH Y1 receptors are significantly increased (Stricker-Krongrad and Beck, 2004c). We also found a suggestion of upregulation of Y1 receptor subtype within the PVH and PFA, two targets of NPY that are known to contribute to the control of both appetitive and consummatory ingestive behaviors in the present study. This apparent upregulation in Y1 receptor occurred in the PVH and PFA with no upregulation in other hypothalamic areas (*eg.* dorsomedial hypothalamus and lateral hypothalamus) known to be involved in ingestive behaviors. Presumably, the upregulation of Y receptors would allow for any remaining NPY fibers from the ARC or other NPY sources to stimulate an increased number of receptors that would result in downstream behavioral exaggerations.

Although MSG-treated animals have exaggerated increases in food intake after food deprivation or central NPY injection (Stricker-Krongrad et al., 1996a; Stricker-Krongrad and Beck, 2004b), we did not find the same supersensitivity to food intake when our food deprived MSG- or NPY-SAP-treated hamsters were refed. It has been suggested that this sensitivity to NPY after MSG treatment is due to an upregulation of NPY receptors in the PVH, specifically, NPY Y1 and Y5 receptors (Stricker-Krongrad and Beck, 2004a). As stated previously, we also found a suggested upregulation of Y1 receptor within the PVH and PFA in our animals, however we only found an exaggerated increase in food hoarding and not food intake when the food

deprived hamsters were refed, likely because Siberian hamsters (Bartness et al., 1995) and other hamster species (Borer et al., 1979; Lea and Tarpy, 1986; Schneider and Buckley, 2001) do not increase their food intake after food deprivation or if they do, the magnitude is small compared with laboratory rats and mice (Bartness and Demas, 2004).

Although both the NPY-SAP and MSG treatments to destroy the ARC have been useful tools in attempting to understand the function of ARC-NPY neurons, there are caveats with both methods. First, the ARC lesions produced by both NPY-SAP and MSG treatments are not selective to destroying only NPY-producing neurons. Any ARC cell that expresses Y receptors are potentially destroyed by NPY-SAP and any neuron that expresses glutamate receptors are potentially destroyed by MSG, including the proopiomelanocortin neurons that produce α -MSH (Alessi et al., 1988) and are known to decrease appetitive and consummatory ingestive behaviors in a variety of species (Murphy et al., 1998; Benoit et al., 2001). Second, the lesions do not destroy all neurons of the ARC. The remaining neurons may be responsible for or contribute to the behavioral changes. Also, MSG treatment not only produces lesions of the ARC but also the area postrema, subfornical organ and retinal ganglion cells (Olney, 1969). Because we have converging evidence of the effect of ARC destruction on ingestive behavior using both the MSG and NPY-SAP treatment, we do not believe that destruction of these additional areas with the MSG treatment have contributed significantly to the results. However, a role of these other areas cannot be excluded.

In summary, anatomical and behavioral results demonstrate that NPY-SAP and MSG treatment produces lesions of ARC cells and undoubtedly a decrease in NPY neurons. The loss of ARC cells causes marked differences in both appetitive and consummatory ingestive behaviors, but does not demonstrate that ARC is necessary for any of these behaviors. Even

though NPY-SAP and MSG treatments caused ARC cell loss they did not completely disrupts baseline ingestive behaviors and food deprivation-induced increases in food hoarding are even more enhanced after MSG or NPY-SAP treatment. Compensatory mechanisms that play a role in this supersensitization include an upregulation of Y receptors, but also could include increased expression of NPY from other sources such as the catecholamine cell groups A1, C1-C3 (Everitt et al., 1984b). Collectively, these results show that ARC may be sufficient to control appetitive and consummatory ingestive behaviors, but other NPY sources may be just as important.

Figure Captions

Figure 4.0. Representative coronal sections of the brain showing effects of Blank-Saporin (B-SAP; *left*) or NPY-Saporin (NPY-SAP; *right*) on cresyl violet (a), NPY in the ARC (b) and NPY in the PVH (c).

Figure 4.1. Mean \pm SEM baseline foraging for the 10 Revolutions/pellet group (10 Rev; a) and wheel revolutions for the Free Wheel group (FW; b) 8-21 d after injection of Blank-Saporin (B-SAP) or NPY-Saporin (NPY-SAP).

Figure 4.2. Mean \pm SEM baseline food hoarding for 10 Revolutions/pellet group (10 Rev; a), Free Wheel group (FW; b) and Blocked Wheel group (BW; c) 8-21 d after injection of Blank-Saporin (B-SAP) or NPY-Saporin (NPY-SAP).

Figure 4.3. Mean \pm SEM baseline food intake for 10 Revolutions/pellet group (10 Rev; a), Free Wheel group (FW; b) and Blocked Wheel group (BW; c) 8-21 d after injection of Blank-Saporin (B-SAP) or NPY-Saporin (NPY-SAP). * $P_s < 0.05$ compared with B-SAP animals.

Figure 4.4. Mean \pm SEM foraging for the 10 Revolutions/pellet group (10 Rev; a) and wheel revolutions for the Free Wheel group (FW; b) after food deprivation for Blank-Saporin (B-SAP) and NPY-Saporin (NPY-SAP) animals. * $P_s < 0.05$ compared with B-SAP/Food deprived animals. # $P_s < 0.05$ compared with B-SAP animals.

Figure 4.5. Mean \pm SEM food hoarding for the 10 Revolutions/pellet group (10 Rev; a), Free Wheel group (FW; b) and Blocked Wheel group (BW; c) after food deprivation for Blank-Saporin (B-SAP) and NPY-Saporin (NPY-SAP) animals. $P_s < 0.05$ compared with B-SAP/Food deprived animals.

Figure 4.6. Mean \pm SEM food intake for the 10 Revolutions/pellet group (10 Rev; a), Free Wheel group (FW; b) and Blocked Wheel group (BW; c) after food deprivation for Blank-Saporin (B-SAP) and NPY-Saporin (NPY-SAP) animals. * $P_s < 0.05$ compared with B-SAP/Food deprived animals.

Figure 4.7. Representative coronal sections of the brain showing effects of Phosphate Buffered Saline (PBS; *left*) or monosodium glutamate (MSG; *right*) on cresyl violet (a), tryptophan hydroxylase (TH; b) and NPY (c).

Figure 4.8. Mean \pm SEM baseline wheel revolutions after Phosphate Buffered Saline (PBS) or monosodium glutamate (MSG) treatment.

Figure 4.9. Mean \pm SEM baseline food hoarding after Phosphate Buffered Saline (PBS) or monosodium glutamate (MSG) treatment.

Figure 4.10. Mean \pm SEM baseline food intake after Phosphate Buffered Saline (PBS) or monosodium glutamate (MSG) treatment. * $P_s < 0.05$ compared with PBS animals.

Figure 4.11. Mean \pm SEM wheel revolutions after food deprivation for the Phosphate Buffered Saline (PBS) or monosodium glutamate (MSG) groups. *Ps<0.05 compared with PBS/Food deprived animals.

Figure 4.12. Mean \pm SEM food hoarding after food deprivation for the Phosphate Buffered Saline (PBS) or monosodium glutamate (MSG) groups. *Ps<0.05 compared with PBS/Food deprived animals.

Figure 4.13. Mean \pm SEM food intake after food deprivation for the Phosphate Buffered Saline (PBS) or monosodium glutamate (MSG) groups.

Figure 4.14. Representative coronal sections of the brain showing compensatory effects after destruction of the ARC on NPY Y1 receptor in the PVH (a), NPY Y1 receptor in the PFA (b) and α -Melanocyte Stimulating Hormone (α -MSH; c).

Figure 4.0a

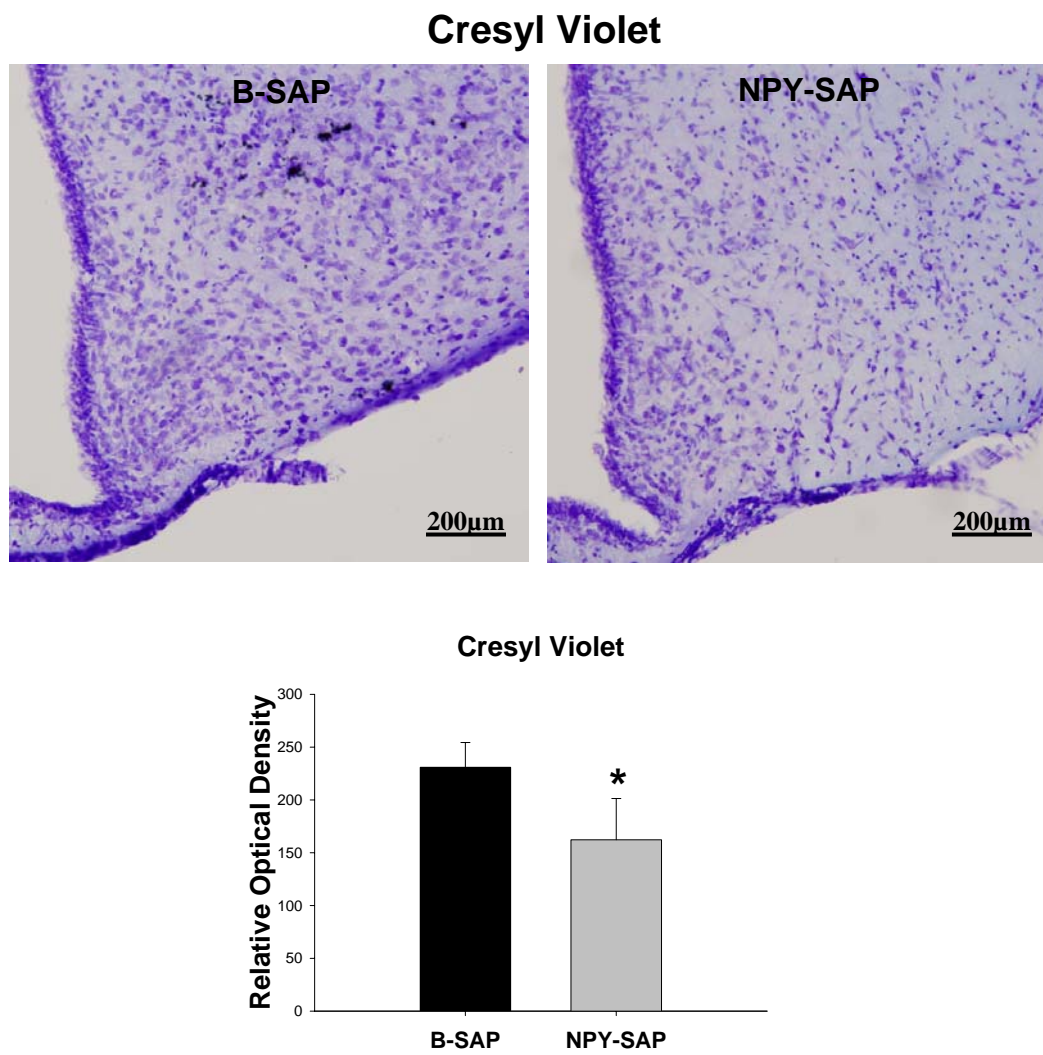


Figure 4.0b

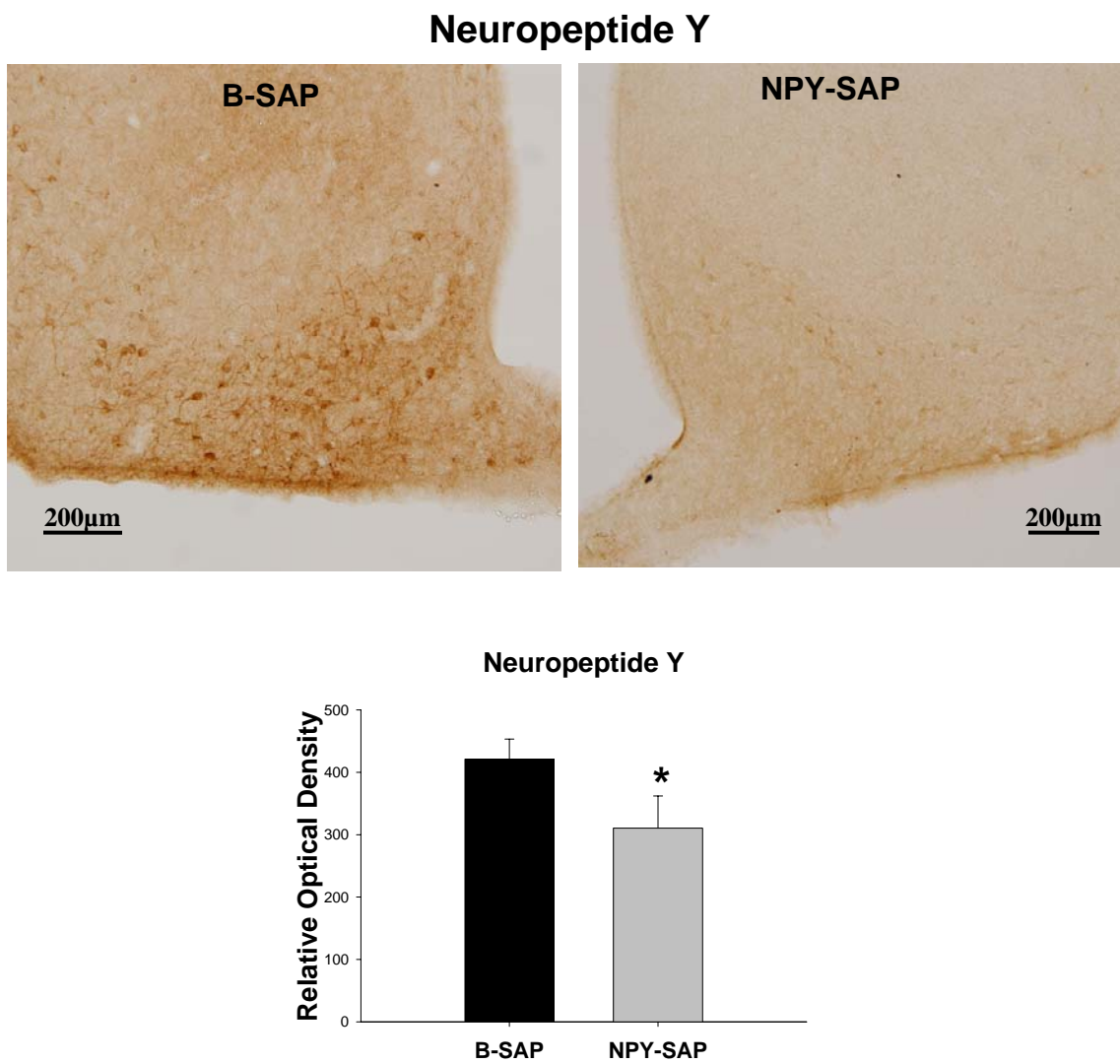


Figure 4.0c

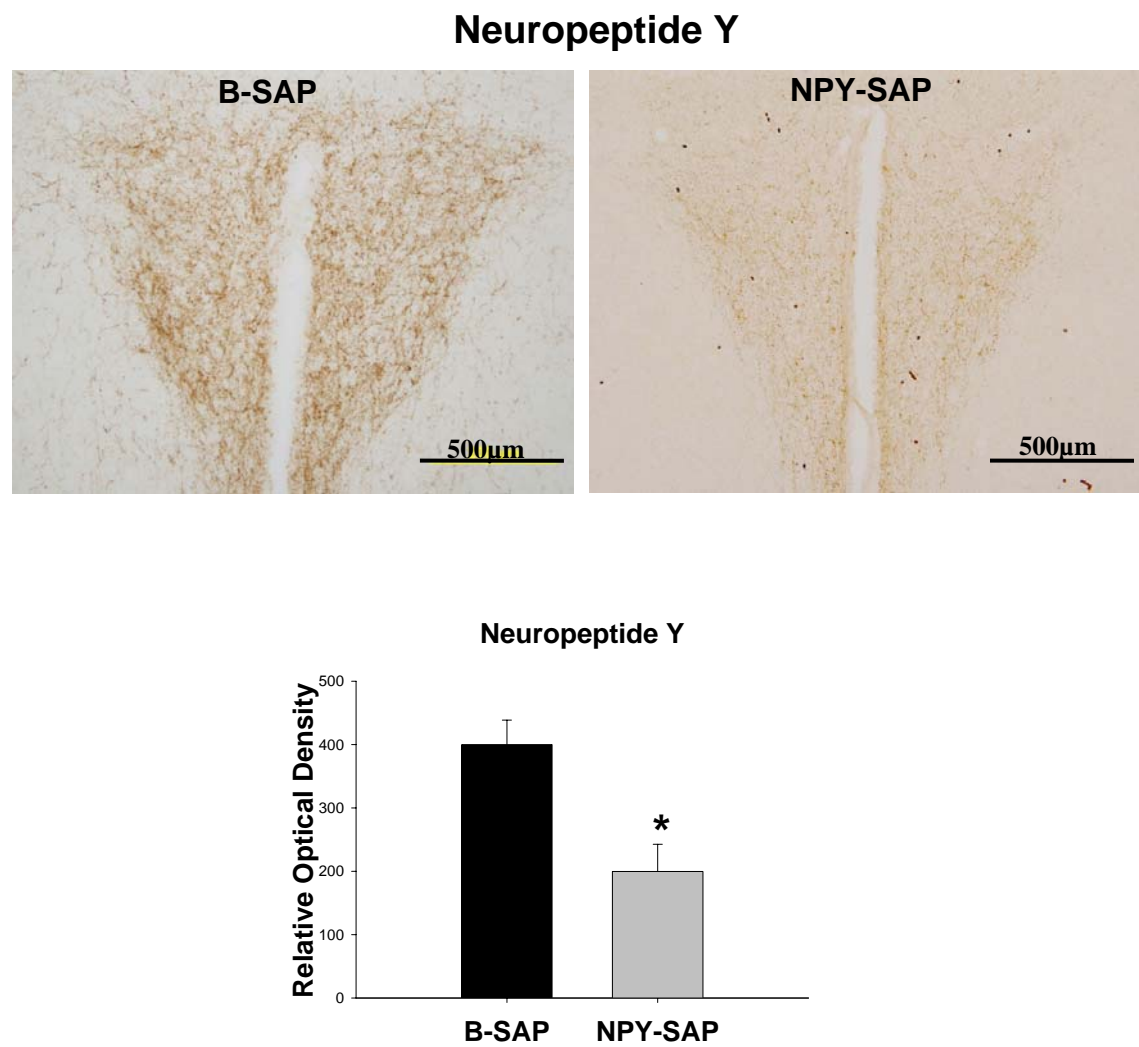


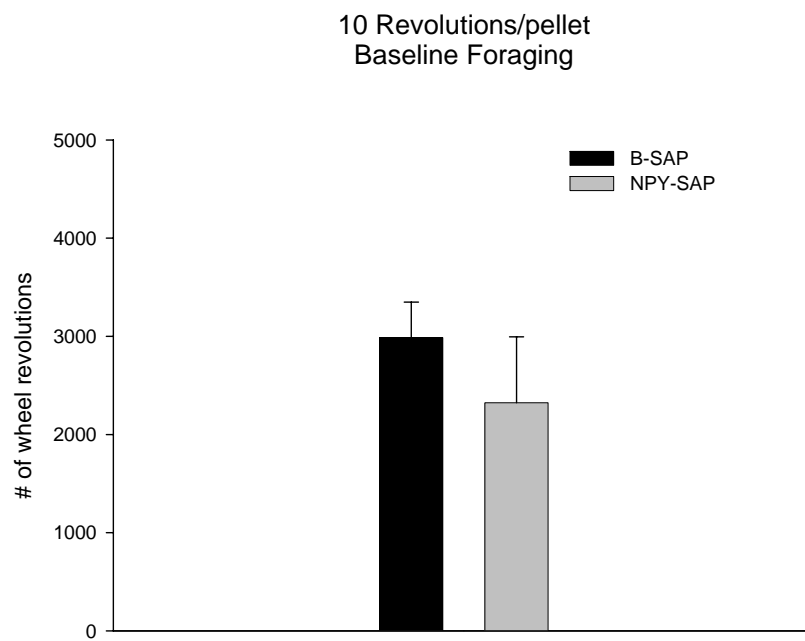
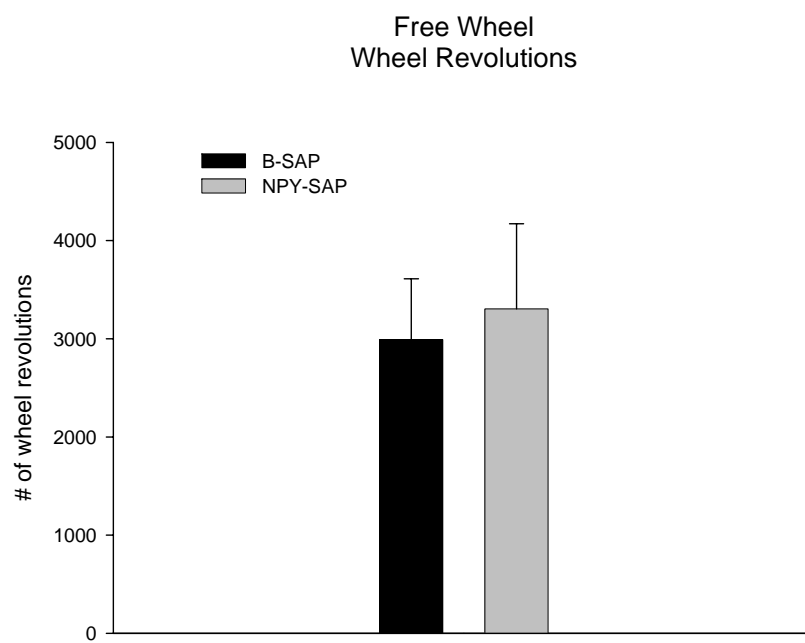
Figure 4.1a**Figure 4.1b**

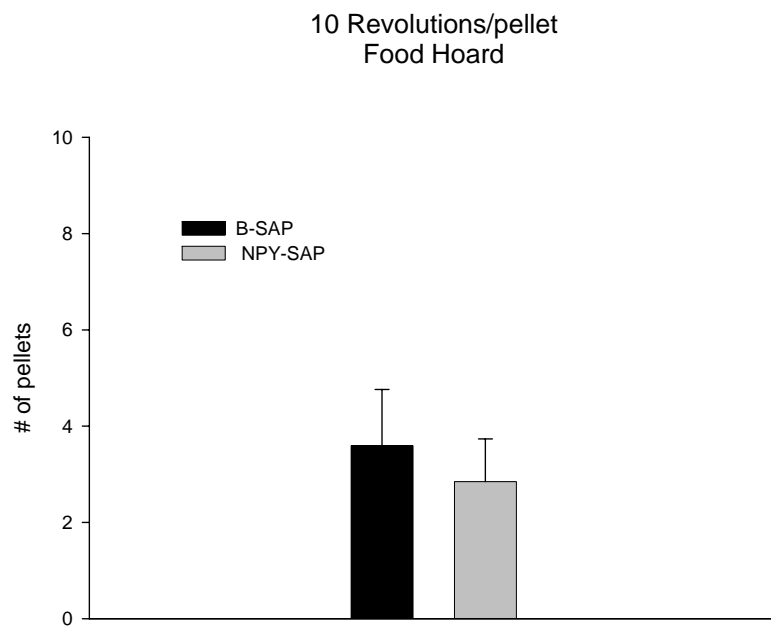
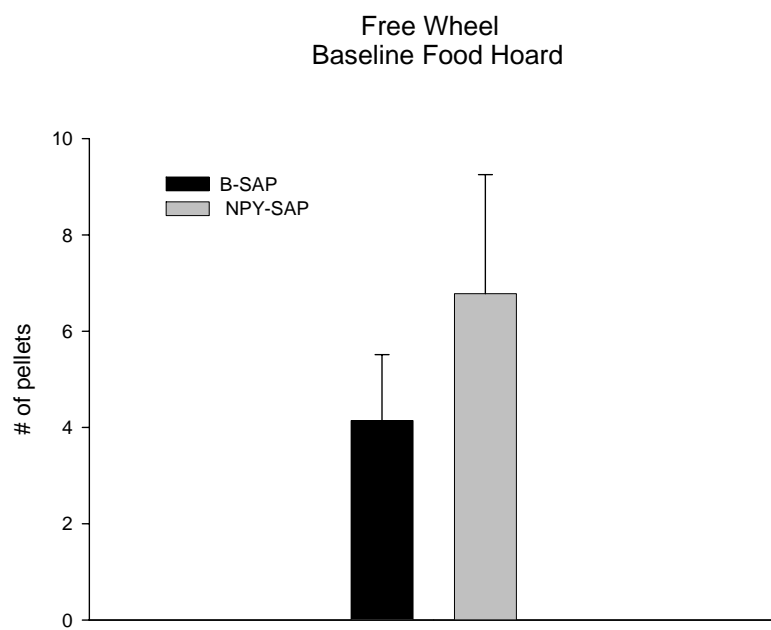
Figure 4.2a**Figure 4.2b**

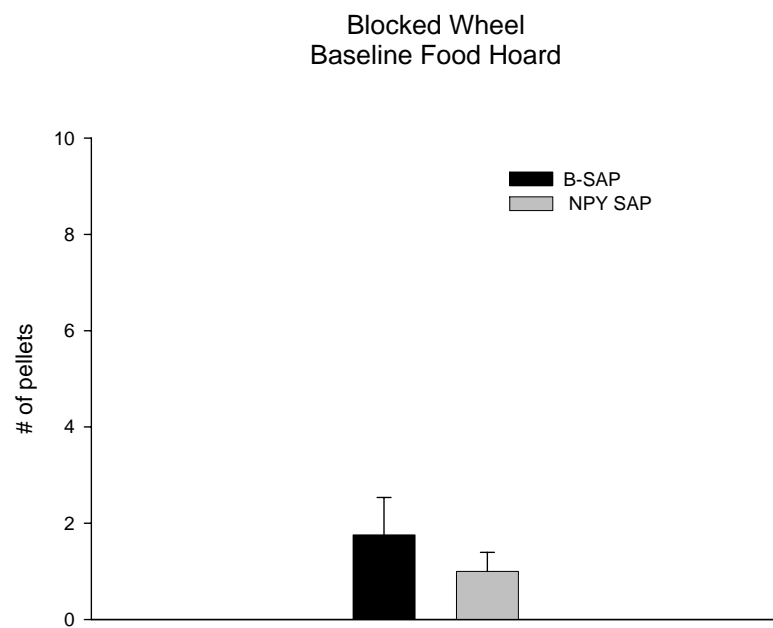
Figure 4.2c

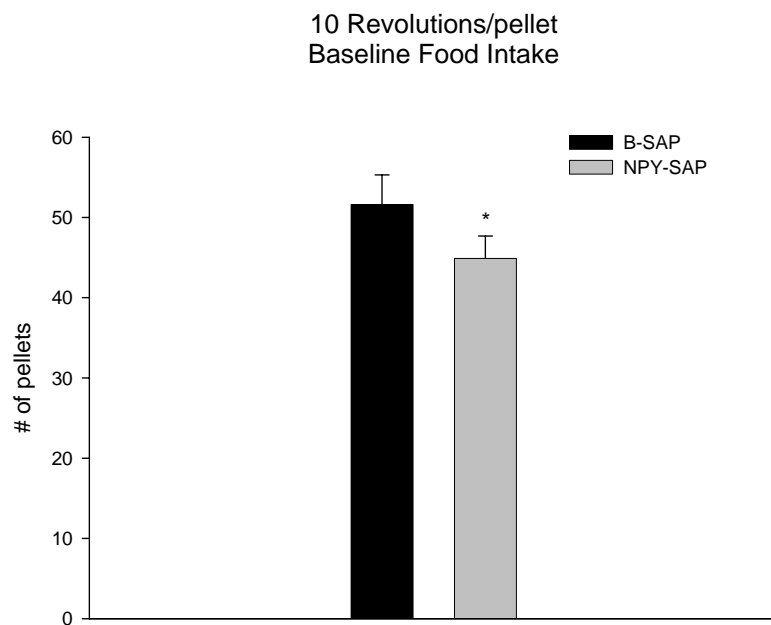
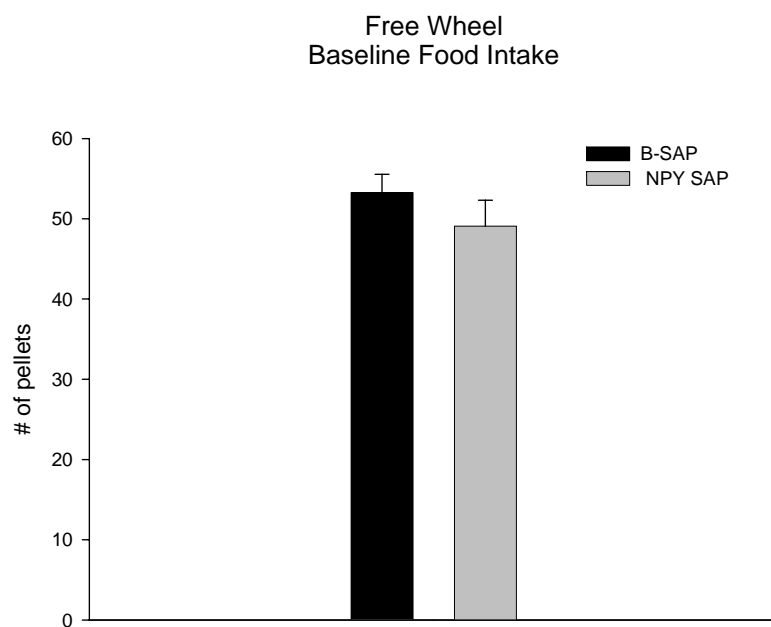
Figure 4.3a**Figure 4.3b**

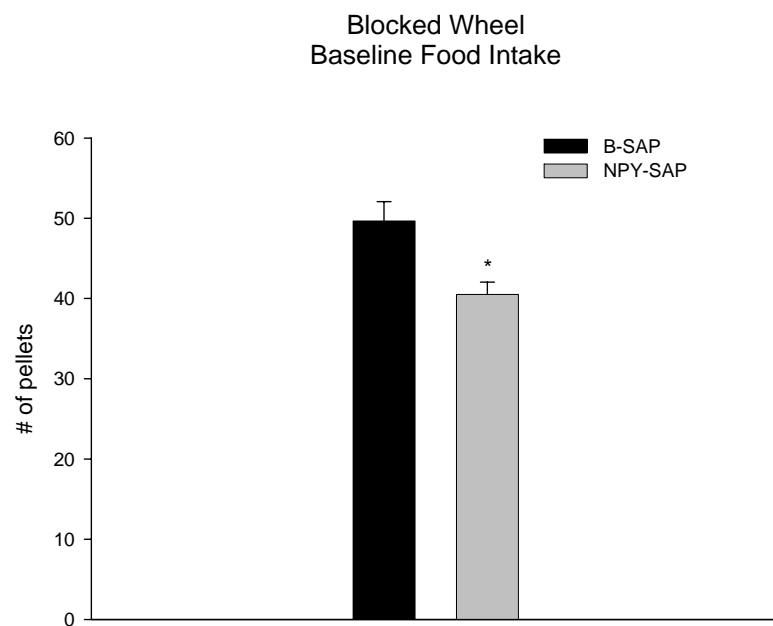
Figure 4.3c

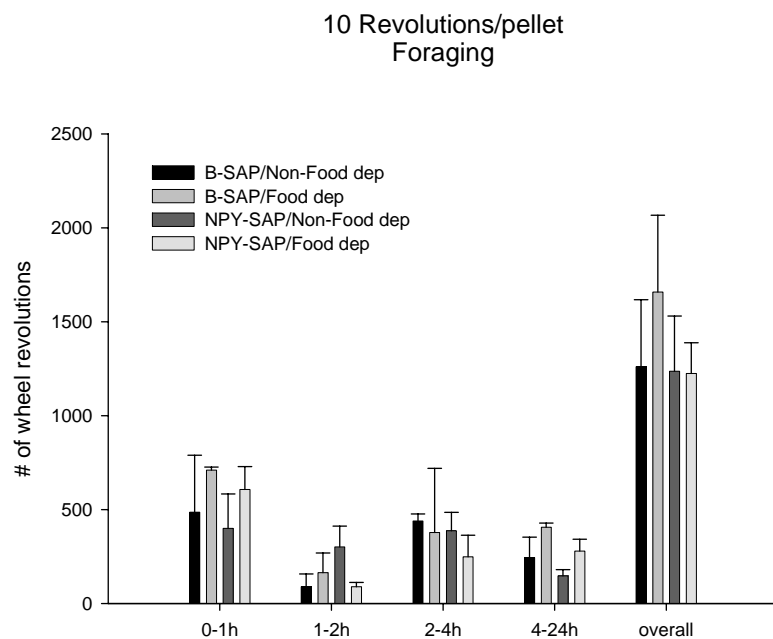
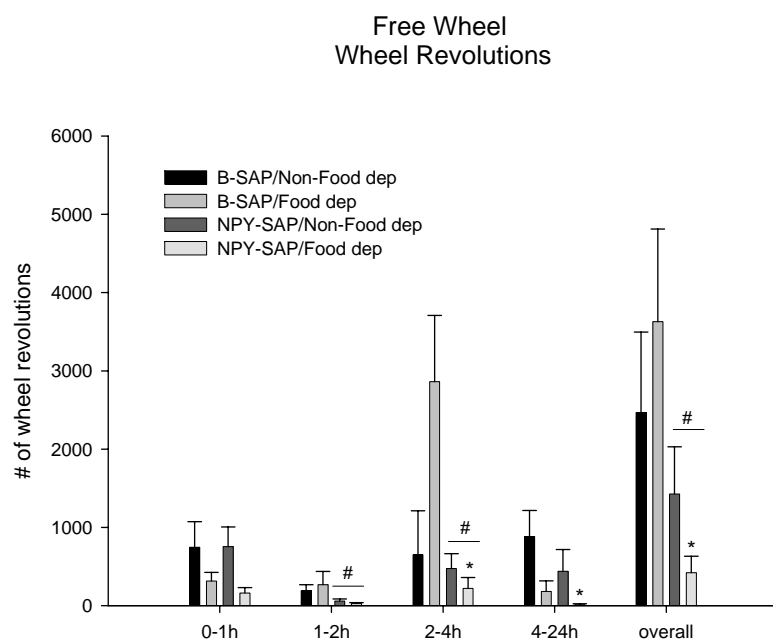
Figure 4.4a**Figure 4.4a**

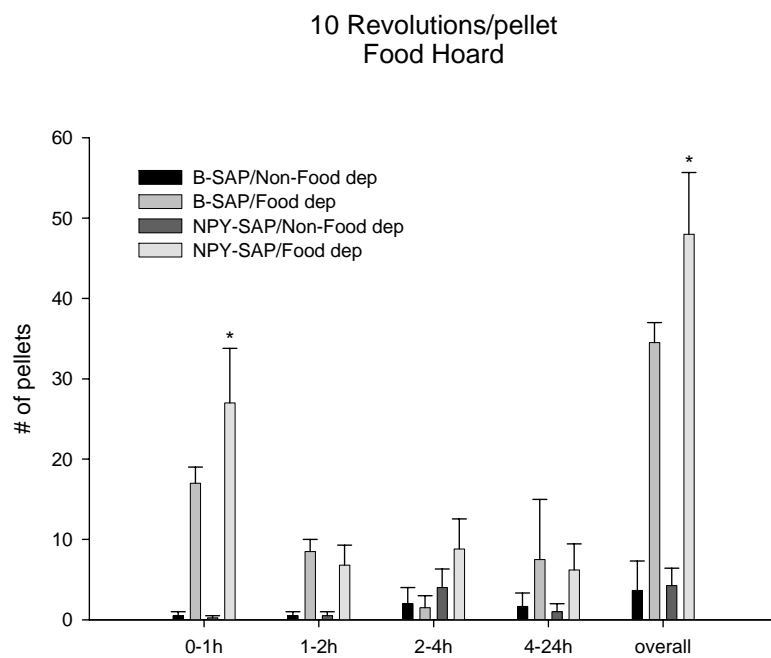
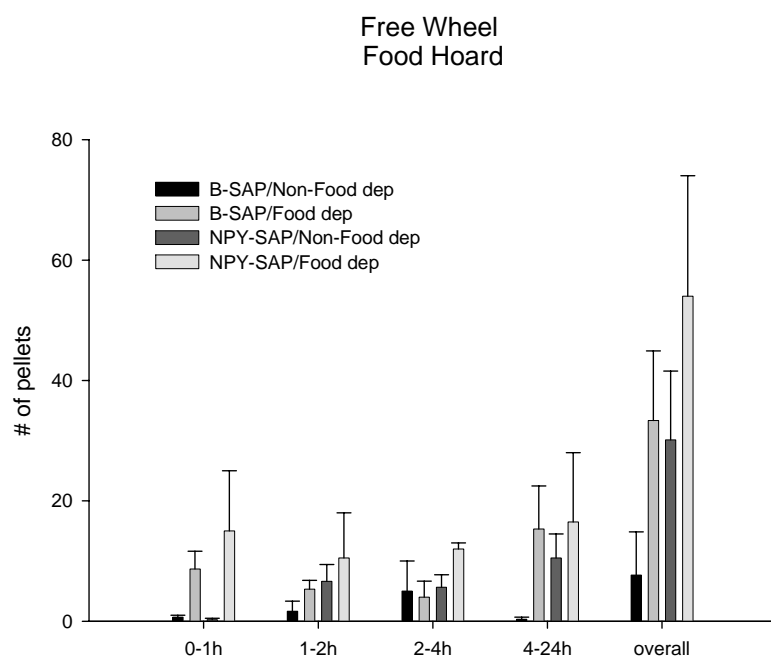
Figure 4.5a**Figure 4.5b**

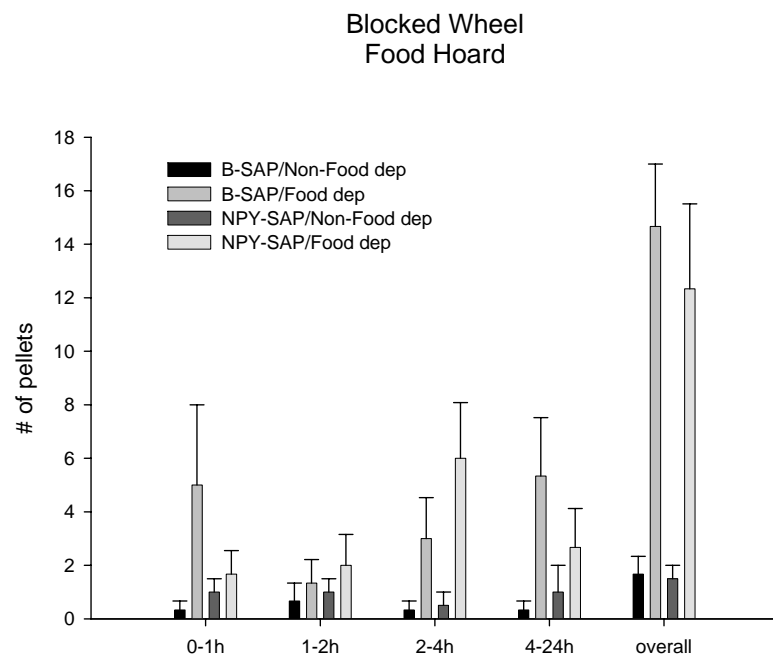
Figure 4.5c

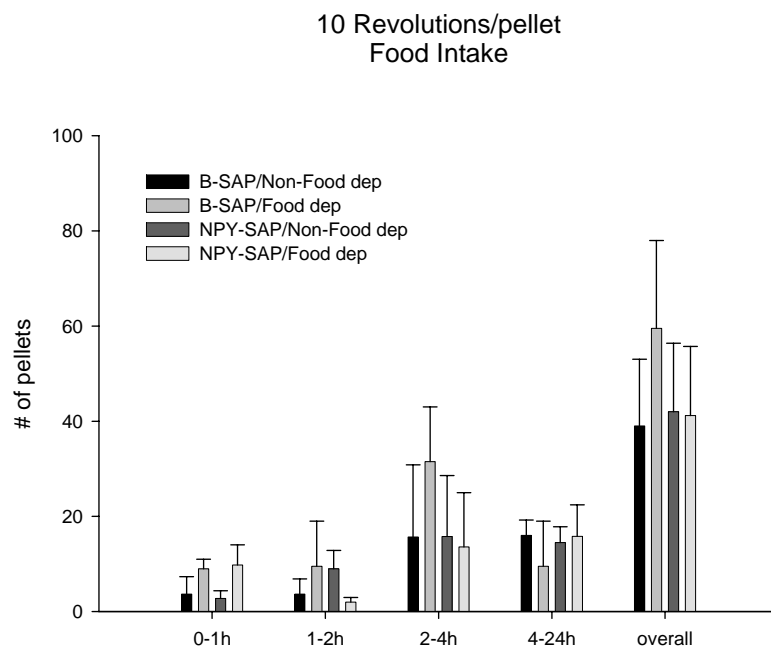
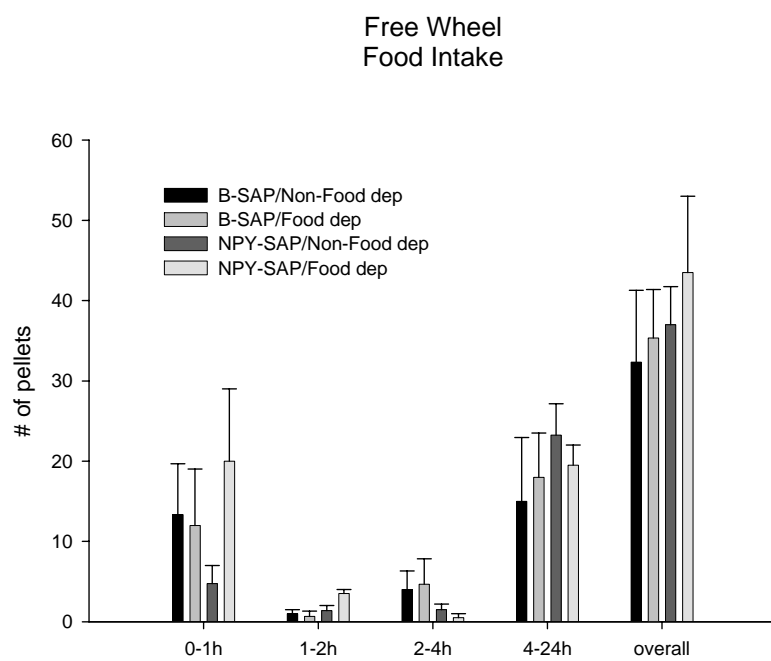
Figure 4.6a**Figure 4.6b**

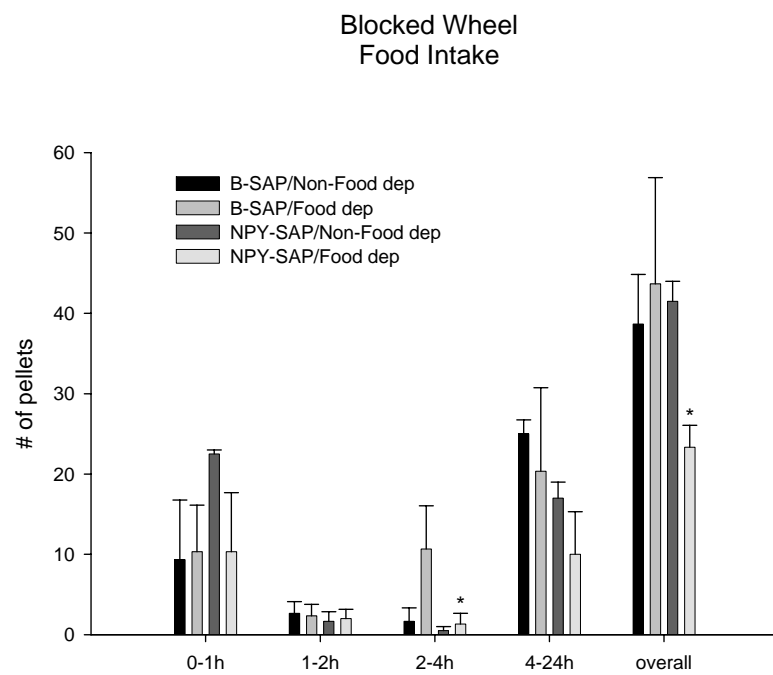
Figure 4.6c

Figure 4.7a

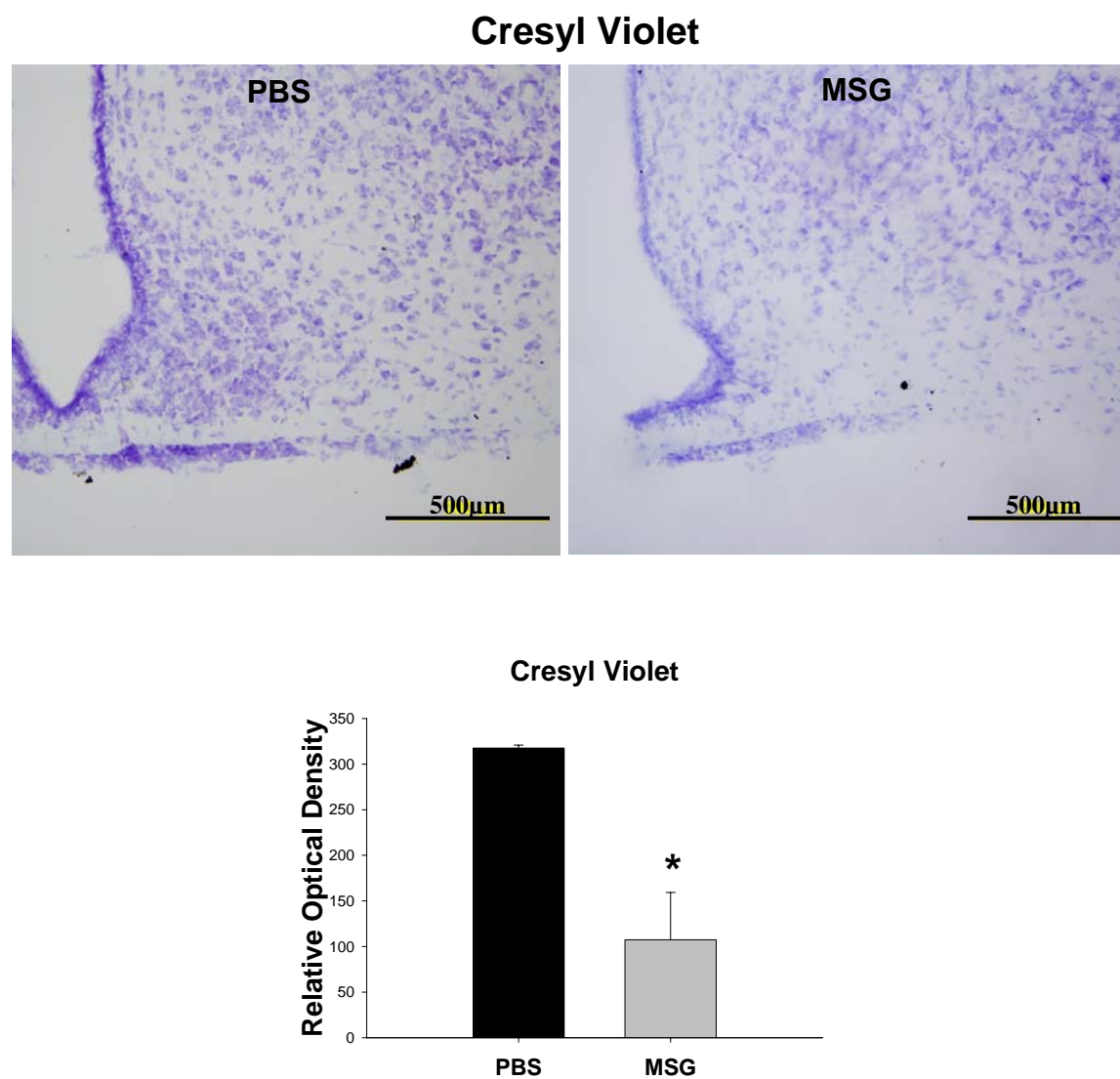


Figure 4.7b

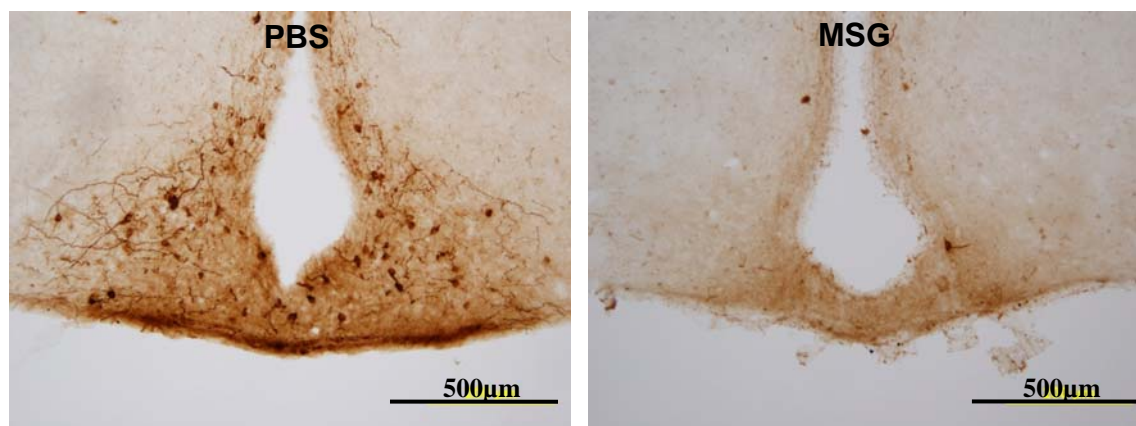
Tyrosine Hydroxylase

Figure 4.7c

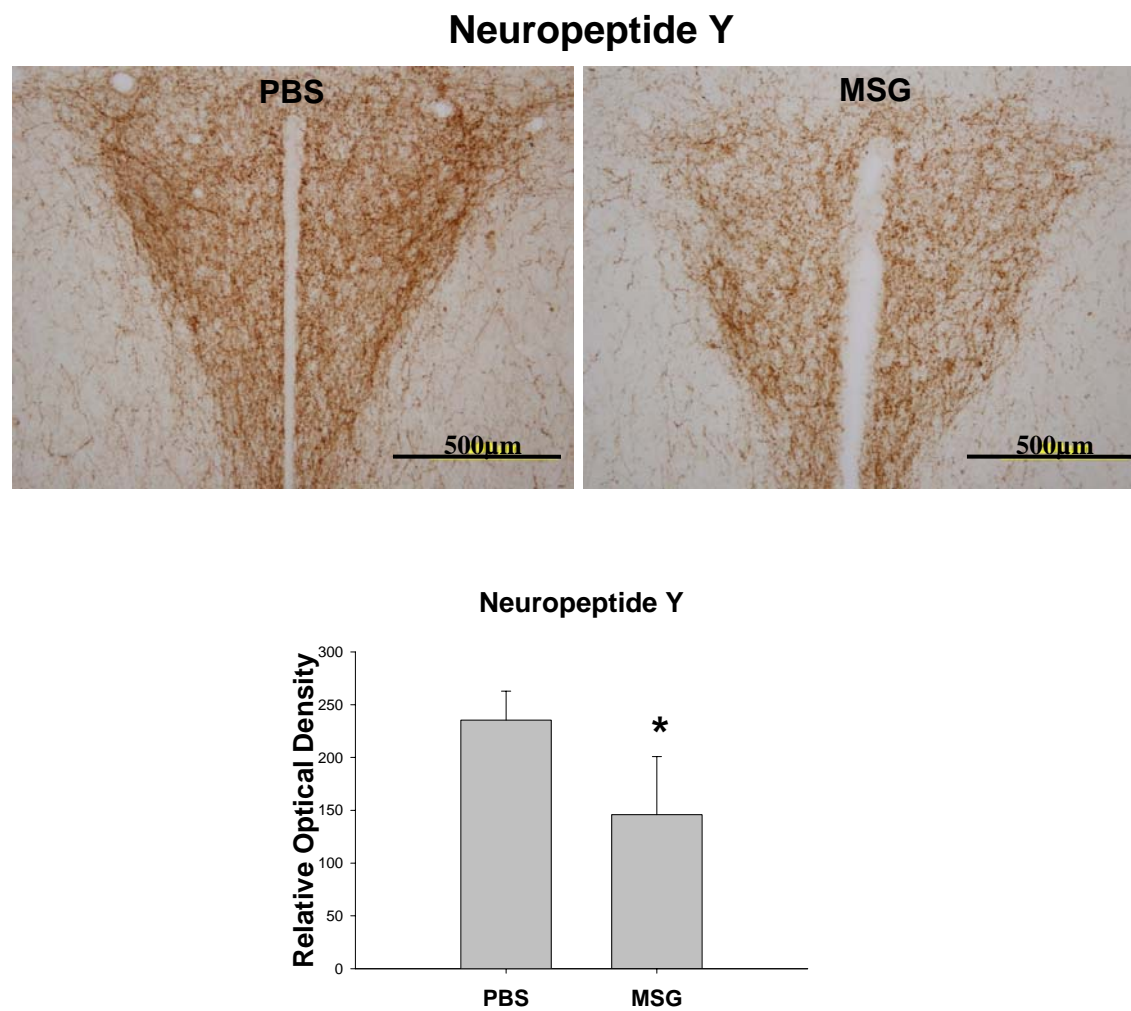


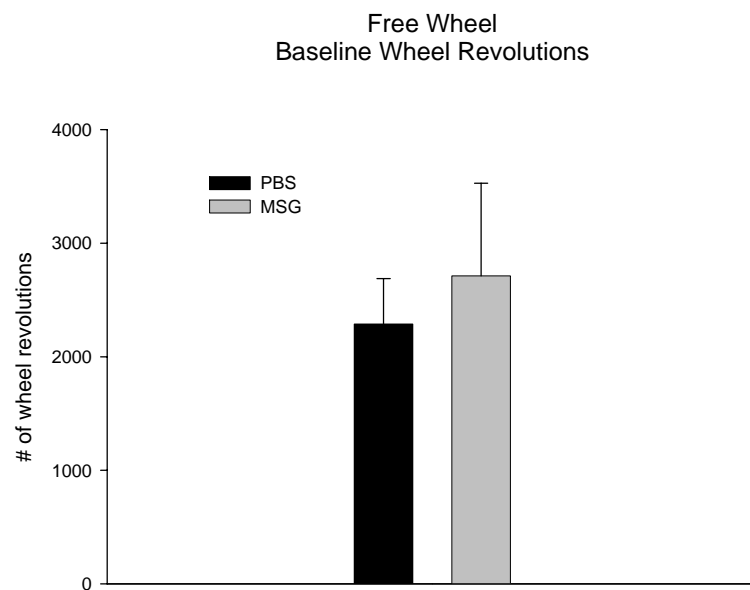
Figure 4.8

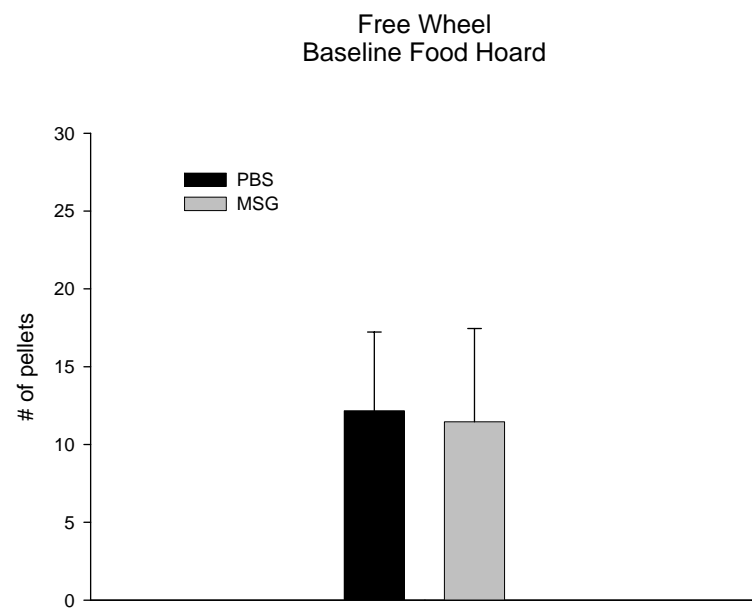
Figure 4.9

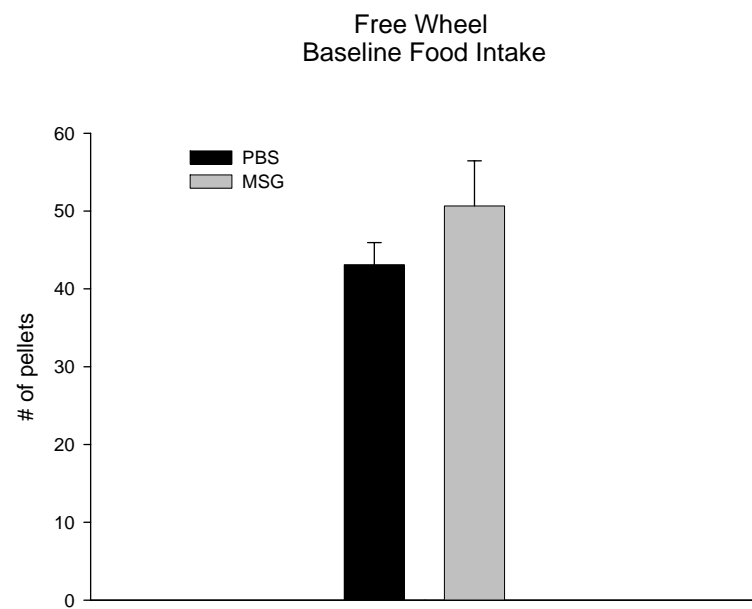
Figure 4.10

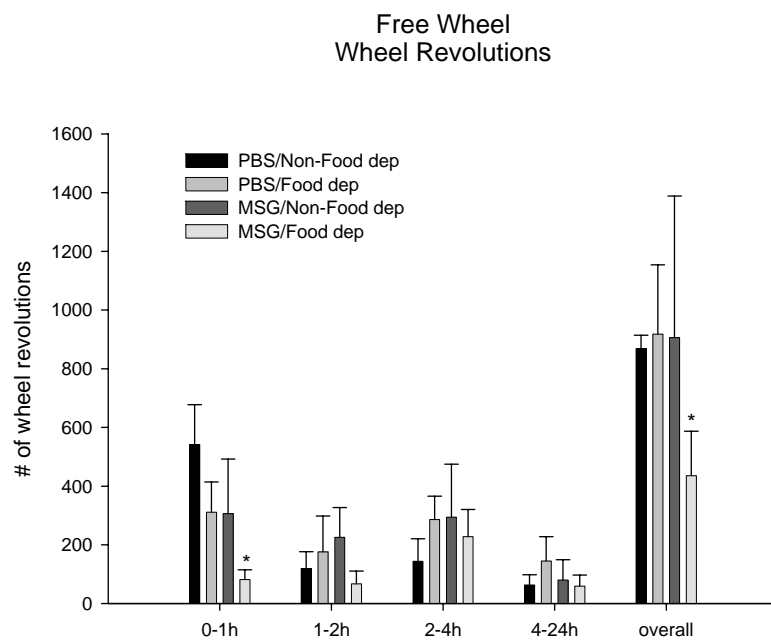
Figure 4.11

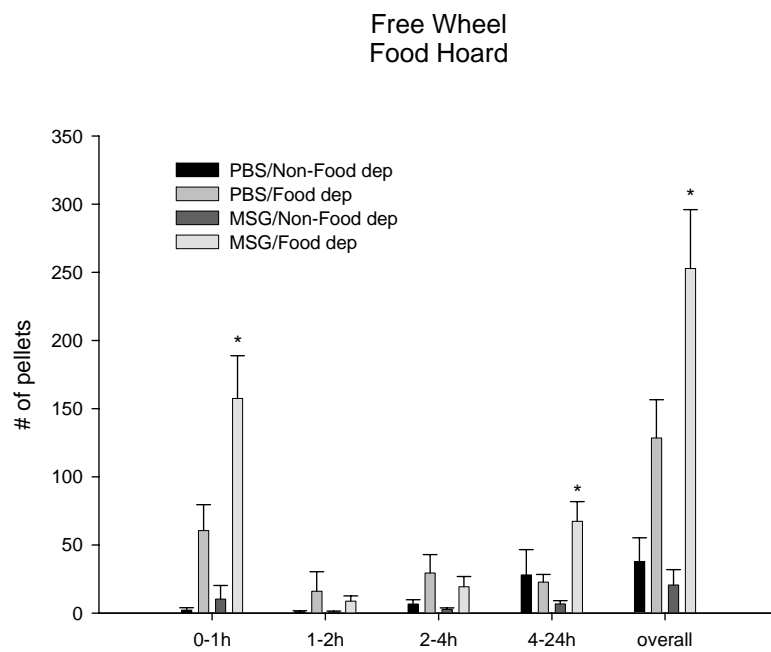
Figure 4.12

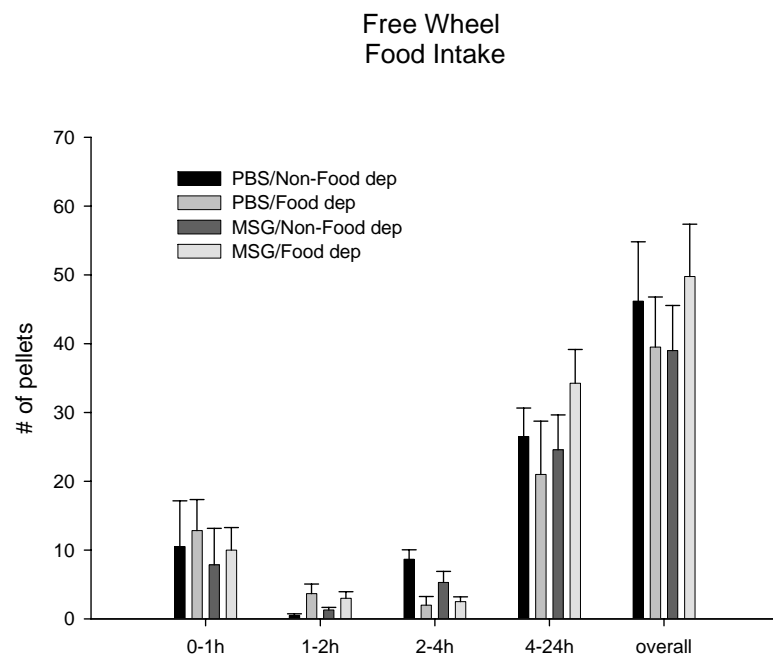
Figure 4.13

Figure 4.14a

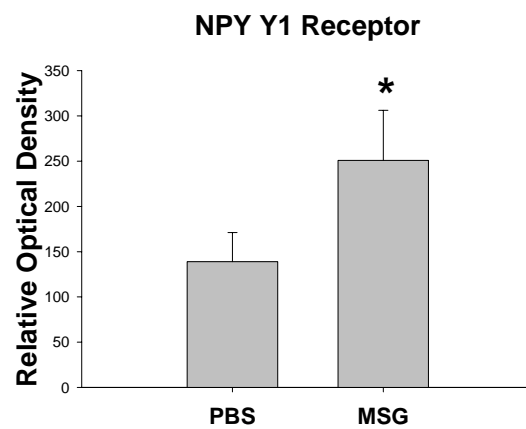
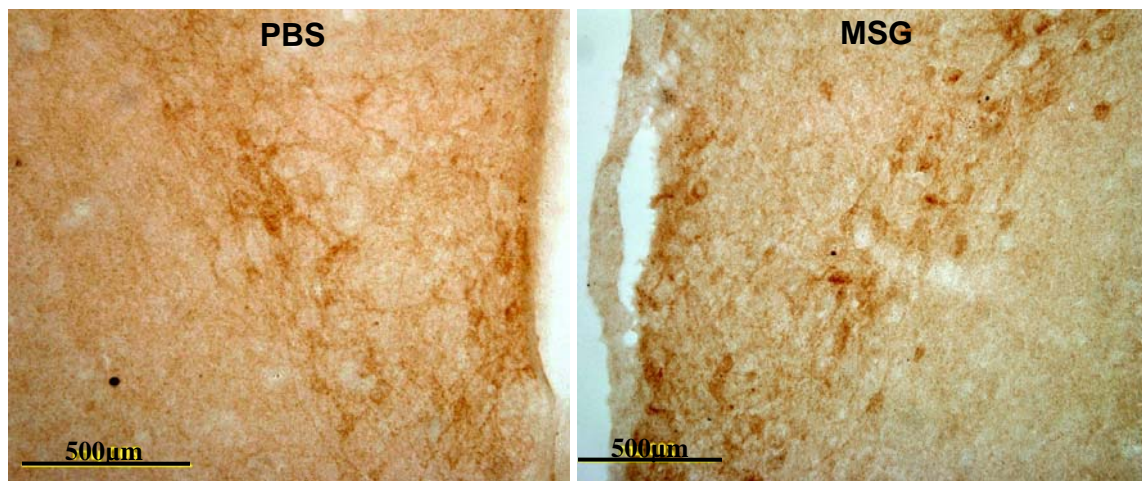
NPY Y1 Receptor in the PVH

Figure 4.14b

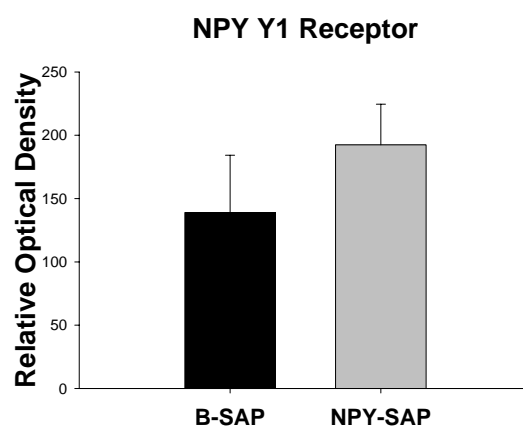
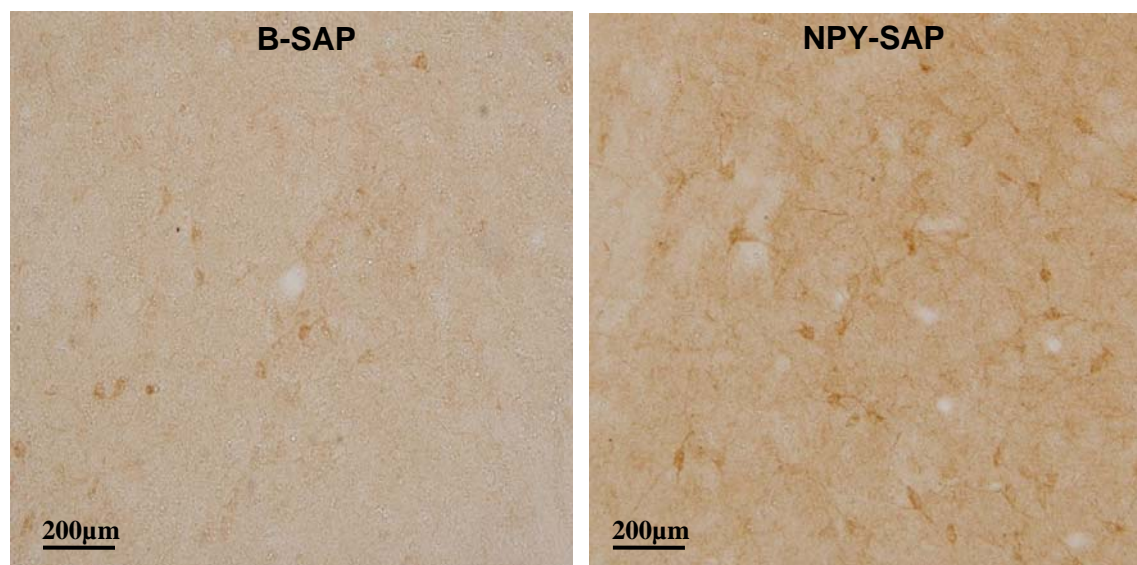
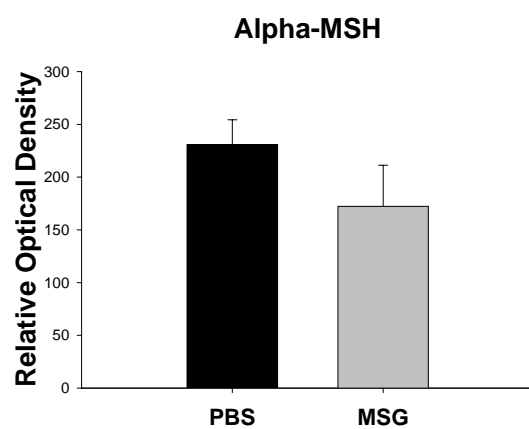
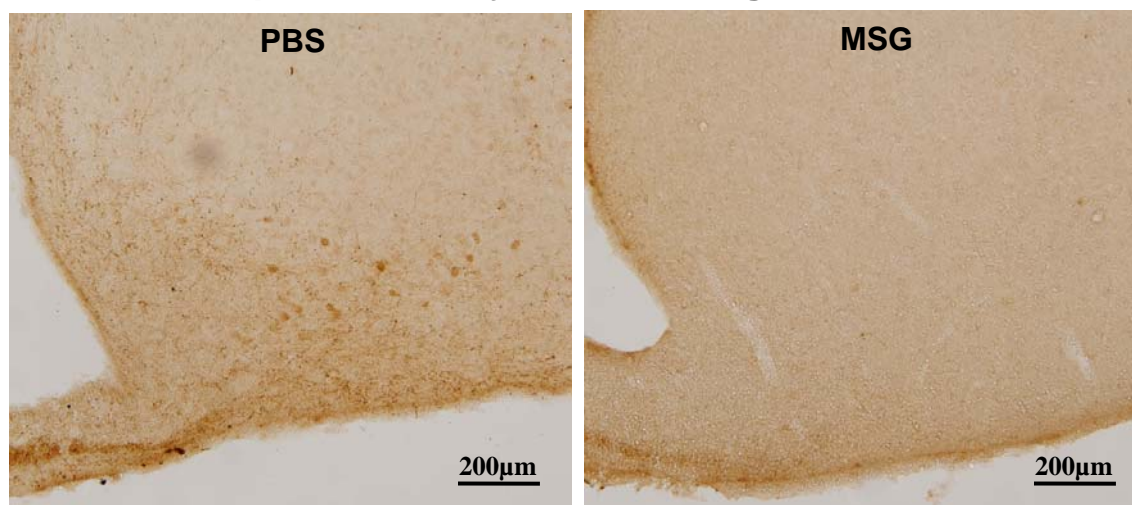
NPY Y1 Receptor in the PFA

Figure 4.14c

Alpha-Melanocyte Stimulating Hormone

CHAPTER 5

CONCLUSION

Obesity is a disease of literally and figuratively enormous proportions. Even with a growing number of advertisements for “weight loss solutions,” a constant flow of new diets and more individuals than ever adopting a fitness regimen, there is an ever increasing rate of obesity within our society. This increase in obesity is a major cause for concern because obesity is linked to many secondary health consequences that include type II diabetes, heart disease, and cancer (Vague et al., 1980; Satcher, 2001; Gasteyger and Tremblay, 2002). Much attention has been paid to decreasing energy intake through dieting or increasing energy expenditure through exercise as a means for combating obesity. This approach has not been very effective because people have a difficult time committing to such changes. We live in an environment where we are constantly bombarded with visual and auditory stimuli that motivate us to acquire (appetitive ingestive behavior) and eat (consummatory ingestive behavior) more food than is needed to maintain normal energy balance. Understanding the mechanisms that regulate such behavior would enable researchers to devise alternate means, such as drug therapy, to fight the obesity epidemic. The subsequent conclusion discusses findings from this dissertation and the contribution it adds to the understanding of the regulatory mechanisms of appetitive and consummatory ingestive behaviors that may ultimately lead to a behavioral or pharmaceutical treatment to combat obesity.

Even though ingestive behavior is comprised of both appetitive and consummatory phases, most research has focused on the regulatory mechanisms controlling the consummatory ingestive behavior of food intake, with little attention focused on the control of appetitive ingestive behaviors. This dissertation defined new roles of NPY in controlling the appetitive ingestive behaviors of foraging and food hoarding and expanded the current knowledge of the NPY-mediation of the

consummatory ingestive behavior of food intake. We knew from previous studies in our laboratory (Day et al., 2005) that 3rd ventricular NPY injections increase food hoarding more than food intake in Siberian hamsters, but we did not know the specific sites of action of NPY that may be driving this change. This dissertation supports previous research in laboratory rats showing the PVH and PFA as two key nuclei in controlling NPY-induced consummatory ingestive behavior (Stanley and Leibowitz, 1984; Stanley et al., 1993), but defines a greater role of these two areas in controlling the appetitive ingestive behavior of food hoarding in Siberian hamsters. The PFA was found to play an additional role in increasing foraging and food intake, albeit to a lesser extent than increasing food hoarding. Because previous research on the site-specific effect of NPY used model systems designed to only look at consummatory ingestive behaviors, the significant finding of the effect of NPY on controlling appetitive ingestive behaviors was overlooked. This shows the importance of selecting the right model to answer the question concerning the role of appetitive ingestive behaviors in obesity. A contributing factor to humans overeating and becoming obese is that they are motivated to acquire and hoard food that they are then more likely to eat (Ransley et al., 2003b). Using a model system like laboratory rats and mice that do not naturally hoard food will not provide the most comprehensive analysis of ingestive behaviors. Therefore, some neuropeptides thought to stimulate food intake in home-cage tests may also, or instead, trigger appetitive ingestive behaviors if the model allows for the expression of foraging and hoarding.

Changes in appetitive and consummatory ingestive behaviors may be controlled by separate NPY receptors. Of the five receptor subtypes that have been cloned for NPY, four have been localized within the rodent brain, including the NPY Y1, Y2, Y4 and Y5 (Parker and Herzog, 1999). Although the exact role of each of the NPY receptor subtypes in regulating ingestive behaviors is unclear, the Y1 and Y5 receptor subtypes appear to be the most directly involved in the regulation of

appetitive and consummatory ingestive behaviors (Chamorro et al., 2002b). A NPY Y1 receptor-specific agonist predominantly increases food hoarding, whereas the Y5 receptor specific agonist predominantly increases food intake after third ventricular injections in Siberian hamsters (Day et al., 2005). This dissertation defined the NPY Y1 receptor subtype in the PVH and PFA as specific sites of action of the NPY-mediated increase in food hoarding. Given that humans hoard food and that this leads to an increased likelihood to eat the hoarded food (Ransley et al., 2003a), the Y1 receptor may be a key point of attack in the pharmaceutical treatment of obesity.

Food deprivation increases foraging and food hoarding in Siberian hamsters, with no change in food intake ((Day and Bartness, 2003). Because antagonism of the Y1 receptor within the PVH was found here only to inhibit the post-food deprivation increase in food hoarding and not foraging, it is likely that other Y receptor subtypes are responsible for the NPY-mediation of foraging in Siberian hamsters. A direct test of the role of NPY Y2, Y4 or Y5 receptor subtypes using specific antagonists to block post-food deprivation increases in foraging would be beneficial, but the current antagonists available for these NPY receptor subtypes either have agonistic properties, are not specific for just one receptor subtype or they do not readily dissolve in solution. Therefore, the effect of NPY in the PVH was blocked by destroying all Y receptor-containing neurons located there using NPY conjugated to the immunotoxin saporin. We were able to show that NPY Y receptors, other than the Y1, mediate the post-food deprivation increase of foraging, as well as, mediate changes in food intake after the energetic challenge of food deprivation. In addition, we showed that Y receptors in the PVH are important for the normal control of the appetitive ingestive behaviors of foraging and hoarding, but not food intake. Not only does this method of using NPY-SAP to destroy Y receptor-containing neurons in the PVH offer insight into the direct affect of NPY on ingestive behaviors within this nuclei, but it also provides insight into how NPY may be able to convey signals

of energy status in the PVH to affect other downstream homeostatic mechanisms. The PVH serves as an integrator and link between the neuroendocrine and autonomic nervous systems where NPY may be able to affect numerous circuits involved in energy homeostasis and generate appropriate responses to modulate ingestive behaviors. NPY Y receptors are colocalized with many other neuropeptides or hormones that are important regulators of energy balance and endocrine axes (Parker and Herzog, 1999), including CRH (Campbell, 2000; Li, 2000), TRH (Broberger et al., 1999c), OT and AVP (Campbell, 2001). These data provide morphological evidence for the role of NPY in the PVH influencing energy metabolism through actions affecting downstream hypothalamic-pituitary axes and the autonomic nervous system through Y receptor-mediated mechanisms. NPY-SAP could be used as tool to investigate the role of NPY in mediating downstream effects on these systems. If we could define the role of each Y receptor within the PVH, combined with the phenotyping of neurons where each NPY Y receptor subtype is expressed in the PVH, we could better understand how NPY simultaneously could affect ingestive behaviors and stimulate or inhibit these other networks.

The hypothalamic view of the NPY-mediated effect on ingestive behaviors has hampered the investigation into the mechanisms involved in the chronic positive energy balance of obesity because NPY may mediate changes in ingestive behaviors in areas outside the hypothalamus. Because NPY is predominantly produced in the ARC, research has focused on the response of ARC-NPY to various peripheral signals (*e.g.*, leptin, ghrelin, insulin) that may ultimately affect the expression of appetitive and consummatory ingestive behaviors. Most research has neglected the fact that these same signals also could directly or indirectly alter the expression of brainstem NPY (*e.g.*, catecholaminergic cell groups A1, C1-C3; (Everitt et al., 1984a)), that then could mediate changes of ingestive behaviors by activating local Y receptors in the brainstem (Dumont et al., 1993) or through

NPY afferent projections (Broberger and Hokfelt, 2001) to Y receptors in forebrain nuclei (Dumont et al., 1993). Results from this dissertation showed that the ARC, one source of NPY production (Chronwall et al., 1985b; White and Kershaw, 1989), is not needed for appetitive ingestive behaviors under baseline conditions or after food deprivation, or for consummatory ingestive behaviors after food deprivation. After lesions of the ARC and presumably ARC-NPY producing neurons produced by both NPY-SAP or MSG treatment used in the present studies, the PVH still had an extensive innervation of NPY fibers, suggesting that the remaining brainstem NPY population may be sufficient to stimulate ingestive behaviors even with the loss of the ARC. Other studies also suggest that there may be a local effect of NPY within the brainstem. For example, fourth ventricular injections of NPY stimulate food intake in rats to the same extent as third ventricular injections (Corp et al., 2001). In decerebrate rats, when all connections between the forebrain and brainstem are severed, the isolated brainstem is still capable of altering consummatory ingestive behaviors when energetically challenged (Grill and Kaplan, 2001; Harris et al., 2006a). Although these data suggest that the brainstem may contain local circuits that are capable of solely controlling ingestive behaviors, no one has looked at the brainstem control of foraging and food hoarding and if site-specific injections of NPY within the brainstem affects these appetitive ingestive behaviors. More research on the brainstem NPY-mediated control of ingestive behaviors could help to better understand if this population plays a role in the regulation of ingestive behaviors under normal or energetically challenging conditions.

The sustainability of ingestive behaviors after the loss of the ARC points to the fact that other mechanisms are able to compensate to drive changes in both appetitive and consummatory ingestive behaviors after the destruction of a predominant source of NPY (Chronwall et al., 1985a; White and Kershaw, 1989). After lesions of the ARC and decreases in NPY fiber-ir that

presumably resulted from loss of ARC-NPY, we saw compensatory increases in NPY Y1 receptors in the PVH and a substantial NPY innervation of the PVH remaining. Even though these compensatory mechanisms of the NPY system may contribute to the sustainability of ingestive behaviors after the presumed loss of ARC-NPY neurons, the destruction of the ARC may have caused damage to additional neurochemical systems that may result in other compensatory mechanisms to maintain energy balance. Two melanocortin peptides, agouti-related protein (AgRP) and α -melanotan stimulating hormone (α -MSH), produced by ARC neurons also would presumably be destroyed by NPY-SAP or MSG treatments. A decrease in either or both peptides may result in additional compensatory mechanisms, beyond NPY-mediated alterations, in both appetitive and consummatory ingestive behaviors. AgRP is colocalized with ARC-NPY neurons (Mercer et al., 2000a) and is another potent stimulator of ingestive behaviors (Day and Bartness, 2004). α -MSH, produced by proopiomelanocortin (POMC) neurons of the ARC (Chronwall, 1985) and nucleus of the solitary tract (Lichtensteiger et al., 1996) is an anorexigenic peptide that exerts an opposite effect of NPY or AgRP on ingestive behaviors (Cowley et al., 1999; Morton and Schwartz, 2001; Dhillo et al., 2002; Mercer and Tups, 2003). When animals are faced with negative energy balance, such as in food deprivation, both NPY and AgRP gene expression are increased (Brady et al., 1990; Hahn et al., 1998; Mizuno et al., 1999; Mercer et al., 2000b), whereas POMC mRNA levels are decreased (Brady et al., 1990; Hahn et al., 1998; Mizuno et al., 1999; Mercer et al., 2000b). The opposite gene expression profile is seen when animals are in positive energy balance, as in diet-induced obesity (Ziotopoulou et al., 2000; Torri et al., 2002). The differential action of NPY/AgRP and α -MSH also are reflected in their effect on appetitive and consummatory ingestive behaviors after central injections of each peptide. Whereas, central injections of NPY or AgRP increases

food intake in laboratory rats, they increase food hoarding to a greater extent than food intake in Siberian hamsters (Morley et al., 1987b; Kalra and Kalra, 2000; Wirth and Giraudo, 2000; Day and Bartness, 2004; Day et al., 2005). Central injection of MTII, the synthetic analogue of α -MSH, markedly reduces food intake in laboratory rats, but also food hoarding in Siberian hamsters (Brown et al., 1998; Schuhler et al., 2003). Thus, NPY, AgRP and α -MSH function together in the control of appetitive and consummatory ingestive behaviors to maintain energy balance. After destruction of the ARC in the present studies, there were decreases in NPY and α -MSH-ir that suggests a decrease in NPY/AgRP and POMC neurons that produce these two peptides, respectively. Because the concentration of this dissertation was limited to the NPY-mediated control of ingestive behaviors, the compensatory mechanisms that may have resulted from the presumed loss of AgRP and α -MSH neurons also may have contributed to the sustainability of ingestive behaviors. Understanding how these different mechanisms interact in future studies is essential to finding a therapy that will be effective at eliminating the increasing obesity trend.

Collectively, the results from this dissertation indicate that the control of appetitive ingestive behaviors under normal conditions is mediated by the effect of NPY in the PVH. Because an ARC lesion that presumably destroyed ARC-NPY neurons did not affect normal foraging or food hoarding, it suggests that the NPY fibers to the PVH that mediate the effect of foraging and hoarding originate from a source other than ARC-NPY. By contrast, a lesion of the ARC and a presumed loss of ARC-NPY decreased food intake under normal conditions, but there was no effect on normal food intake when the effect of NPY in the PVH was blocked by NPY-SAP. This suggests that normal feeding may be controlled by ARC-NPY projections to a brain area other than the PVH. In Chapter 2, we showed PFA-NPY increased food intake, albeit not to the same extent as food hoarding. In laboratory rats, however, PFA-NPY produced robust

increases in food intake that were far greater than NPY injected into other hypothalamic areas (Stanley et al., 1993). There also was a distinct contrast in the effect of NPY in mediating changes in appetitive and consummatory ingestive behaviors after food deprivation between the study in Chapter 3 blocking the effect of NPY in the PVH or the study in Chapter 4 producing a lesion of the ARC and presumably ARC-NPY neurons. When the ARC was destroyed, all three behaviors measured were able to compensate after food deprivation. By contrast, blocking the effect of NPY in the PVH affects all three behaviors such that post-food deprivation increases in foraging and food hoarding were inhibited and food intake was decreased (there is no post-fast increase in food intake). This suggests that there are many neurochemicals that may be able to compensate for a decrease in NPY, but that these neurochemicals have the PVH as a primary site of action. Because the PVH is an integration site for numerous homeostatic systems (Watts, 2000; Campbell, 2001), it is not surprising that destroying the Y receptors in this area would produce such a robust effect on both appetitive and consummatory ingestive behaviors.

Obesity is caused by an imbalance between energy intake and expenditure and is influenced by both genetic and environmental factors. Although humans appear to be genetically programmed to conserve energy and eat beyond current energy requirements whenever food is plentiful (Poston and Foreyt, 1999; Illius et al., 2002), the rapid increase in obesity cannot simply be explained by genetics alone. We are constantly bombarded with visual and auditory stimuli that motivate us to acquire and eat more food than is needed to maintain normal energy balance. An environment with easy access to high calorie, low cost foods combined with decreased physical activity has resulted in the chronic positive energy balance that leads to weight gain. The majority of obesity cases are not the result of improper satiety signaling. Most of us get the feeling of fullness after a meal at our favorite restaurant, but we just can't pass up the dessert cart. It is in understanding what motivates

us to eat beyond what is necessary that will help to alleviate the increasing rate of obesity. Overall, this dissertation has provided insight into additional roles of NPY, Y receptors and specific nuclei in mediating changes in the motivation to acquire and eat food, as well as expanded the current knowledge of the mechanisms underlying food intake. Hopefully future research that incorporates the study of appetitive ingestive behaviors may lead to a pharmaceutical or behavioral manipulation that will stop us from grabbing that piece of cake from the dessert cart.

REFERENCES

1. Alessi, N.E., Quinlan, P., Khachaturian, H., 1988. MSG effects on beta-endorphin and alpha-MSH in the hypothalamus and caudal medulla. *Peptides* 9, 689-695.
2. Bai, F.L., Yamano, M., Shiotani, Y., Emson, P.C., Smith, A.D., Powell, J.F., Tohyama, M., 1985. An arcuato-paraventricular and -dorsomedial hypothalamic neuropeptide Y-containing neurons in the rat hypothalamus. *Brain Res.* 331, 172-175.
3. Bartness, T.J., 1997. Food hoarding is increased by pregnancy, lactation and food deprivation in Siberian hamsters. *Am.J.Physiol.* 272, R118-R125.
4. Bartness, T.J., Clein, M.R., 1994. Effects of food deprivation and restriction, and metabolic blockers on food hoarding in Siberian hamsters. *Am.J.Physiol.* 266, R1111-R1117.
5. Bartness, T.J., Day, D.E., 2003. Food hoarding: a quintessential anticipatory appetitive behavior. *Progress in Psychobiology and Physiological Psychology* 18, 69-100.
6. Bartness, T.J., Demas, G.E., 2004. Comparative studies of food intake: Lessons from non-traditionally studied species, in: E.M. Stricker, S.C. Woods (Eds.), *Food and Fluid Intake*. Plenum, New York, pp. 423-467.
7. Bartness, T.J., Morley, J.E., Levine, A.S., 1995. Effects of food deprivation and metabolic fuel utilization on the photoperiodic control of food intake in Siberian hamsters. *Physiol.Behav.* 57, 61-68.
8. Batterham, R.L., Cowley, M.A., Small, C.J., Herzog, H., Cohen, M.A., Dakin, C.L., Wren, A.M., Brynes, A.E., Low, M.J., Ghatei, M.A., Cone, R.D., Bloom, S.R., 2002. Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature* 418, 650-654.
9. Beck, B., Jhanwar-Uniyal, M., Burlet, A., Chapleur-Chateau, M., Leibowitz, S.F., Burlet, C., 1990. Rapid and localized alterations of neuropeptide Y (NPY) in discrete hypothalamic nuclei with feeding status. *Brain Res.* 528, 245-249.
10. Beck, B., Richy, S., Dimitrov, T., Stricker-Krongrad, A., 2001. Opposite regulation of hypothalamic orexin and neuropeptide Y receptors and peptide expressions in obese Zucker rats. *Biochem.Biophys.Res.Comm.* 286, 518-523.
11. Beneke, W.M., Davis, C.H., 1985. Relationship of hunger, use of a shopping list and obesity to food purchases. *Int.J.Obes.* 9, 391-399.
12. Benoit, S.C., Tracy, A.L., Air, E.L., Kinzig, K., Seeley, R.J., Davidson, T.L., 2001. The role of the hypothalamic melanocortin system in behavioral appetitive processes. *Pharmacol.Biochem.Behav.* 69, 603-609.
13. Borer, K.T., Rowland, N.E., Mirow, A., Borer, R.C.Jr., Kelch, R.P., 1979. Physiological and behavioral responses to starvation in the golden hamster. *Am.J.Physiol.* 236, E105-E112.

14. Brady, L.S., Smith, M.A., Gold, P.W., Herkenham, M., 1990. Altered expression of hypothalamic neuropeptide mRNAs in food-restricted and food-deprived rats. *Neuroendocrinology* 52, 441-447.
15. Broberger, C., Hokfelt, T., 2001. Hypothalamic and vagal neuropeptide circuitries regulating food intake. *Physiol Behav.* 74, 669-682.
16. Broberger, C., Johansen, J., Brismar, H., Johansson, C., Schalling, M., Hokfelt, T., 1999b. Changes in neuropeptide Y receptors and pro-opiomelanocortin in the anorexia (anx/anx) mouse hypothalamus. *J Neurosci.* 19, 7130-7139.
17. Broberger, C., Johansen, J., Brismar, H., Johansson, C., Schalling, M., Hokfelt, T., 1999a. Changes in neuropeptide Y receptors and pro-opiomelanocortin in the anorexia (anx/anx) mouse hypothalamus. *J Neurosci.* 19, 7130-7139.
18. Broberger, C., Johansen, J., Johansson, C., Schalling, M., Hokfelt, T., 1998. The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. *Proc.Natl.Acad.Sci.U.S.A* 95, 15043-15048.
19. Broberger, C., Visser, T.J., Kuhar, M.J., Hokfelt, T., 1999c. Neuropeptide Y innervation and neuropeptide-Y-Y1-receptor-expressing neurons in the paraventricular hypothalamic nucleus of the mouse. *Neuroendocrinology* 70, 295-305.
20. Brown, K.S., Gentry, R.M., Rowland, N.E., 1998. Central injections in rats of alpha-melanocyte-stimulating hormone analog: effects on food intake and brain Fos. *Regul.Pept.* 78, 89-94.
21. Bugarith, K., Dinh, T.T., Li, A.J., Speth, R.C., Ritter, S., 2005h. Basomedial hypothalamic injections of neuropeptide Y conjugated to saporin selectively disrupt hypothalamic controls of food intake. *Endocrinology* 146, 1179-1191.
22. Bugarith, K., Dinh, T.T., Li, A.J., Speth, R.C., Ritter, S., 2005a. Basomedial hypothalamic injections of neuropeptide Y conjugated to saporin selectively disrupt hypothalamic controls of food intake. *Endocrinology* 146, 1179-1191.
23. Bugarith, K., Dinh, T.T., Li, A.J., Speth, R.C., Ritter, S., 2005b. Basomedial hypothalamic injections of neuropeptide Y conjugated to saporin selectively disrupt hypothalamic controls of food intake. *Endocrinology* 146, 1179-1191.
24. Bugarith, K., Dinh, T.T., Li, A.J., Speth, R.C., Ritter, S., 2005c. Basomedial hypothalamic injections of neuropeptide Y conjugated to saporin selectively disrupt hypothalamic controls of food intake. *Endocrinology* 146, 1179-1191.
25. Bugarith, K., Dinh, T.T., Li, A.J., Speth, R.C., Ritter, S., 2005d. Basomedial hypothalamic injections of neuropeptide Y conjugated to saporin selectively disrupt hypothalamic controls of food intake. *Endocrinology* 146, 1179-1191.

26. Bugarith, K., Dinh, T.T., Li, A.J., Speth, R.C., Ritter, S., 2005e. Basomedial hypothalamic injections of neuropeptide Y conjugated to saporin selectively disrupt hypothalamic controls of food intake. *Endocrinology* 146, 1179-1191.
27. Bugarith, K., Dinh, T.T., Li, A.J., Speth, R.C., Ritter, S., 2005f. Basomedial hypothalamic injections of neuropeptide Y conjugated to saporin selectively disrupt hypothalamic controls of food intake. *Endocrinology* 146, 1179-1191.
28. Bugarith, K., Dinh, T.T., Li, A.J., Speth, R.C., Ritter, S., 2005g. Basomedial hypothalamic injections of neuropeptide Y conjugated to saporin selectively disrupt hypothalamic controls of food intake. *Endocrinology* 146, 1179-1191.
29. Bunyan, J., Murrell, E.A., Shah, P.P., 1976. The induction of obesity in rodents by means of monosodium glutamate. *Br.J.Nutr.* 35, 25-39.
30. Chamorro, S., la-Zuana, O., Fauchere, J.L., Feletou, M., Galizzi, J.P., Levens, N., 2002b. Appetite suppression based on selective inhibition of NPY receptors. *Int.J.Obes.Relat Metab Disord.* 26, 281-298.
31. Chamorro, S., la-Zuana, O., Fauchere, J.L., Feletou, M., Galizzi, J.P., Levens, N., 2002a. Appetite suppression based on selective inhibition of NPY receptors. *Int.J.Obes.Relat Metab Disord.* 26, 281-298.
32. Chronwall, B.M., 1985. Anatomy and physiology of the neuroendocrine arcuate nucleus. *Peptides* 6, 1-11.
33. Chronwall, B.M., DiMaggio, D.A., Massari, V.J., Pickel, V.M., Ruggiero, D.A., O'Donohue, T.L., 1985a. The anatomy of neuropeptide-Y-containing neurons in rat brain. *Neuroscience* 15, 1159-1181.
34. Chronwall, B.M., DiMaggio, D.A., Massari, V.J., Pickel, V.M., Ruggiero, D.A., O'Donohue, T.L., 1985b. The anatomy of neuropeptide-Y-containing neurons in rat brain. *Neuroscience* 15, 1159-1181.
35. Chronwall, B.M., DiMaggio, D.A., Massari, V.J., Pickel, V.M., Ruggiero, D.A., O'Donohue, T.L., 1985c. The anatomy of neuropeptide-Y-containing neurons in rat brain. *Neuroscience* 15, 1159-1181.
36. Chronwall, B.M., DiMaggio, D.A., Massari, V.J., Pickel, V.M., Ruggiero, D.A., O'Donohue, T.L., 1985d. The anatomy of neuropeptide-Y-containing neurons in rat brain. *Neuroscience* 15, 1159-1181.
37. Chronwall, B.M., DiMaggio, D.A., Massari, V.J., Pickel, V.M., Ruggiero, D.A., O'Donohue, T.L., 1985e. The anatomy of neuropeptide-Y-containing neurons in rat brain. *Neuroscience* 15, 1159-1181.

38. Chronwall, B.M., DiMaggio, D.A., Massari, V.J., Pickel, V.M., Ruggiero, D.A., O'Donohue, T.L., 1985f. The anatomy of neuropeptide-Y-containing neurons in rat brain. *Neuroscience* 15, 1159-1181.
39. Chronwall, B.M., DiMaggio, D.A., Massari, V.J., Pickel, V.M., Ruggiero, D.A., O'Donohue, T.L., 1985g. The anatomy of neuropeptide-Y-containing neurons in rat brain. *Neuroscience* 15, 1159-1181.
40. Clark, J.T., Kalra, P.S., Crowley, W.R., Kalra, S.P., 1984. Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. *Endocrinology* 115, 427-429.
41. Corp, E.S., McQuade, J., Krasnicki, S., Conze, D.B., 2001. Feeding after fourth ventricular administration of neuropeptide Y receptor agonists in rats. *Peptides* 22, 493-499.
42. Cowley, M.A., Pronchuk, N., Fan, W., Dinulescu, D.M., Colmers, W.F., Cone, R.D., 1999. Integration of NPY, AGRP, and melanocortin signals in the hypothalamic paraventricular nucleus: evidence of a cellular basis for the adipostat. *Neuron* 24, 155-163.
43. Craig, W., 1918. Appetites and aversions as constituents of instincts. *Biol.Bull.* 34, 91-107.
44. Day, D.E., Bartness, T.J., 2001. Effects of foraging effort on body fat and food hoarding by Siberian hamsters. *J.Exp.Zool.* 289, 162-171.
45. Day, D.E., Bartness, T.J., 2003. Fasting-induced increases in hoarding are dependent on the foraging effort level. *Physiol.Behav.* 78, 655-668.
46. Day, D.E., Bartness, T.J., 2004. Agouti-related protein increases food hoarding, but not food intake by Siberian hamsters. *Am.J.Physiol.* 286, R38-R45.
47. Day, D.E., Keen-Rhinehart, E., Bartness, T.J., 2005. Role of NPY and its receptor subtypes in foraging, food hoarding and food intake by Siberian hamsters. *Am.J.Physiol.* 289, R29-R36.
48. Day, D.E., Mintz, E.M., Bartness, T.J., 1999. Diet self-selection and food hoarding after food deprivation by Siberian hamsters. *Physiol.Behav.* 68, 187-194.
49. Day, D.E., Mintz, E.M., Bartness, T.J., 2002. Diet choice exaggerates food hoarding, intake and pup survival across reproduction. *Physiol.Behav.* 75, 143-157.
50. de Quidt, M.E., Emson, P.C., 1986. Distribution of neuropeptide Y-like immunoreactivity in the rat central nervous system--II. Immunohistochemical analysis. *Neuroscience* 18, 545-618.
51. Dhillon, W.S., Small, C.J., Stanley, S.A., Jethwa, P.H., Seal, L.J., Murphy, K.G., Ghatei, M.A., Bloom, S.R., 2002. Hypothalamic interactions between neuropeptide y, agouti-related protein, cocaine- and amphetamine-regulated transcript and alpha-melanocyte-stimulating hormone in vitro in male rats. *J.Neuroendocrinol.* 14, 725-730.

52. Dodd, D.K., Stalling, R.B., Bedell, J., 1977. Grocery purchases as a function of obesity and assumed food deprivation. *Int.J Obes.* 1, 43-47.
53. Dumont, Y., Fournier, A., St-Pierre, S., Quirion, R., 1993. Comparative characterization and autoradiographic distribution of neuropeptide Y receptor subtypes in the rat brain. *J.Neurosci.* 13, 73-86.
54. Ebling, F.J., Arthurs, O.J., Turney, B.W., Cronin, A.S., 1998. Seasonal neuroendocrine rhythms in the male Siberian hamster persist after monosodium glutamate-induced lesions of the arcuate nucleus in the neonatal period. *J.Neuroendocrinology* 10, 701-712.
55. Everitt, B.J., Hokfelt, T., Terenius, L., Tatemoto, K., Mutt, V., Goldstein, M., 1984e. Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. *Neuroscience* 11, 443-462.
56. Everitt, B.J., Hokfelt, T., Terenius, L., Tatemoto, K., Mutt, V., Goldstein, M., 1984f. Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. *Neuroscience* 11, 443-462.
57. Everitt, B.J., Hokfelt, T., Terenius, L., Tatemoto, K., Mutt, V., Goldstein, M., 1984g. Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. *Neuroscience* 11, 443-462.
58. Everitt, B.J., Hokfelt, T., Terenius, L., Tatemoto, K., Mutt, V., Goldstein, M., 1984h. Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. *Neuroscience* 11, 443-462.
59. Everitt, B.J., Hokfelt, T., Terenius, L., Tatemoto, K., Mutt, V., Goldstein, M., 1984i. Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. *Neuroscience* 11, 443-462.
60. Everitt, B.J., Hokfelt, T., Terenius, L., Tatemoto, K., Mutt, V., Goldstein, M., 1984a. Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. *Neuroscience* 11, 443-462.
61. Everitt, B.J., Hokfelt, T., Terenius, L., Tatemoto, K., Mutt, V., Goldstein, M., 1984b. Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. *Neuroscience* 11, 443-462.
62. Everitt, B.J., Hokfelt, T., Terenius, L., Tatemoto, K., Mutt, V., Goldstein, M., 1984c. Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. *Neuroscience* 11, 443-462.
63. Everitt, B.J., Hokfelt, T., Terenius, L., Tatemoto, K., Mutt, V., Goldstein, M., 1984d. Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. *Neuroscience* 11, 443-462.

64. Flint, W.E., 1966. Die Zwerghamster der Palaarktischen Fauna. Ziemsen-Verlag, Wittenberg, FRG.
65. Fraley, G.S., Ritter, S., 2003. Immunolesion of norepinephrine and epinephrine afferents to medial hypothalamus alters basal and 2-deoxy-d-glucose-induced neuropeptide y and agouti gene-related protein messenger ribonucleic Acid expression in the arcuate nucleus. *Endocrinology* 144, 75-83.
66. Gasteyger, C., Tremblay, A., 2002. Metabolic impact of body fat distribution. *J Endocrinol.Invest* 25, 876-883.
67. Grill, H.J., Kaplan, J.M., 2001. Interoceptive and integrative contributions of forebrain and brainstem to energy balance control. *Int.J.Obes.Relat Metab Disord.* 25 Suppl 5, S73-S77.
68. Hahn, T.M., Breininger, J.F., Baskin, D.G., Schwartz, M.W., 1998. Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. *Nat.Neurosci.* 1, 271-272.
69. Harris, R.B., Kelso, E.W., Flatt, W.P., Bartness, T.J., Grill, H.J., 2006a. Energy expenditure and body composition of chronically maintained decerebrate rats in the fed and fasted condition. *Endocrinology* 147, 1365-1376.
70. Harris, R.B., Kelso, E.W., Flatt, W.P., Bartness, T.J., Grill, H.J., 2006b. Energy expenditure and body composition of chronically maintained decerebrate rats in the fed and fasted condition. *Endocrinology* 147, 1365-1376.
71. Hollopeter, G., Erickson, J.C., Seeley, R.J., Marsh, D.J., Palmiter, R.D., 1998. Response of neuropeptide Y-deficient mice to feeding effectors. *Regul.Pept.* 75-76, 383-389.
72. Illius, A.W., Tolkamp, B.J., Yearsley, J., 2002. The evolution of the control of food intake. *Proc.Nutr.Soc.* 61, 465-472.
73. Inui, A., 1999. Neuropeptide Y feeding receptors: are multiple subtypes involved? *Trends Pharmacol.Sci.* 20, 43-46.
74. Jain, M.R., Dube, M.G., Kalra, S.P., Kalra, P.S., 1998. Neuropeptide Y release in the paraventricular nucleus is decreased during transient hyperphagia induced by microinjection of colchicine into the ventromedial nucleus of rats. *Neurosci.Lett.* 256, 21-24.
75. Kalra, P.S., Kalra, S.P., 2000. Use of antisense oligodeoxynucleotides to study the physiological functions of neuropeptide Y. *Methods* 22, 249-254.
76. Kalra, S.P., Dube, M.G., Fournier, A., Kalra, P.S., 1991a. Structure-function analysis of stimulation of food intake by neuropeptide Y: effects of receptor agonists. *Physiol.Behav.* 50, 5-9.
77. Kalra, S.P., Dube, M.G., Sahu, A., Phelps, C.P., Kalra, P.S., 1991b. Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. *Proc.Natl.Acad.Sci.USA* 88, 10931-10935.

78. Kalra, S.P., Dube, M.G., Shuye, P., Bin, X., Horvath, T.L., Kalra, P.S., 1999. Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. *Endocrine Rev.* 20, 68-100.
79. Kask, A., Rago, L., Harro, J., 1998. Evidence for involvement of neuropeptide Y receptors in the regulation of food intake: studies with Y1-selective antagonist BIBP3226. *Br.J.Pharmacol.* 124, 1507-1515.
80. Keen-Rhinehart, E., Bartness, T.J., 2005. Peripheral ghrelin injections stimulate food intake, foraging and food hoarding in Siberian hamsters. *Am.J.Physiol.* 288, R716-R722.
81. Kushi, A., Sasai, H., Koizumi, H., Takeda, N., Yokoyama, M., Nakamura, M., 1998. Obesity and mild hyperinsulinemia found in neuropeptide Y-Y1 receptor- deficient mice. *Proc.Natl.Acad.Sci.U.S.A* 95, 15659-15664.
82. Lea, S.E.G., Tarpy, R.M., 1986. Hamsters' demand for food to eat and hoard as a function of deprivation and cost. *Anim.Behav.* 34, 1759-1768.
83. Li, C., Chen, P., Smith, M.S., 1998. Neuropeptide Y (NPY) neurons in the arcuate nucleus (ARH) and dorsomedial nucleus (DMH), areas activated during lactation, project to the paraventricular nucleus of the hypothalamus (PVH). *Regul.Pept.* 75-76, 93-100.
84. Lichtensteiger, W., Hanimann, B., Siegrist, W., Eberle, A.N., 1996. Region- and stage-specific patterns of melanocortin receptor ontogeny in rat central nervous system, cranial nerve ganglia and sympathetic ganglia. *Brain Res Dev.Brain Res* 91, 93-110.
85. Marsh, D.J., Hollopeter, G., Kafer, K.E., Palmiter, R.D., 1998. Role of the Y5 neuropeptide Y receptor in feeding and obesity. *Nat.Med.* 4, 718-721.
86. Mela, D.J., Aaron, J.I., Gatenby, S.J., 1996. Relationships of consumer characteristics and food deprivation to food purchasing behavior. *Physiol Behav.* 60, 1331-1335.
87. Mercer, J.G., Lawrence, B., Beck, B., Burlet, A., Atkinson, T., Barrett, P., 1995. Hypothalamic NPY and preproNPY mRNA in Djungarian hamsters: effects of food deprivation and photoperiod. *Am.J.Physiol.* 269, R1099-R1106.
88. Mercer, J.G., Moar, K.M., Ross, A.W., Hoggard, N., Morgan, P.J., 2000a. Photoperiod regulates arcuate nucleus POMC, AGRP, and leptin receptor mRNA in Siberian hamster hypothalamus. *Am.J.Physiol.* 278, R271-R281.
89. Mercer, J.G., Moar, K.M., Ross, A.W., Morgan, P.J., 2000b. Regulation of leptin receptor, POMC and AGRP gene expression by photoperiod and food deprivation in the hypothalamic arcuate nucleus of the male Siberian hamster (*Phodopus sungorus*). *Appetite* 34, 109-111.
90. Mercer, J.G., Tups, A., 2003. Neuropeptides and anticipatory changes in behaviour and physiology: seasonal body weight regulation in the Siberian hamster. *Eur.J.Pharmacol.* 480, 43-50.

91. Mizuno, T.M., Makimura, H., Silverstein, J., Roberts, J.L., Loppingco, T., Mobbs, C.V., 1999. Fasting regulates hypothalamic neuropeptide Y, agouti-related peptide, and proopiomelanocortin in diabetic mice independent of changes in leptin or insulin. *Endocrinology* 140, 4551-4557.
92. Morgan, D.G., Small, C.J., Abusnana, S., Turton, M., Gunn, I., Heath, M., Rossi, M., Goldstone, A.P., O'Shea, D., Meeran, K., Ghatei, M., Smith, D.M., Bloom, S., 1998. The NPY Y1 receptor antagonist BIBP 3226 blocks NPY induced feeding via a non-specific mechanism. *Regul. Pept.* 75-76, 377-382.
93. Morley, J.E., Hernandez, E.N., Flood, J.F., 1987a. Neuropeptide Y increases food intake in mice. *Am.J.Physiol.* 253, R516-R522.
94. Morley, J.E., Levine, A.S., Gosnell, B.A., Kneip, J., Grace, M., 1987b. Effect of neuropeptide Y on ingestive behaviors in the rat. *Am.J.Physiol* 252, R599-R609.
95. Morton, G.J., Schwartz, M.W., 2001. The NPY/AgRP neuron and energy homeostasis. *Int.J. Obes.Relat Metab Disord.* 25 Suppl 5, S56-S62.
96. Murphy, B., Nunes, C.N., Ronan, J.J., Harper, C.M., Beall, M.J., Hanaway, M., Fairhurst, A.M., Van Der Ploeg, L.H., MacIntyre, D.E., Mellin, T.N., 1998. Melanocortin mediated inhibition of feeding behavior in rats. *Neuropeptides* 32, 491-497.
97. Nikolettseas, M.M., 1977. Obesity in exercising, hypophagic rats treated with monosodium glutamate. *Physiol Behav.* 19, 767-773.
98. O'Shea, D., Morgan, D., Meeran, K., Edwards, C., Turton, M., Choi, S., Heath, M., Gunn, I., Taylor, G., Howard, J., Bloom, C., Small, C., Haddo, O., Ma, J., Callinan, W., Smith, D., Ghatei, M., Bloom, S., 1997a. Neuropeptide Y induced feeding in the rat is mediated by a novel receptor. *Endocrinology* 138, 196-202.
99. O'Shea, D., Morgan, D.G., Meeran, K., Edwards, C.M., Turton, M.D., Choi, S.J., Heath, M.M., Gunn, I., Taylor, G.M., Howard, J.K., Bloom, C.I., Small, C.J., Haddo, O., Ma, J.J., Callinan, W., Smith, D.M., Ghatei, M.A., Bloom, S.R., 1997b. Neuropeptide Y induced feeding in the rat is mediated by a novel receptor. *Endocrinology* 138, 196-202.
100. O'Shea, D., Morgan, D.G., Meeran, K., Edwards, C.M., Turton, M.D., Choi, S.J., Heath, M.M., Gunn, I., Taylor, G.M., Howard, J.K., Bloom, C.I., Small, C.J., Haddo, O., Ma, J.J., Callinan, W., Smith, D.M., Ghatei, M.A., Bloom, S.R., 1997c. Neuropeptide Y induced feeding in the rat is mediated by a novel receptor. *Endocrinology* 138, 196-202.
101. O'Shea, D., Morgan, D.G., Meeran, K., Edwards, C.M., Turton, M.D., Choi, S.J., Heath, M.M., Gunn, I., Taylor, G.M., Howard, J.K., Bloom, C.I., Small, C.J., Haddo, O., Ma, J.J., Callinan, W., Smith, D.M., Ghatei, M.A., Bloom, S.R., 1997d. Neuropeptide Y induced feeding in the rat is mediated by a novel receptor. *Endocrinology* 138, 196-202.

102. Ogden, C.L., Carroll, M.D., Curtin, L.R., McDowell, M.A., Tabak, C.J., Flegal, K.M., 2006a. Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA* 295, 1549-1555.
103. Ogden, C.L., Carroll, M.D., Curtin, L.R., McDowell, M.A., Tabak, C.J., Flegal, K.M., 2006b. Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA* 295, 1549-1555.
104. Olney, J.W., 1969. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science* 164, 719-721.
105. Parker, R.M., Herzog, H., 1999. Regional distribution of Y-receptor subtype mRNAs in rat brain. *Eur.J Neurosci.* 11, 1431-1448.
106. Perrigo, G., Bronson, F.H., 1985. Behavioral and physiological responses of female house mice to foraging variation. *Physiol.Behav.* 34, 437-440.
107. Poston, W.S., Foreyt, J.P., 1999. Obesity is an environmental issue. *Atherosclerosis* 146, 201-209.
108. Ransley, J.K., Donnelly, J.K., Botham, H., Khara, T.N., Greenwood, D.C., Cade, J.E., 2003b. Use of supermarket receipts to estimate energy and fat content of food purchased by lean and overweight families. *Appetite* 41, 141-148.
109. Ransley, J.K., Donnelly, J.K., Botham, H., Khara, T.N., Greenwood, D.C., Cade, J.E., 2003a. Use of supermarket receipts to estimate energy and fat content of food purchased by lean and overweight families. *Appetite* 41, 141-148.
110. Ransley, J.K., Donnelly, J.K., Botham, H., Khara, T.N., Greenwood, D.C., Cade, J.E., 2003g. Use of supermarket receipts to estimate energy and fat content of food purchased by lean and overweight families. *Appetite* 41, 141-148.
111. Ransley, J.K., Donnelly, J.K., Botham, H., Khara, T.N., Greenwood, D.C., Cade, J.E., 2003f. Use of supermarket receipts to estimate energy and fat content of food purchased by lean and overweight families. *Appetite* 41, 141-148.
112. Ransley, J.K., Donnelly, J.K., Botham, H., Khara, T.N., Greenwood, D.C., Cade, J.E., 2003e. Use of supermarket receipts to estimate energy and fat content of food purchased by lean and overweight families. *Appetite* 41, 141-148.
113. Ransley, J.K., Donnelly, J.K., Botham, H., Khara, T.N., Greenwood, D.C., Cade, J.E., 2003d. Use of supermarket receipts to estimate energy and fat content of food purchased by lean and overweight families. *Appetite* 41, 141-148.
114. Ransley, J.K., Donnelly, J.K., Botham, H., Khara, T.N., Greenwood, D.C., Cade, J.E., 2003c. Use of supermarket receipts to estimate energy and fat content of food purchased by lean and overweight families. *Appetite* 41, 141-148.

115. Sahu, A., Dube, M.G., Kalra, S.P., Kalra, P.S., 1988a. Bilateral neural transections at the level of mesencephalon increase food intake and reduce latency to onset of feeding in response to neuropeptide Y. *Peptides* 9, 1269-1273.
116. Sahu, A., Dube, M.G., Kalra, S.P., Kalra, P.S., 1988b. Bilateral neural transections at the level of mesencephalon increase food intake and reduce latency to onset of feeding in response to neuropeptide Y. *Peptides* 9, 1269-1273.
117. Sahu, A., Kalra, S.P., Crowley, W.R., Kalra, P.S., 1988c. Evidence that NPY-containing neurons in the brainstem project into selected hypothalamic nuclei: implication in feeding behavior. *Brain Res.* 457, 376-378.
118. Sahu, A., Kalra, S.P., Crowley, W.R., Kalra, P.S., 1988d. Evidence that NPY-containing neurons in the brainstem project into selected hypothalamic nuclei: implication in feeding behavior. *Brain Res.* 457, 376-378.
119. Satcher, D., 2001. Overweight and obesity threaten U.S. health gains, pp. 1-3.
120. Sawchenko, P.E., Swanson, L.W., Grzanna, R., Howe, P.R., Bloom, S.R., Polak, J.M., 1985. Colocalization of neuropeptide Y immunoreactivity in brainstem catecholaminergic neurons that project to the paraventricular nucleus of the hypothalamus. *J Comp Neurol.* 241, 138-153.
121. Schaffhauser, A.O., Stricker-Krongrad, A., Brunner, L., Cumin, F., Gerald, C., Whitebread, S., Criscione, L., Hofbauer, K.G., 1997. Inhibition of food intake by neuropeptide Y Y5 receptor antisense oligodeoxynucleotides. *Diabetes* 46, 1792-1798.
122. Schneider, J.E., Buckley, C.A., 2001. Food hoarding is increased by food deprivation and decreased by leptin treatment in Syrian hamsters. *Am.J.Physiol.* 285, R1021-R1029.
123. Schuhler, S., Horan, T.L., Hastings, M.H., Mercer, J.G., Morgan, P.J., Ebling, F.J., 2003. Decrease of food intake by MC4-R agonist MTII in Siberian hamsters in long and short photoperiods. *Am.J Physiol* 284, R227-R232.
124. Schwartz, M.W., Woods, S.C., Porte, D., Jr., Seeley, R.J., Baskin, D.G., 2000. Central nervous system control of food intake. *Nature* 404, 661-671.
125. Stanley, B.G., Leibowitz, S.F., 1985. Neuropeptide Y injected in the paraventricular hypothalamus: a powerful stimulant of feeding behavior. *Proc.Natl.Acad.Sci.U.S.A* 82, 3940-3943.
126. Stanley, B.G., Leibowitz, S.F., 1984. Neuropeptide Y: stimulation of feeding and drinking by injection into the paraventricular nucleus. *Life Sci.* 35, 2635-2642.
127. Stanley, B.G., Magdalin, W., Seirafi, A., Nguyen, M.M., Leibowitz, S.F., 1992. Evidence for neuropeptide Y mediation of eating produced by food deprivation and for a variant of the Y1 receptor mediating this peptide's effect. *Peptides* 13, 581-587.

128. Stanley, B.G., Magdalin, W., Seirafi, A., Thomas, W.J., Leibowitz, S.F., 1993. The perifornical area: the major focus of (a) patchily distributed hypothalamic neuropeptide Y-sensitive feeding system(s). *Brain Res.* 604, 304-317.
129. Steinman, J.L., Gunion, M.W., Morley, J.E., 1994a. Forebrain and hindbrain involvement of neuropeptide Y in ingestive behaviors of rats. *Pharmacol.Biochem.Behav.* 47, 207-214.
130. Steinman, J.L., Gunion, M.W., Morley, J.E., 1994b. Forebrain and hindbrain involvement of neuropeptide Y in ingestive behaviors of rats. *Pharmacol.Biochem.Behav.* 47, 207-214.
131. Steinman, J.L., Gunion, M.W., Morley, J.E., 1994c. Forebrain and hindbrain involvement of neuropeptide Y in ingestive behaviors of rats. *Pharmacol.Biochem.Behav.* 47, 207-214.
132. Stricker-Krongrad, A., Beck, B., 2004e. Up-regulation of neuropeptide Y receptors in the hypothalamus of monosodium glutamate-lesioned Sprague-Dawley rats. *Nutr.Neurosci.* 7, 241-245.
133. Stricker-Krongrad, A., Beck, B., 2004f. Up-regulation of neuropeptide Y receptors in the hypothalamus of monosodium glutamate-lesioned Sprague-Dawley rats. *Nutr.Neurosci.* 7, 241-245.
134. Stricker-Krongrad, A., Beck, B., 2004d. Up-regulation of neuropeptide Y receptors in the hypothalamus of monosodium glutamate-lesioned Sprague-Dawley rats. *Nutr.Neurosci.* 7, 241-245.
135. Stricker-Krongrad, A., Beck, B., 2004c. Up-regulation of neuropeptide Y receptors in the hypothalamus of monosodium glutamate-lesioned Sprague-Dawley rats. *Nutr.Neurosci.* 7, 241-245.
136. Stricker-Krongrad, A., Beck, B., 2004b. Up-regulation of neuropeptide Y receptors in the hypothalamus of monosodium glutamate-lesioned Sprague-Dawley rats. *Nutr.Neurosci.* 7, 241-245.
137. Stricker-Krongrad, A., Beck, B., 2004a. Up-regulation of neuropeptide Y receptors in the hypothalamus of monosodium glutamate-lesioned Sprague-Dawley rats. *Nutr.Neurosci.* 7, 241-245.
138. Stricker-Krongrad, A., Beck, B., Burlet, C., 1996a. Enhanced feeding response to neuropeptide Y in hypothalamic neuropeptide Y-depleted rats. *Eur.J.Pharmacol.* 295, 27-34.
139. Stricker-Krongrad, A., Beck, B., Burlet, C., 1996b. Enhanced feeding response to neuropeptide Y in hypothalamic neuropeptide Y-depleted rats. *Eur.J.Pharmacol.* 295, 27-34.
140. Stricker-Krongrad, A., Beck, B., Nicolas, J.P., Burlet, C., 1992. Central effects of monosodium glutamate on feeding behavior in adult Long-Evans rats. *Pharmacol.Biochem.Behav.* 43, 881-886.

141. Torri, C., Pedrazzi, P., Leo, G., Muller, E.E., Cocchi, D., Agnati, L.F., Zoli, M., 2002. Diet-induced changes in hypothalamic pro-opio-melanocortin mRNA in the rat hypothalamus. *Peptides* 23, 1063-1068.
142. Vague, J., Vague, P., Tramon, M., Vialettes, B., Mercier, P., 1980. Obesity and diabetes. *Acta Diabetol.Lat.* 17, 87-99.
143. Vander Wall, S.B., 1990. Food hoarding in animals. Univ. Chicago Press, Chicago.
144. Weiner, J., 1987. Limits to energy budget and tactics in energy investments during reproduction in the Djungarian hamster (*Phodopus sungorus sungorus* Pallas 1770). *Symp.zool.Soc.Lond.* 57, 167-187.
145. White, J.D., Kershaw, M., 1989. Increased hypothalamic neuropeptide Y expression following food deprivation. *Mol.Cell.Neurosci.* 1: 41-48.
146. Wieland, H., Engel, W., Eberlein, W., Rudolf, K., Doods, H., 1998. Subtype selectivity of the novel nonpeptide neuropeptide Y Y1 receptor antagonist BIBO 3304 and its effect on feeding in rodents. *Br.J Pharmacol.* 125, 549-555.
147. Wiley, R.G., Kline IV, R.H., 2000. Neuronal lesioning with axonally transported toxins. *J.Neurosci.Methods* 103, 73-82.
148. Wirth, M.M., Giraudo, S.Q., 2000. Agouti-related protein in the hypothalamic paraventricular nucleus: effect on feeding. *Peptides* 21, 1369-1375.
149. Wood, A.D., Bartness, T.J., 1996. Food deprivation-induced increases in hoarding by Siberian hamsters are not photoperiod-dependent. *Physiol Behav.* 60, 1137-1145.
150. Yokosuka, M., Dube, M.G., Kalra, P.S., Kalra, S.P., 2001. The mPVN mediates blockade of NPY-induced feeding by a Y5 receptor antagonist: a c-FOS analysis. *Peptides* 22, 507-514.
151. Yoshihara, T., Honma, S., Honma, K., 1996b. Prefeeding release of paraventricular neuropeptide Y is mediated by ascending noradrenergic neurons in rats. *Am.J.Physiol* 270, E596-E600.
152. Yoshihara, T., Honma, S., Honma, K., 1996c. Prefeeding release of paraventricular neuropeptide Y is mediated by ascending noradrenergic neurons in rats. *Am.J.Physiol* 270, E596-E600.
153. Yoshihara, T., Honma, S., Honma, K., 1996a. Effects of restricted daily feeding on neuropeptide Y release in the rat paraventricular nucleus. *Am.J.Physiol.Endocrinol.Metab.* 270, E589-E595.
154. Ziotopoulou, M., Mantzoros, C.S., Hileman, S.M., Flier, J.S., 2000. Differential expression of hypothalamic neuropeptides in the early phase of diet-induced obesity in mice. *Am.J.Physiol Endocrinol.Metab* 279, E838-E845.